A Comprehensive Dna Test For The Detection Of Translocations In Acute Leukemia

Van den Berg-De Ruiter, E.; Alimohamed, M. Z.; Johansson, L. F.; de Boer, E. N.; Splinter, E.; Klous, P.; Bosga, A. G.; van Min, M.; Mulder, A. B.; Vellenga, E.

Published in:
Haematologica

EUROPEAN HEMATOLOGY ASSOCIATION

haematologica

Journal of the European Hematology Association
Owned & published by the Ferrata Storti Foundation

22nd Congress of the European Hematology Association
Madrid, Spain, June 22 - 25, 2017

ABSTRACT BOOK

ISSN 0390-6078

Volume 102
JUNE
2017|s2

EUROPEAN HEMATOLOGY ASSOCIATION

haematologica
Journal of the European Hematology Association
Owned & published by the Ferrata Storti Foundation

22nd Congress of the European Hematology Association

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haematologica

Journal of the European Hematology Association
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ISSN 0390-6078

The abstract book of the 22nd Congress of the European Hematology Association is published as a supplement of Haematologica/the Hematology Journal in one volume per year.

All business correspondence and purchase and reprint requests should be addressed either to Haematologica Journal Office, via Giuseppe Belli 4, 27100 Pavia, Italy; phone: +39 0382 27129; fax: +39 0382 394705; e-mail: office@haematologica.org or to the European Hematology Association, Koninginnegracht 12b, 2514 AA The Hague, The Netherlands; phone: +31 (0)70 345 55 63; fax: + 31 (0)70 392 36 63; e-mail: info@ehaweb.org.

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Article Citations
Cite articles in this volume as follows:

TITLE. AUTHORS. JOURNAL YEAR; VOLUME(SUPPLEMENT NO):PAGE. Abstract n. XXX
Example: RITUXIMAB CONSOLIDATION AND MAINTENANCE THERAPY PROLONG RESPONSE DURATION IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA
G. Del Poeta, M.I. Del Principe, A. Siniscalchi, L. Maurillo, F. Buccisano, A. Venditti, F. Luciano, P. Niscola, A. Zucchetto, V. Gattei, A.P. Perrotti, P. De Fabritiis, S. Amadori
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Word of Welcome

On behalf of the EHA Board and the Scientific Program Committee we are pleased to introduce to you this year's Abstract Program. The richness of the program is a testament to EHA's spirit: unity through diversity.

The Scientific Program Committee has compiled an exciting program of Simultaneous Oral and Poster Sessions from close to 2500 submitted abstracts representing all fields of hematology. For the second year, a number of presenters will have the opportunity to pitch their abstract. These Poster pitches are an exciting opportunity to promote basic science and research, and to invite delegates to the poster walks.

The six Best Abstracts will be presented during the Presidential Symposium on Friday afternoon. This will be a session not to miss. During this plenary session EHA is also awarding, for the first time, the best abstracts by trainees in four categories in basic and clinical hematology research. These awardees and the travel grant winners can be found on the next page. YoungEHA are the future of hematology!

The late breaking abstract submission is an integral part of the scientific program. The late breaking submission is intended for abstracts with "hot" data that were not available by the time of the regular submission deadline. Only few abstracts, with the most exciting results are selected for a presentation in the Late Breaking Oral Session on Sunday morning.

A selection of abstracts will be presented during the regular Poster Walks. The Poster Session consists of two parts: the Poster Walk and dedicated Poster Browsing Time. This setup guarantees sufficient time for discussion of the important research presented, so look out for the Poster Walk Moderators in their red baseball caps! There will also be E-posters available on the E-poster screens, for which a specific time is allocated during the Poster Browsing Time at the end of each Walk. The Simultaneous Oral Sessions are spread over three days (Friday to Sunday) providing you with ample opportunity to attend a number of these important sessions.

All posters can be viewed on the E-poster screens from Friday morning to Saturday evening. All the abstracts are also available on the EHA Learning Center, for which you have complimentary access after the congress: learningcenter.ehaweb.org.

On behalf of the EHA Board, the committees and all the people involved in this year's EHA Congress, we thank you for coming to Madrid and wish you a great meeting.

Shai Izraeli

Chair Scientific Program Committee 22nd Congress

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Travel Grant Winners

For this Congress 140 travel grants have been awarded to junior members of EHA, based on the mean score of their abstracts.
EHA congratulates the following persons with their travel grants:

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YoungEHA Best Abstract Awards

One of the primary missions of the European Hematology Association is to support young hematology clinicians and researchers. This year we are proud to announce the launching of the YoungEHA Best Abstract Awards. These will be awarded to the highest ranking abstracts in the following four categories: Clinicians or medical students training for a PhD degree, PhD research students, postdoctoral fellows and clinical hematology trainees. We are honored that these outstanding YoungEHA trainees will be presenting during the EHA congress – they are the future of Hematology!

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Direttore responsabile: Prof. Edoardo Ascari; Autorizzazione del Tribunale di Pavia n. 63 del 5 marzo 1955.
Printing: Tipografia PI-ME, via Vigentina 136, Pavia, Italy. Printed in June 2017.

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Late Breaking Oral Session

The best abstracts selected from the late breaking abstract submission are presented during this oral session.

A complete session overview is available via the mobile app or the online program at ehaweb.org

SIMULTANEOUS SESSIONS I

New advances in plasma cell disorders and implications for therapy

S100

NEXT GENERATION SEQUENCING METHODOLOGY FOR DETERMINING CYTOGENETIC RISK STATUS IN THE DARATUMUMAB PHASE 3 CASTOR AND POLLUX STUDIES IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA

C. Chiu[1,*], D. Soong[1], I. Spicka[2], M. Beksac[3], M. Schaffer[1], J. Schecter[4], N. J. Bahlis[5], M. A. Dimopoulos[6]
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Background: Cytogenetic risk status in multiple myeloma (MM) studies is traditionally determined by using fluorescence *in situ* hybridization (FISH) or karyotyping to assess chromosomal abnormalities. However, these technologies have limited resolution and a narrow target range, and reproducible interpretation may be confounded by inter-laboratory variation.
Aims: To describe the NGS methodology used to determine cytogenetic risk status in the daratumumab phase 3 CASTOR and POLLUX studies in RRMM.
Methods: Bone marrow aspirates were collected at screening and assessed centrally via NGS. Whole exome sequencing (exome-seq) and RNA sequencing (RNA-seq) was performed using the Illumina HiSeq platform to identify the presence or absence of defined risk markers: t(4;14), t(14;16), or del17p. The use of RNA-seq allowed for investigation of chromosomal translocations in expressed genomic locations at a higher resolution than FISH, and exome-seq data was used to derive the copy number status in coding regions across the genome. RNA-seq was performed using total RNA and rRNA removal to capture translocations involving coding and intronic regions. Translocation calls were made using two fusion callers, and gene expression was quantified to allow for evaluation of genes associated with translocation events. For t(4;14) translocations, the detected events involved RNA-seq reads fused between IgH and WHSC1 or FGFR3. For t(14;16), the detected translocations involved IgH and WWOX. Manual inspection of patients with t(4;14) showed higher WHSC1 or FGFR3 expression, whereas t(14;16) patients showed higher MAF and CCND2 expression. For del17p detection, exome data of each tumor was compared against 100 peripheral blood mononuclear cell (PBMC) control samples from CASTOR and POLLUX studies. Copy number variation data from two callers were combined to utilize information on relative read depth, systematic biases (observed in pooled normal controls), as well as SNP allele frequency (indicative of loss of heterozygosity events). A del17p event was detected when >50% of the 17p region was deleted.
Results: Based on the RNA-Seq and exome results, cytogenetic risk status in the CASTOR and POLLUX studies was defined as high risk with having either t(4;14), t(14;16), or del17p, and standard risk with the confirmed absence of these molecular abnormalities. Comparisons of NGS with FISH showed high concordance for t(4;14), t(14;16), and del17p in both studies (Table 1).

Table 1.

Concordance rate between FISH and NGS	POLLUX	CASTOR
t(4;14)	96%	92%
t(14;16)	98%	97%
del17p	88%	90%

PFS analyses investigating differences between treatment groups and between risk groups using FISH-derived risk and NGS-derived risk showed consistent results between FISH and NGS, with improvements in PFS being associated with the addition of daratumumab to standard-of-care regimens in both high- and standard-risk subgroups (Figure 1).
Summary/Conclusions: These studies represent the first, comprehensive use of NGS in global phase 3 clinical trials in RRMM. The NGS methodology accurately identified the presence of defined risk populations t(4;14), t(14;16), and del17p and showed good concordance with FISH. As FISH was performed locally with different probes and pathologists, the high degree of concordance between FISH and NGS is notable and supports the use of NGS for determining cytogenetic risk in patients with RRMM. The utility of NGS in these clinical studies extends far beyond the detection of cytogenetic abnormalities and additional analysis are planned to interrogate these datasets in the identification of novel biomarkers.

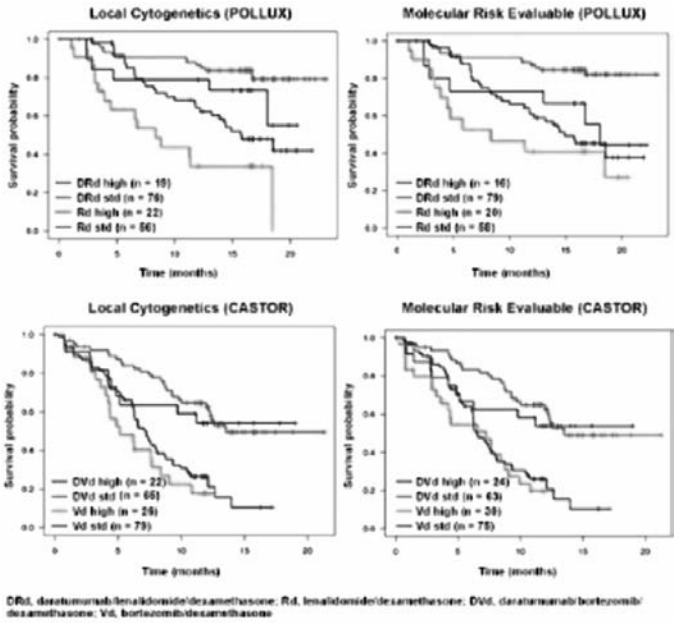

Figure 1.

S101

EFFICACY BY CYTOGENETIC RISK STATUS FOR DARATUMUMAB IN COMBINATION WITH LENALIDOMIDE AND DEXAMETHASONE OR BORTEZOMIB AND DEXAMETHASONE IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA

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Background: Daratumumab (D) is a human CD38-targeting monoclonal antibody that exerts its antimyeloma activity through both direct (on-tumor) and indirect (immunomodulatory) mechanisms of action. Two randomized phase 3 trials in patients with relapsed or refractory multiple myeloma (RRMM) demonstrated that combining D with the standard-of-care regimens lenalidomide +dexamethasone (Rd, POLLUX) or bortezomib +dexamethasone (Vd, CASTOR)

significantly improved progression-free survival (PFS) and achieved higher overall response rates (ORRs) compared with the respective standard-of-care regimen alone (Dimopoulos MA *et al.*, *N Engl J Med* 2016;375(14):1319-1331; Palumbo A *et al.*, *N Engl J Med* 2016;375(8):754-766.). Due to its novel mechanisms of action, addition of D to standard-of-care regimens may benefit RRMM patients who have poor prognoses resulting from high-risk cytogenetic abnormalities.

Aims: To examine the efficacy of DRd and DVd in RRMM patients with standard or high cytogenetic risk status.

Methods: Bone marrow aspirates were collected at screening visits from 311/569 patients from POLLUX and from 353/498 patients from CASTOR, and cytogenetic abnormalities were detected via next-generation sequencing (NGS). Patients were considered to be of high cytogenetic risk status if they had ≥1 of the following abnormalities: t(4;14), t(14;16), or del17p; patients were considered to be of standard cytogenetic risk if they lacked these abnormalities. Minimal residual disease (MRD) was assessed at suspected complete response (CR) at 3 sensitivity thresholds (10^{-4}, 10^{-5}, and 10^{-6}) using the ClonoSEQ™ NGS-based assay (Adaptive Biotechnologies, Seattle, WA). Efficacy analyses included PFS, ORR, and MRD-negative rates.

Results: For POLLUX, the median follow-up was 17.3 months. Treating high-risk patients with DRd significantly prolonged median PFS *vs* Rd (top panel Figure 1) and numerically increased ORR (85% *vs* 67%; *P*=0.14). Responses to DRd *vs* Rd included CR or better in 33% *vs* 6% of these patients, and very good partial responses (VGPR) or better in 63% *vs* 31%. In standard-risk patients, DRd *vs* Rd also resulted in significant improvements in median PFS (Figure 1) as well as ORR (95% *vs* 82%; *P*=0.0020). Responses to DRd *vs* Rd included CR or better in 52% *vs* 24% of these patients, and VGPR or better in 84% *vs* 51%. At 10^{-5} sensitivity threshold, MRD-negative rates for DRd *vs* Rd were 18% *vs* 0% (*P*=0.0027) among high-risk patients and 30% *vs* 10% (*P*<0.0001) for standard-risk patients. For CASTOR, the median follow-up was 13.0 months. Treating both high- and standard-risk patients with DVd *vs* Vd significantly prolonged median PFS (bottom panel Figure 1) and increased ORR (high risk: 82% *vs* 62%; *P*=0.039; standard risk: 85% *vs* 64%; *P*=0.0003). Responses to DVd *vs* Vd among high-risk patients included CR or better in 30% *vs* 9% of patients and VGPR or better in 64% *vs* 34%; among standard-risk patients, responses included CR or better in 25% *vs* 8% of patients and VGPR or better in 64% *vs* 27%. At 10^{-5} sensitivity threshold, MRD-negative rates for DVd *vs* Vd were 14% *vs* 0% (*P*=0.0018) among high-risk patients and 12% *vs* 2% (*P*=0.0011) for standard-risk patients.

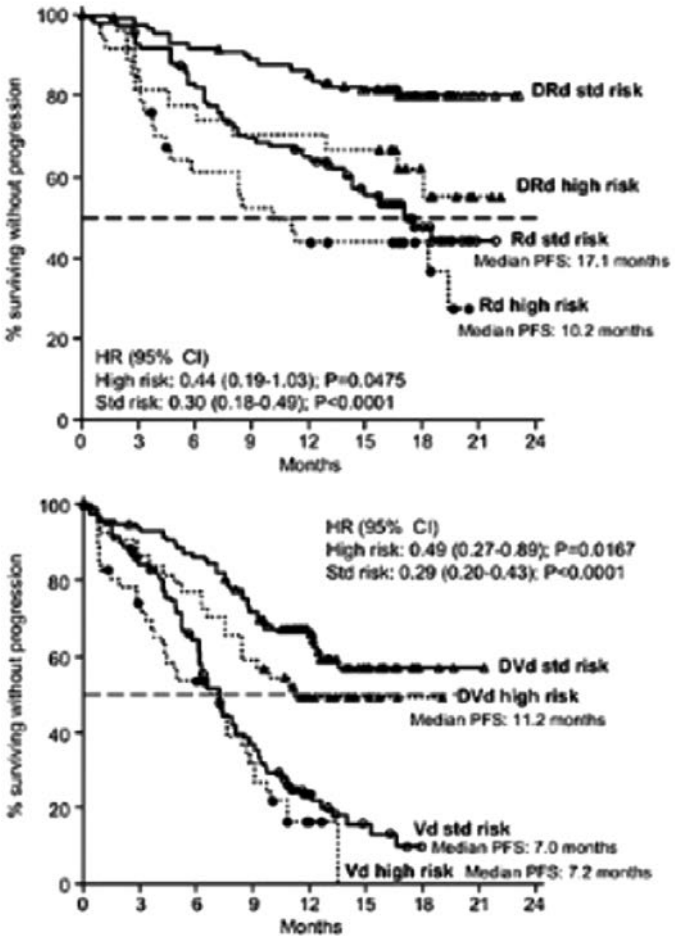

Figure 1.

Summary/Conclusions: Adding D to Rd or Vd improved treatment outcomes irrespective of cytogenetic risk status in patients with RRMM. Both DRd and DVd appear to benefit RRMM patients who have poor prognoses due to high-risk cytogenetic abnormalities. Updated data, including analyses based on individual cytogenetic abnormalities, will be presented at the meeting based on longer follow-up.

S102

MINIMAL RESIDUAL DISEASE BY MULTIPARAMETER FLOW CYTOMETRY IN TRANSPLANT ELIGIBLE PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA: RESULTS FROM THE EMN02/HO95 PHASE 3 TRIAL

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Background: Multiple myeloma (MM) is still an incurable disease and patients may relapse despite achievement of complete remission (CR). Available data show that MRD detection is a sensitive strategy to appropriately measure response in MM patients.

Aims: We evaluated MRD by MFC in patients with newly diagnosed MM enrolled in the EMN02/HO95 phase 3 trial.

Methods: Patients were ≤65 years of age and treatment consisted of Bortezomib-Cyclophosphamide-Dexamethasone (VCD) induction, mobilization and stem cell collection, intensification with Bortezomib-Melphalan-Prednisone (VMP) *vs* High-Dose-Melphalan (HDM) followed by stem cell transplant, consolidation with Bortezomib-Lenalidomide-Dexamethasone (VRD) *vs* no consolidation, and Lenalidomide maintenance. MRD was assessed in patients achieving at least a very good partial response (VGPR) before starting maintenance (after HDM, VMP or VRD) and during maintenance every 6-12 months; samples were centralized to 3 European labs. MFC was performed on bone marrow according to Euroflow-based methods (8 colors, 2 tubes) with a sensitivity of 10^{-5}. Quality checks were done to compare sensitivity and to show correlation between protocols (Hofste op Bruinink D, *ASH* 2016 abstract 2072).

Results: A total of 316 patients could be evaluated before maintenance: median age was 57 years (IQR: 52-62), 18% (57/316) had ISS III and 22% (70/316) had high risk cytogenetic abnormalities defined as presence of either one among del17, t(4;14) or t(14;16); 63% (199/316) had received HDM and 37% (117/316) VMP; thereafter 51% (160/316) had received VRD. After a median follow-up of 30 months from MRD enrolment, 76% (239/316) patients were MRD-negative: 64% (153/239) in the HDM *vs* 36% (86/239) in the VMP groups. The 3-year PFS was 50% in MRD-positive *vs* 77% in MRD-negative patients (HR 2.87, 95% CI: 1.75 - 4.72; p<0.001). Subgroup analyses were carried out to assess the risk factors for MRD-positivity according to baseline characteristics and therapies: high risk cytogenetic abnormalities were the most important risk factors (HR 9.87, 95% CI: 4.3 – 22.63; interaction-p=0.001). Finally, 48% of MRD positive patients at pre-maintenance who had a second MRD evaluation after at least 1 year of lenalidomide became MRD-negative.

Summary/Conclusions: MRD by MFC is a strong prognostic factor in MM patients receiving intensification with novel agents or transplant; lenalidomide maintenance further improved depth of response; high risk cytogenetic abnormalities are the most important prognostic factors in MRD-positive patients.

S103

PHASE I, OPEN-LABEL TRIAL OF ANTI-BCMA CHIMERIC ANTIGEN RECEPTOR T CELLS IN PATIENTS WITH RELAPSED/ REFRACTORY MULTIPLE MYELOMA

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Background: Immunotherapy has emerged as a potentially curative treatment in hematological malignancies. Uniformly expressed in plasma cells, B-cell maturation antigen (BCMA) is an appropriate target antigens for CAR T-cell therapies in multiple myeloma.

Aims: This phase I, open-label trial was conducted to assess the efficacy and

safety profile of LCAR-B38M anti-BCMA CAR T cells in patients with relapsed/refractory multiple myeloma.
Methods: All patients underwent leukapheresis to obtain peripheral blood mononuclear cells and their T cells were engineered to express anti-BCMA CAR. Three doses of 300 mg/m^2 cyclophosphamide were administered on day -5, -4, and -3 (before the recruitment, patients took the same chemotherapy to identify they were refractory to cyclophosphamide monotherapy) and engineered-T cells were reinfused on day 0, 2, and 6. This trial was divided into the dose escalation stage and expansion cohort. Toxicity and responses were assessed according to the Common Terminology Criteria for Adverse Events (version 4.0) and International Myeloma Working Group (IMWG) Uniform Response Criteria for Multiple Myeloma, respectively.
Results: As of the February 20th, 2017 data cut-off, 22 patients had been enrolled, two of whom were diagnosed as plasma cell leukemia. The male: female ratio was 11:11 and median age was 53.5 years. Chromosomal abnormalities were detectable by FISH in eight patients, two of whom involved in the deficiency of p53. Eleven patients were triple refractory (chemotherapy, proteasome inhibitors, and immunomodulatory drugs), 11 resisted to double prior treatments (chemotherapy and proteasome inhibitors/ immunomodulatory drugs), and four relapsed after autologous hematopoietic stem cell transplant. The median number of infused CAR T cells was 4.0×10E6 (range, 1.5×10E6-7.0×10E6) per kg. The median follow-up was 131.5 (range, 29-327) days. 100% of patients achieved an objective response. The first six patients achieved complete responses with flow MRD-negative; 14 patients achieved very good partial responses; one patient, with renal failure, achieved partial response; all these 22 patients had kept their best response at the end of follow-up. The pictures we enclosed were the subcutaneous nodules in one patient with extramedullary plasmacytoma. We found that the nodules were obviously decreased after the infusion and disappeared finally. Another one achieved transient partial response, which last for 12 days. He then took the secondary infusion but failed since the post-operation large-dose administration of corticosteroid for spinal meningioma. He terminally died of the progression of myeloma. The most common toxicity attributable to CAR T cells was cytokine release syndrome (CRS). Toxicities were minimal except for two grade 3 CRS and one grade 4 CRS. All CRSs were controllable with nonsteroidal anti-inflammatory drugs (NSAIDs) or tocilizumab and no dose-limiting toxicities or treatment-related deaths were observed (Figure 1).

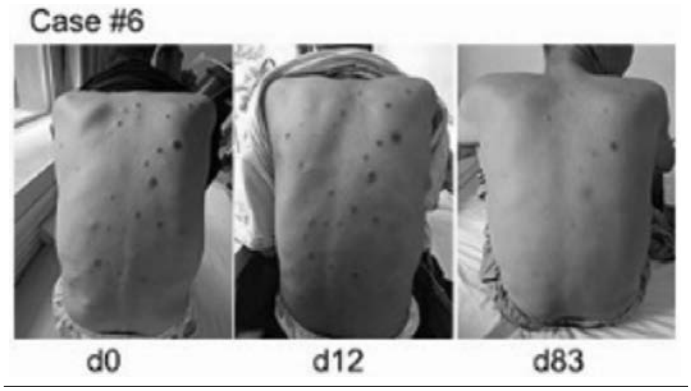

Figure 1.

Summary/Conclusions: Our findings demonstrated the safety and antimyeloma activity of LCAR-B38M anti-BCMA CAR T cells.

S104

PATIENTS WITH LIGHT CHAIN AMYLOIDOSIS TREATED WITH NEOD001 ACHIEVE RAPID ORGAN RESPONSES THAT ARE INDEPENDENT OF PREVIOUS PLASMA CELL–DIRECTED THERAPIES

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Background: Light chain (AL) amyloidosis is a rare and often fatal disease caused by the accumulation of misfolded light chain (LC) aggregates that can lead to progressive failure of critical organs, causing significant morbidity and mortality. Patients' survival depends upon rapid suppression of the misfolded LC and stabilization or recovery of organ function. Current therapies limit LC production; however, ~75% of patients have persistent organ dysfunction. NEOD001 is a novel investigational monoclonal antibody that targets misfolded LC and may neutralize circulating LC aggregates and clear insoluble deposits.
Aims: To assess the association between responses and time, depth, number or type of previous plasma cell–directed (PCD) treatments and organ response.
Methods: Inclusion criteria for this trial were: completed ≥1 PCD treatment before enrollment, attained partial hematologic response (HR) or better to any previous therapy, and have persistent organ dysfunction. NEOD001 was administered intravenously every 28 days. During the dose-escalation phase, 27 patients received NEOD001 at 0.5, 1, 2, 4, 8, 16, or 24 mg/kg in a 3+3 study design. In the expansion phase, 42 additional patients with renal, cardiac, or nerve involvement were enrolled and treated (24 mg/kg). We assessed cardiac and renal best responses based on consensus criteria. Peripheral nervous system (PN) responses were assessed at month 10 (after 9 infusions) using the Neuropathy Impairment Score–Lower Limbs (NIS-LL). We explored the potential impact on organ response of the number and type of organs affected and the number of, type of, and time since previous therapies at baseline.
Results: In the overall population (N=69), the median age was 61 years (61% male). Median (range) time since diagnosis was 2.9 (0.4-16.0) years, and 45% of patients underwent ≥3 previous PCD regimens. Median time to first best response was 1.8 (cardiac), 3.7 (renal), and 1.0 (PN) months. Best response rate indicating organ response was observed in 53% of cardiac-evaluable patients (n=19/36) and 64% of renal-evaluable patients (n=23/36). PN responses were observed in 82% (n=9/11) of PN-evaluable patients. Time from patients' best HR to previous PCD treatment was not related to the attainment of NEOD001 organ response (responder/stable: 35.6/36/6 months [cardiac] and 30.6/32.5 months [renal]; P>0.05). Depth of patients' best HR also was not related to the attainment of NEOD001 organ response (percentage of patients with organ response in CR/VGPR/PR after PCD: 47.1/66.7/42.9% [cardiac] and 68.8/63.6/62.5% [renal]; P>0.05). Similarly, time or depth of patients' last HR did not impact the NEOD001 organ response rate (P>0.05). Patients with NEOD001 organ responses were no more likely to have had their last PCD therapy <6 than ≥6 months from their first NEOD001 dose. Patients' previous PCD treatment type was not related to the attainment of NEOD001 organ response (percentage of patients undergoing: stem cell transplantation, 55.6/61.1% [cardiac/renal]; bortezomib-based therapy, 52.0/68.8%; or other chemotherapy, 50.0/57.1%; P>0.05). Exploratory analyses showed no association between the time to response or percentage of responders and the number of previous PCD treatments.
Summary/Conclusions: NEOD001 specifically targets disease-causing, misfolded LC aggregates in AL amyloidosis. Organ responses in patients treated with monthly NEOD001 infusions were achieved rapidly and independently of time since previous chemotherapy, depth of hematologic response, or predominant type of PCD treatment.

Aggressive Non-Hodgkin lymphoma - 1st line

S105

RITUXIMAB MAINTENANCE AFTER AUTOLOGOUS TEM CELL TRANSPLANTATION PROLONGS SURVIVAL IN YOUNGER PATIENTS WITH MANTLE CELL LYMPHOMA: FINAL RESULTS OF THE LYMA TRIAL OF THE LYSA/GOELAMS GROUP

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Background: Mantle cell lymphoma (MCL) is currently an incurable disease. In spite of high complete response rates (CR) after initial immunochemotherapy induction followed by autologous stem cell transplantation (ASCT), MCL patients experience iterative relapses.
Aims: We investigated whether or not rituximab maintenance (RM; 375mg/m^2 every 2 months for 3 years) after ASCT prolongs response duration.
Methods: This phase III trial included 299 patients (<66y) at diagnosis, of whom 240 were randomly assigned to RM or observation after ASCT. The primary end point was event-free survival (EFS) (progression, relapse, death, severe infection during RM) after ASCT.
Results: After 4 courses of immunochemotherapy induction (R-DHAP; Rituximab, dexamethasone, cytarabine, platinium derivative), overall response and CR rates were 89.3% and 77.3%, respectively. ASCT was performed in 257 patients. Median follow-up from randomization after ASCT was 50.2 (46.4-54.2) months. Starting from randomization, 4-year EFS was 78.9% (95%CI; 69.5 to 85.6) for RM (n=120) *versus* 61.4% (95%CI; 51.3 to 69.9) for observation (n=120) (p=0.0012), 4-year progression-free survival (PFS) was 82.2% (95%CI; 73.2 to 88.4) for RM *versus* 64.6% (95%CI; 54.6 to 73) for observation (p=0.0005) and OS was 88.7% (95%CI; 80.7 to 93.5) for RM *versus* 81.4% (95%CI; 72.3 to 87.7) for observation (p=0.0413). The death rate was lower for patients in the RM arm were less likely to die (hazard ratio (HR)=0.5; 95%CI, 0.255 to 0.986) than for patients in the observation arm.
Summary/Conclusions: The LyMa trial demonstrates for the first time that RM after ASCT prolongs EFS, PFS and OS. Thus, 4 courses of R-DHAP plus ASCT (without TBI) followed by RM maintenance (one infusion every 2 month for 3 years) is a new standard of care for young MCL patients.

S106

POLA-R-CHP: POLATUZUMAB VEDOTIN COMBINED WITH RITUXIMAB, CYCLOPHOSPHAMIDE, DOXORUBICIN, PREDNISONE FOR PATIENTS WITH PREVIOUSLY UNTREATED DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Polatuzumab vedotin (pola) is an antibody drug conjugate containing the anti-mitotic MMAE targeting CD79b, an antigen expressed ubiquitously in DLBCL. Pola as monotherapy and in combination with anti-CD20 antibodies demonstrated encouraging efficacy in r/r DLBCL.[1,2] The initial dose-escalation portion of this multicenter, open-label Ph Ib/II study of pola in combination with rituximab, cyclophosphamide, doxorubicin, and prednisone (pola-R-CHP) showed an acceptable safety profile and established a recommended Ph II dose of pola at 1.8 mg/kg.[3] We report updated safety and efficacy results for the Ph II dose in 45 previously untreated DLBCL patients (pts) (ClinicalTrials.gov NCT01992653).
Aims: To evaluate the safety and efficacy of pola-R-CHP as first-line treatment in patients with DLBCL.
Methods: Five pts of the dose escalation phase and the 40 pts of the expansion phase were included in this analysis. All pts provided informed consent to participate in the study. All had newly diagnosed DLBCL and were treated with pola at 1.8 mg/kg and R-CHP at standard doses every 21 days for 6 or 8 cycles. Investigator assessments for anti-tumor activity were performed according to IWG 2007 following 4 cycles and at the end of study treatment (EOT).
Results: All 45 pts received at least one dose of study drug. The median age was 69 years; 93% were >60 years, 33% ECOG >1, 82% Stage III/IV, and 78% IPI 3-5. Of the 29 pts with cell of origin (COO) status by digital gene expression, 11 (38%) were ABC, 14 (48%) were GCB, while 4 (14%) were unclassified. Forty patients completed 6 or 8 cycles (23 and 17 pts respectively). All pts experienced at least one AE. Grade (Gr) 3/4 AEs occurred in 58%, and one pt experienced a Gr 5 atrial fibrillation. Gr 3/4 neutropenia and febrile neutropenia (FN) occurred in 27% and 11%. Serious adverse events (SAEs) were reported in 17 pts (38%) including 3 FN, and 2 each of neutropenia, pneumonia, pulmonary embolism and influenza A. Peripheral neuropathy (PN) occurred in 18 (40%) patients. Among these pts with PN, 12 were Gr 1, 4 were Gr 2, and 2 were Gr 3. All Gr 2/3 PN attributed to pola occurred at C5 or later. Four pts discontinued pola early for the following reasons: Gr 5 atrial fibrillation (after C2, not attributed to pola by investigator), E. coli UTI (C5), worsening essential tremor (C3), PN (C7). During treatment, 6 pts had dose reductions in pola and 1 pt had cyclophosphamide and doxorubicin dose reductions. ORR by PET at EOT was 91%; 78% had a CR and 13% PR. 3 pts progressed and 1 was unevaluable. In the COO determined population, CR was 91% in ABC and 86% in GCB pts. At the data cutoff of November 4, 2016 with a median study duration of 9.5 months, (range 1.3-28 months), only 1 pt had a disease progression in follow up.
Summary/Conclusions: Pola at 1.8 mg/kg in combination with R-CHP in 1L DLBCL has an acceptable safety profile and produced promising response rates at the end of treatment. The majority of the patients in this trial represented a poor prognosis group by age and IPI. In this context, treatment response to this regimen may warrant further exploration.

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S107

RITUXIMAB SC AND IV PLUS CHOP SHOW SIMILAR EFFICACY AND SAFETY IN THE RANDOMIZED MABEASE STUDY IN FIRST-LINE DLBCL

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Background: Intravenous (IV) rituximab plus chemotherapy is standard treatment for diffuse large B-cell lymphoma (DLBCL). A subcutaneous (SC) formulation of rituximab may simplify treatment and reduce burden.
Aims: MabEase (NCT01649856) studied efficacy, safety and patient (pt) satisfaction with rituximab SC or IV plus CHOP as first-line DLBCL treatment.
Methods: Pts were randomized 2:1 to rituximab SC (IV 375mg/m^2 cycle 1; SC 1400mg cycles 2–8) or IV (375mg/m^2 cycles 1–8) plus CHOP every 14 or 21 days. The primary endpoint was investigator-assessed complete response (CR)/unconfirmed CR (CRu) at the end of induction (EOI). Secondary endpoints included safety, survival, treatment satisfaction (Cancer Treatment Satisfaction Questionnaire [CTSQ], Rituximab Administration Satisfaction Questionnaire [RASQ]) and time savings. Follow-up continued until at least 24 months after EOI in the last patient recruited.
Results: Of 576 pts (381 SC; 195 IV), 572 (378 SC; 194 IV) received treatment. EOI CR/CRu rates were 50.6% (95% CI 45.3–55.9) and 42.4 (95% CI 35.1–49.7) in the SC and IV groups, respectively (Table 1). After 35 months' median follow-up, median progression-free survival (PFS), event-free survival (EFS) and overall survival (OS) were not reached in either arm and no statistically significant differences were observed between treatment arms. PFS, EFS and OS rates were also similar at 24 months' follow-up (non-significant differences; Table 1). Grade ³3 adverse events (58.3% SC; 54.3% IV) and administration-related reactions (21% in both groups) were similar between arms. Of SC recipients, 5.7% had injection site reactions *vs* none in the IV group (p<0.001). Febrile neutropenia occurred more often in the SC arm (12.5% *vs* 6.9% in IV, p=0.06). RASQ scores for 'impact on activities of daily living', 'convenience' and 'satisfaction' were improved with SC *vs* IV; CTSQ scores were similar between arms (Figure 1).

When pts in the SC group were asked, if given the option, which treatment they would prefer, 90.8% stated a preference for SC over IV. Median administration time (6 minutes SC *vs* 2.6–3.0 hours IV) and chair/bed and overall hospital times were shorter with SC than with IV treatment.

Table 1. Efficacy endpoints in the intent-to-treat population.

Efficacy endpoint	Rituximab SC plus CHOP		Rituximab IV plus CHOP	
	N	% (95% CI)	N	% (95% CI)
End of induction treatment				
CR/CRu	342	50.6 (45.3–55.9)	177	42.4 (35.1–49.7)
PR	342	31.6 (26.7–36.8)	177	35.6 (28.6–43.1)
PD	342	3.8 (2.0–6.4)	177	6.2 (3.1–10.8)
ORR	342	82.2 (77.7–86.1)	177	78.0 (71.1–83.8)
24 months' follow-up				
PFS*	342	67.5 (62.6–72.5)	177	72.3 (65.7–78.9)
EFS†	342	61.7 (56.5–66.8)	177	66.7 (59.7–73.6)
OS	342	87.4 (83.2–90.5)	177	88.0 (82.0–92.1)

*p=0.264.
†p=0.265.
ORR, overall response rate; PD, progressive disease; PR, partial response.

Figure. Patient satisfaction at cycle 3 and cycle 7 of treatment

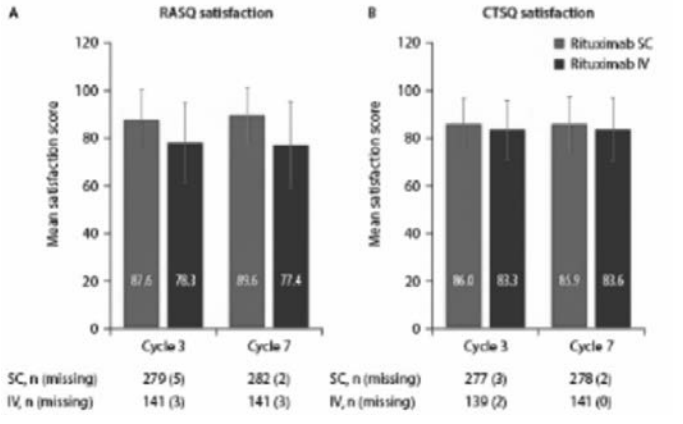

Figure 1. Patient satisfaction at cycle 3 and cycle 7 of treatment.

Summary/Conclusions: Rituximab SC had similar efficacy and safety to the IV form, with improvements in patient satisfaction ratings, and administration/hospital time savings. Our findings support the use of rituximab SC in this setting.

S108

ANALYSIS AND CHARACTERIZATION OF HEMATOLOGIC CANCERS USING A COMPREHENSIVE NGS PANEL COMPRISED OF DNA AND RNA BAITS TARGETING 704 GENES

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Background: As next-generation sequencing (NGS) methodologies improve, so does the ability to characterize hematopoietic and lymphoid neoplasm genomes. This promises to revolutionize oncology, allowing more accurate and precise classification of patients and potentially leading to novel targeted and combination therapies with improved outcomes.
Aims: We constructed a custom targeted sequencing panel, MyHEME™, to comprehensively identify and characterize DNA and RNA changes in a broad range of hematologic malignancies, including Non-Hodgkin lymphoma (NHL).
Methods: The MyHEME targeted sequencing panel is comprised of two independent bait sets that target a combined 704 genes known or predicted to contribute to hematologic cancers (DNA baits for 571 genes and RNA baits for 361 genes; 228 genes are found in common between the two bait sets). Libraries were constructed using 1µg of DNA or 0.1µg of RNA and sequenced on an Illumina platform. Sequenced reads are analyzed using proprietary MyInformatics™ software to identify single nucleotide variants (SNVs), indels and structural variants (SVs). Both the MyHEME panel and MyInformatics software were created under ISO13485 design control. To characterize the performance metrics of the MyHEME panel, we used the NIST human reference sample NA12878 along with combinations of hematologic cancer derived cell lines with known pathogenic variants at various allelic frequencies.
Results: Analytical validation of the MyHEME panel established an average read depth of 1,175x (with a median read depth of 1,088x) for the DNA targets and an average transcripts per million (TPM) of 2,256 (with a median TPM of 743) for the RNA targets. For the DNA targets, we establish sensitivity >95% (99.8% for SNVs at a 2.5% limit of detection (LOD); 100% for coding indels at a 5.0% LOD) and specificity >95% (95.5% for SNVs at a 2.5% LOD; 97.7% for coding indels at a 5.0% LOD). We also show the ability to cross-confirm results between the 228 genes common to both the DNA and RNA targets. Importantly, novel gene fusions, which are generally difficult to detect and validate, were cross-confirmed when observed in both the DNA and RNA targets. For example, we identified a novel t(9;22) translocation causing a *NUP214-XKR3* gene fusion using both the DNA and RNA targets. Additionally, while RNA data provides the fused exons of the transcripts, DNA data gives the precise genomic breakpoint coordinat
Summary/Conclusions: MyHEME is an extensive panel for sensitively and specifically identifying SNV, indel and SV mutations in 704 target genes. This panel can comprehensively characterize mutations in multiple diverse hematologic cancer samples, including Non-Hodgkin Lymphoma, AML, ALL, and Multiple Myeloma. By utilizing a high depth of coverage, MyHEME can accurately detect clones present down to 5% of a patient's sample. In addition, by targeting both DNA and RNA, MyHEME contains a built in validation method to cross-confirm novel variants of interest.

S109

TP53 MUTATIONS, BUT NOT DELETION OF TP53 AND CDKN2A, HAVE INDEPENDENT PROGNOSTIC VALUE IN MANTLE CELL LYMPHOMA TREATED BY THE NORDIC (MCL2 AND MCL3) REGIMEN

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Background: During the past decades, the outcome of MCL treatment has improved substantially in younger patients. However, the course of disease remains heterogeneous, and there is a need for better stratification of patients with poor responses from those with durable responses. The Nordic trials, MCL2 and MCL3, represent standard-of-care regimens for younger MCL patients.
Aims: Preliminary analyses of diagnostic samples from MCL2 and MCL3, show that TP53 mutations are associated with significantly poorer outcome. Recently, deletions of TP53 and CDKN2A was shown to confer negative impact in a cohort similar to the Nordic.(Delfau-Larue *et al.,* 2015) Thus, in this study we aim to describe the prevalence and impact of deletions of TP53 and CDKN2A in the light of TP53 mutations.
Methods: Fresh frozen DNA from diagnostic bone marrow samples from MCL2 and MCL3 were analyzed. In both trials, patients received intensified first-line induction therapy with alternating courses of R-CHOP and R-hd-Cytarabine and consolidation with high-dose therapy and ASCT. (Geisler *et al.,* 2008; Kolstad *et al.,* 2014). Targeted NGS of *ATM, CCND1, TP53, KMT2D, NOTCH1, NOTCH2, WHSC1 and BIRC3* was performed by Ion Torrent Technology. Cut-off for calling a mutation was set to a variant allele frequency >3%. Median coverage was >2700X. Copy Number Variations (CNVs) of TP53 and CDKN2A were measured by droplet digital PCR by commercially available assays, and RPP30 used as a standard control.
Results: We investigated the presence of CDKN2A and TP53 deletions in diagnostic samples from 175 and 157 patients, respectively. Patients were untreated and <66 years (median 58, range 37-65). Fifty-three percent were either MIPI intermediate- or high-risk, 17% had blastoid morphology and 42% had Ki67≥30%, and 83% had bone marrow involvement at diagnosis. After a median follow-up of 9.2 years, median overall (OS), progression-free survival (PFS) and cumulated incidence of relapse (CIR) of all patients were 12.4 and 8.2 and 10.2 years, respectively. In our mutational analyses (n=147), only TP53 had prognostic impact in multivariate analyses (MVAs). Outcome of the 15 patients (10%) with TP53-mutations was poor with a median OS, PFS and CIR of 1.8, 1.0 and 1.2 years (p<0.0001 for all three outcomes), respectively. Preliminary data shows deletions of TP53 in 28 patients (18%) and deletion of CDKN2A in 38 (22%). Eight patients carried both deletions. Del-CDKN2A was significantly associated with mutations of TP53, MIPI high risk, blastoid morphology and Ki67>30%. Del-TP53 was associated with Ki67>30%, but no other high risk markers. Altogether, 31 (25%) of 122 patients harbored a deletion and/or mutation in TP53 and 4 (3%) carried both aberrations. In univariate analyses, del-TP53 was significantly associated with poor OS (p=0.01), but not PFS and CIR, whereas del-CDKN2A was significant for CIR (p=0.02), but not OS and PFS. Patients with both deletions did significantly worse for all three endpoints. In MVA, (including all factors with significance in univariate analyses: MIPI, blastoid morphology, Ki67-index>30%, NOTCH1 mutations, TP53 mutations, del-TP53 and del-CDKN2A) only mutations of TP53 remained a significant predictor of outcome.
Summary/Conclusions: Here we evaluate the impact of TP53- and CDKN2A-deletions in the context of TP53 mutations of younger, optimally treated MCL patients. In line with previous reports, both deletions were associated with poorer outcome; however, in multivariate analyses only TP53 mutations was an independent prognostic factor, substantiating its role as a biomarker for response to the standard-of-care immune-chemotherapy.

MRD directed treatment in AML

S110

DEEP MOLECULAR RESPONSE TO GILTERITINIB IMPROVES SURVIVAL IN FLT3 MUTATION-POSITIVE RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA

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Background: Mutations in Fms-like tyrosine kinase 3 (FLT3) are common in patients with acute myeloid leukemia (AML) and are associated with an aggressive disease course and a poor prognosis. Notably, FLT3 internal tandem duplications (ITD) predict early relapse and short overall survival (OS) after chemotherapy. Gilteritinib, a highly selective FLT3/AXL inhibitor, has displayed antileukemic activity in FLT3 mutation-positive (FLT3^{mut+}) relapsed/refractory (R/R) AML in the CHRYSALIS Phase 1/2 study (NCT02014558), specifically at doses ≥80 mg/d.
Aims: To assess molecular response to gilteritinib in a CHRYSALIS subpopulation.
Methods: This exploratory analysis evaluated molecular response in patients aged ≥18 years with FLT3^{mut+}R/R AML who had been treated with 120 or 200 mg/d gilteritinib. These doses were identified due to their ability to induce high clinical response rates, and consistent, potent FLT3 inhibition in correlative assays. Molecular response was assessed in patients who had bone marrow aspirates obtained at baseline and at ≥1 additional time point. *FLT3-ITD* and total *FLT3* were quantified by next-generation sequencing to assess molecular response. A Cox regression model of OS by Kaplan-Meier estimation established a *FLT3-ITD*:total *FLT3* ratio (ITD signal ratio) of 10^{-2} as the threshold for improved survival.
Results: Of the 147 FLT3-ITD^{mut+}patients who had received gilteritinib 120 or 200 mg/d, 80 patients had bone marrow aspirates at baseline and at ≥1 additional time point, and were included in this analysis. The composite response rate (defined as CR plus CRi plus CRp) for these 80 patients was 55%. During response, 20 patients (25%) had an ITD signal ratio of $\leq 10^{-2}$. Of these 20 patients, 18 had an ITD signal ratio of $\leq 10^{-3}$ (major molecular response [MMR]) and 13 had an ITD signal ratio of $\leq 10^{-4}$ (minimal residual disease [MRD] negative). The median time to achieve minimum ITD signal ratio was 54 days. Elimination of morphologic leukemia was observed in 80% of patients with ITD signal ratios $<10^{-2}$. Patients who had an ITD signal ratio $\leq 10^{-2}$, MMR, or were MRD negative had significantly longer median OS than those who did not (Table 1 and Figure 1).

Table 1. Overall survival in subjects who achieved a molecular response compared with those who did not by depth of response.

Molecular Response	Achieved a Molecular Response		Did not Achieve a Molecular Response		*P*-value
	n	Median OS, Days (95% CI)	n	Median OS, Days (95% CI)	
ITD signal ratio $\leq 10^{-2}$	20	417 (246–NA)	60	199 (142–234)	<.001
MMR	18	417 (228–NA)	62	213 (143–264)	.003
MRD negative	13	417 (228–NA)	67	213 (144–264)	.002

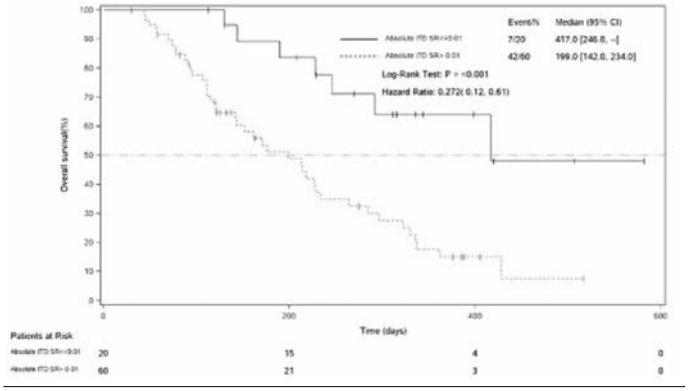

Figure 1.

Summary/Conclusions: Molecular responses to gilteritinib in FLT3-ITD^{mut+}R/R AML correlated with clinical response and improved OS. This is the first demonstration of a robust molecular response to a FLT3 inhibitor in AML. These data suggest that the ITD signal ratio may predict a durable clinical benefit of gilteritinib therapy and validate FLT3 as a critical therapeutic target in AML.

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RISK-ADAPTED, MRD-DIRECTED THERAPY FOR YOUNG ADULTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA: RESULTS OF THE AML1310 TRIAL OF THE GIMEMA GROUP

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Background: A comprehensive AML risk assessment, based on the integration of cytogenetic/genetic data and minimal residual disease (MRD) status, can help optimize patients' (pts) therapeutic post-remission allocation.
Aims: To evaluate the feasibility and results of a phase II trial of intensive chemotherapy in which risk-assignment and post-remission therapy of young patients with AML was based on pre-treatment cytogenetic/genetic data and post-consolidation levels of MRD.
Methods: Between January 2012 and May 2015, 515 pts with de novo AML, 18 to 60 years old, seen at 55 GIMEMA institutions were enrolled in the trial. Induction consisted of i.v. daunorubicin 50 mg/m^2 daily on days 1,3 and 5; i.v. etoposide 50 mg/m^2 daily on days 1 to 5; i.v. cytarabine 100 mg/m^2 as a daily continuous infusion, days 1 to 10. All pts in CR/CRi after 1-2 induction cycles, received 1 consolidation course consisting of i.v. daunorubicin 50 mg/m^2 daily on days 4,5 and 6 and i.v. cytarabine 500 mg/m^2 every 12 hours on days 1 to 6. In pts belonging to ELN low or intermediate-risk category, peripheral blood stem cell collection was attempted by initiating, on day 20 from the start of consolidation therapy, G-CSF until completion of stem cell collection. Post-consolidation therapy was based on risk-allocation. Low-risk pts (NPM1 positive FLT3-ITD negative or CBF positive without c-Kit mutations) were to receive AuSCT; high-risk pts (adverse karyotype or FLT3-ITD positive) were to receive ASCT; intermediate-risk pts (intermediate karyotype or FLT3-TKD positive or c-kit mutated CBF positive) were to receive AuSCT or ASCT depending on the levels of MRD, measured by flow cytometry after consolidation therapy. Allocation to ASCT required the procedure to be performed whatever the source of stem cells (identical sibling, unrelated, cord blood, haploidentical).
Results: 500/515 pts started treatment and were available for the analysis. Median age was 49 (18-61) years and 52% were males. Of 429 evaluable pts, ELN cytogenetic distribution was: low-risk 11%, intermediate-risk 73% and poor-risk 16%. RUNX1/RUNX1T1 was detected in 5% of 499 evaluable cases, CBFbeta/MYH11 in 7% of 496, FLT3-ITD in 25% of 497 and NPM1 in 37% of 499. In 494 evaluable pts, complete remission rate (CR) was 73% (361), 18% had refractory AML and 9% died early during induction. Three hundred-41 pts completed the consolidation phase and were risk allocated: 114 (33%) to the low-risk category (=AuSCT), 122 (36%) to the high-risk (=ASCT) and 78 (23%) to the intermediate category (=AuSCT or ASCT). In 27 pts (8%) belonging to the intermediate-risk category, a leukemia associated phenotype was not found and they were to receive AuSCT. Overall, 109 (33%) and 123 (36%) of 341 pts received AuSCT and ASCT, respectively. Median follow-up was 27.9 months. At 24 months, overall (OS) and disease free survival (DFS) of the whole series was 55.9% and 54.9%, respectively; cumulative incidence of relapse was 32.9%. At the same time point of 24 months, OS and DFS in the low-risk category was 74.8% and 63.8%, respectively; in the high-risk category 42.5% and 44.8%, respectively; in the intermediate-risk category MRD negative 78.6% and 61.4%, respectively; in the intermediate-risk category MRD positive 69.8% and 66.6%, respectively (Figure 1).
Summary/Conclusions: A program of risk-adapted, MRD-driven therapy is feasible in a multicenter, cooperative setting. In the intermediate-risk category,

ASCT can be avoided if MRD is not detectable; if MRD is positive, ASCT can prolong OS and DFS to equalize those of the low-risk category. ASCT was delivered to 2/3 of pts in the high-risk category, using all the available sources of stem cells.

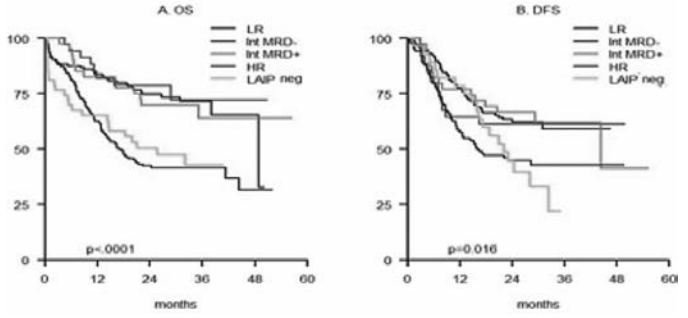

Figure 1.

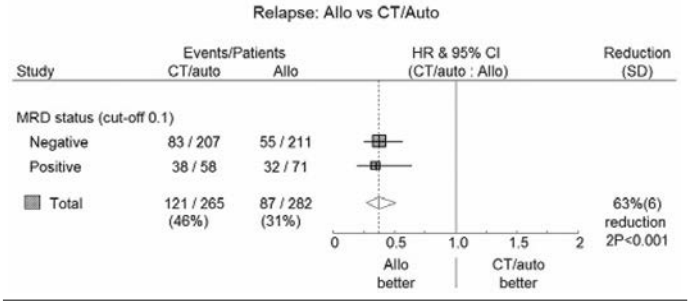

Figure 1.

Summary/Conclusions: The graft-*versus*-leukemia effect of alloHSCT is equally present in MRD positive and MRD negative patients, which advocates a personalized application of alloHSCT taking the risk of relapse determined by AML risk group and MRD status as well as the counterbalancing risk of NRM into account.

S112

GRAFT VERSUS LEUKEMIA EFFECT OF ALLOGENEIC STEM CELL TRANSPLANTATION AND MINIMAL RESIDUAL DISEASE IN PATIENTS WITH AML IN FIRST COMPLETE REMISSION

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Background: The detection of minimal residual disease (MRD) in patients with acute myeloid leukemia (AML) may improve future risk-adapted strategies of AML treatment. The presence of MRD after induction treatment has firmly been shown to predict for relapse and overall outcome, irrespective of type of post-remission treatment (PRT). Currently it is unknown whether and how the presence or absence of MRD should guide the application of allogeneic hematopoietic stem cell transplantation (alloHSCT) as PRT.

Aims: We addressed whether and to what extent alloHSCT quantitatively reduces relapse as compared to conventional post-remission treatment (PRT) in upfront treated patients with MRD positive or MRD negative AML in first hematological complete remission (CR1).

Methods: A total of 1,511 patients were treated in subsequent HOVON-SAKK AML trials of whom 547 patients obtained a CR1, received PRT and had available flow cytometric MRD prior to PRT. MRD positivity was defined by more than 0.1% cells with a leukemia associated phenotype within the white blood cell compartment. MRD status was not known by clinicians during AML treatment. PRT consisted of alloHSCT (n=282), or conventional PRT by a third cycle of chemotherapy (n=160) or autologous HSCT (n=105). Endpoints of the study included overall survival (OS), relapse-free survival (RFS), and cumulative incidences of relapse and non-relapse mortality (NRM) at 4 years. A time-dependent analysis was performed by applying multivariable Cox regression with time-dependent covariate alloHSCT with the cumulative incidence of relapse as primary endpoint.

Results: MRD was positive in 129 (24%) patients after induction chemotherapy before proceeding to PRT. The latest European LeukemiaNET risk classification was similarly distributed among MRD negative and MRD positive patients. No differences were present in transplant characteristics in MRD positive and MRD negative patients. OS and RFS was significantly better in patients without MRD prior to PRT as compared to MRD positive patients (65±2% compared to 50±5% at 4 years, p=0.002, and 58±3% compared to 38±4%, p<0.001, respectively). Improved outcome was mainly caused by a lower cumulative incidence of relapse in MRD negative patients as compared to MRD positive patients (32±2% compared to 54±4% at 4 years, p<0.001, respectively), while NRM was not significantly different and estimated 10±1%. NRM split by EBMT risk score showed less NRM in patients with a low EBMT-risk score as compared to patients with a high EBMT risk score (≤2 compared to >2, 10±2% compared to 22±4%, p=0.005, respectively). Multivariable analysis with adjustment for covariates showed that the incidence of relapse was significantly reduced following alloHSCT as compared to chemotherapy or autologous HSCT (HR 0.36, p<0.001), which was similarly exerted in MRD negative and positive patients (HR 0.38, p<0.001 and HR 0.35, p<0.001). RFS was also improved following alloHSCT as compared to chemotherapy or autoHSCT (HR 0.53, p<0.001), while no significant differences were found for OS (Figure 1).

S113

LEUKEMIC STEM CELL FREQUENCY COMBINED WITH MRD IS AN IMPORTANT BIOMARKER TO PREDICT RELAPSE IN ACUTE MYELOID LEUKEMIA. RESULTS FROM A PROSPECTIVE H102 STUDY

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Background: Despite up-to-date risk algorithms, outcome in acute myeloid leukemia patients is still difficult to predict. Even in good risk patients relapses occur. Further refinement of currently used risk classifications is therefore warranted. Measurable residual disease (MRD) is a well-known risk factor and the independent prognostic impact of MRD was shown for patients independent on risk groups. Nowadays prospective studies are designed on which therapy is adapted based on MRD-positivity or negativity. Although this is a major improvement for risk stratification, relapses occur in a substantial proportion of MRD-negative patients. Previous retrospective studies have shown that the leukemic stem cell (LSC) frequency harbors important prognostic information as well (Bradbury *et al.*, Leukemia 2015), even within MRD-negative patients (Terwijn *et al.* Plos one, 2013).

Aims: In this study we used data of the HOVON/SAKK H102 trial to prospectively define, using flow cytometry, the leukemic CD34+CD38- stem cell frequencies and MRD frequencies to investigate impact on patient outcome.

Methods: In 242 patients who achieved morphologic complete remission, both LSC and MRD data after two cycles of chemotherapy treatment were available. MRD-positivity was defined as a percentage of MRD-positive cells above 0.1% (as compared to total amount of WBCs) and LSC-positivity was defined as a CD34+CD38-LSC percentage above 0.0000% (LSC cut-off 0.0000%; thus no CD34+CD38-LSC events measured).

Results: Cumulative incidence of relapse (CIR) and overall survival (OS) data were investigated for four different MRD/LSC groups: 1. MRD^{neg} +LSC^{neg}patients (n=136) 2. MRD^{pos} +LSC^{neg} patients (n=28) 3. MRD^{neg} +LSC^{pos}patients (n=58) and 4. MRD^{pos} +LSC^{pos} patients (n=20). Results showed that MRD^{pos} +LSC^{pos}patients have the worst prognosis. 3-year CIR for the four above-defined groups was 35% (SE 4), 43% (SE 9), 53% (SE 7), and 100% (SE 0), respectively. Similar results were found for OS: 3-year OS was 66% (SE 4), 68% (SE 9), 53% (SE 8), and 100%, respectively, with 17 patients dead and 3 censored in the latter group. When investigating the impact of MRD/LSC status in the good, intermediate, poor and very poor risk group (according to HOVON), patient numbers were sometimes small; however, results show that MRD^{pos} +LSC^{pos}AML patients in all different risk categories have a very poor prognosis. Moreover, multivariate analyses, containing all well known risk factors including risk group and post remission treatment, showed that MRD^{pos} +LSC^{pos}patients have a significantly worse cumulative incidence of relapse (hazard ratio [HR] 5.89; 95% CI 3.32-10.47) and overall survival (HR 3.62; 95% CI 1.86-7.04) as compared to the MRD^{neg} +LSC^{neg} patient group.

Summary/Conclusions: Overall, we conclude that our prospective results show that CD34+CD38-LSC frequency has important additional value in MRD assessment and that it especially enables to identify very poor risk patients in

all different currently used risk categories. These data urge to include both MRD and LSC in future AML risk classification to better inform post-remission treatment.

S114

DEFINITION OF PARTIAL RESPONSE IN YOUNGER AML PATIENTS AFTER FIRST INDUCTION COURSE MAY BE EXTENDED BY INCLUSION OF IMMUNOPHENOTYPIC DETECTION OF MEASURABLE RESIDUAL DISEASE IN CR

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Background: In AML response by morphology after a first cycle of induction therapy is used to guide further therapy including second cycles of induction and choice of consolidation. It is still uncertain how the quality of response post cycle 1 with inclusion of MRD assessment impacts on outcomes within AML risk subgroups including *NPM1* wild type standard risk and whether this adds information to MRD status in CR post cycle 2.
Aims: To quantify the effect of MRD positivity for response after each cycle of induction therapy in younger patients with AML.
Methods: As part of the UK NCRI AML17 trial (ISRCTN: 55675535) for patients with AML or high risk MDS up to the age of 60, prospective flow cytometric MRD (MFC-MRD) monitoring was performed after each course of induction. Any level of MRD detected was considered MRD+(sensitivity thresholds: ~0.02% by tracking diagnostic leukemic aberrant phenotypes /LAIP, ~0.05-0.1% by "different-from normal" blast LAIP). Clinicians were not informed of MFC-MRD results. Following their first cycle of induction with daunorubicin/ara-C based therapy, patients were allocated a risk group by a validated score (comprising cytogenetics, WBC, age, secondary disease, blast response to cycle 1 and mutation status). Poor risk patients received intensified therapy in cycle 2 with a view of proceeding to SCT.
Results: MFC-MRD results after either induction course are available for 1555 patients randomised from 4/09-12/14 (median age 51, range 0-73). Cycle 1 (C1) response data with MFC-MRD was available for 1,400 patients. 70% achieved morphological CR at this time-point; 14% had resistant disease (RD) and 16% were in partial remission (PR) according to clinician. Of patients in CR (n=984) 56% had detectable MFC-MRD (MRD+). Excluding poor-risk patients 14% of patients did not achieve CR (7% RD, 7% PR), 51% of patients in CR were MRD+. 5 year OS for MRD- *vs* MRD+*vs* PR *vs* RD were 63% *vs* 44% *vs* 37% *vs* 25% for all patients; 69% *vs* 51% *vs* 50% *vs* 30% excluding poor risk patients and 66% *vs* 49% *vs* 49% *vs* 30% for standard risk alone (Figure 1). The similar OS in this group between CR MRD+and PR at C1 was maintained in *NPM1*wt standard risk patients and if censored at stem cell transplant. 771 patients were in CR post cycle 2 (C2) and provided MFC-MRD data. As expected, there were significant differences in 5 year OS between CR MFC MRD+*vs* CR MFC MRD- for all patients (35% *vs* 63%) and excluding poor-risk (38% *vs* 70%, n=512). Importantly post cycle 2 MFC-MRD status also differentiated OS for *NPM1*wt standard risk patients with 5 year OS of 32% *vs* 64% (P=0.002) for MRD+*vs* MRD- (Figure 1). In stratified analyses, there was some evidence that the effect of MRD positivity on OS was lower in poor-risk patients (test for trend p=0.02 for both C1 and C2). The effect of MFC-MRD status on relapse and OS appeared greater at C2 (relapse, OR 2.00(1.56- 2.55), p<0.001; survival, OR 1.80(1.42-2.28) p<0.001) than C1 (relapse, OR 1.69(1.37- 2.07), p<0.001; survival, OR 1.46(1.19-1.79) p<0.001). In patients with data for both time points, C2 MRD remained significant on OS when adjusting for C1 response. 24 patients converted from C1 MRD- to C2 MRD+, with a poor prognosis (15 relapses, 13 deaths). C1 MRD-/C2 MRD- had the best prognosis.

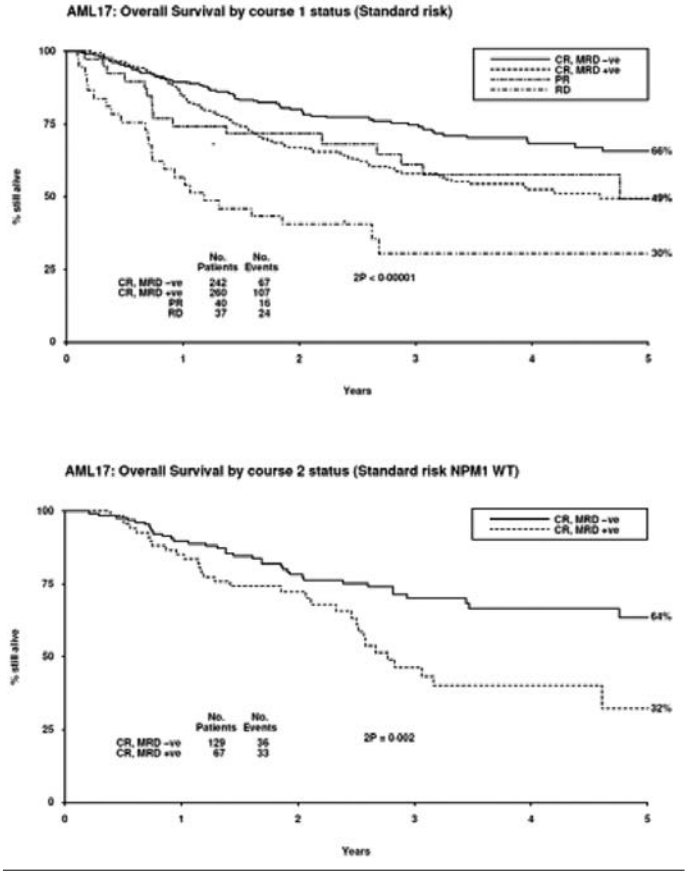

Figure 1.

Summary/Conclusions: MFC-MRD in CR post cycle 1 has similar outcomes to partial remission in younger patients with AML, particularly in patients with good and standard risk disease. Assessment of MFC-MRD post cycle 2 appears to provide additional discrimination to cycle 1: MFC-MRD in courses 1-2 may be useful in further stratifying standard risk patients.

New insights into chronic lymphocytic leukemia biology

S115

CLINICAL IMPACT OF THE SUBCLONAL ARCHITECTURE AND MUTATIONAL COMPLEXITY IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Recent studies have revealed the presence and prognostic impact of small mutated subclones in chronic lymphocytic leukemia (CLL) (Rossi et al 2014, Nadeu et al 2016, Rasi et al 2016). Although these studies focused only on a small subset of 5 genes, their results opened a new perspective where the proportion of cells carrying each specific driver mutation may be relevant to the evolution of this disease. Moreover, the subclonal and mutational complexity estimated by the presence of subclonal driver alterations (Landau et al 2013, Landau et al 2015) or the accumulation of driver alterations (Puente et al 2015) have been proposed as promising indicators of clinical behavior.
Aims: The goal of this study was to determine the relevance of the quantitative subclonal architecture and mutational complexity in the evolution of CLL integrating the deep sequencing analysis of a large panel of driver genes and DNA copy number alterations (CNA).
Methods: The mutational status of 28 driver genes was investigated in 406 previously untreated CLL patients by targeted-deep next-generation sequencing (NGS). Mutations present in less than 1% of tumor cells were identified. All low frequency mutations were verified by allele-specific PCR or a second round of NGS. CNA were analyzed by SNP-arrays. Alterations were classified as clonal if their CCF was ≥85%, and subclonal otherwise. All patients gave informed consent.
Results: Using a highly sensitive NGS strategy we observed that small subclonal mutations were the sole alteration in 22% of the mutated cases, and were frequently detected in nearly all investigated genes. We identified three gene-specific patterns that linked the magnitude of the mutated clones (or mutated cancer cell fraction, CCF) with the prognosis of the patients: i) *CCF-independent pattern*: mutations at any CCF had prognostic value, ii) *CCF-gradual pattern*: the poor prognostic impact was a continuous variable directly related to the size of the clone, and iii) *CCF-clonal pattern*: only mutations with a CCF above a certain threshold impacted the outcome of the patients. Combining mutations and driver CNA, 86% of the patients carried at least one driver alteration, which was clonal in 66%. On the other hand, subclonal driver alterations were present in 60% of the patients. The *mutational complexity* (accumulation of 1 to ≥4 driver alterations), but not the presence of subclonal driver populations, gradually shortened the time to first treatment independently of the IGHV mutational status and Binet stage. Conversely, the *subclonal complexity,* defined as the accumulation of driver alterations with the presence of at least one driver subclone, predicted for a worse overall survival independently of the IGHV and Binet stage. Patients with a *pure clonal population* (presence of one or more driver alterations in all tumor cells) had a similar overall survival than patients without any alteration.
Summary/Conclusions: Our study shows that the prognostic impact of different driver mutations is related to the size of the mutated population. Therefore, the clinical evaluation of gene mutations should consider the quantitative representation of the mutations and not only their presence or absence. In addition, the *mutational complexity* predicts for shorter time to first treatment independently of the IGHV and Binet stage, whereas the *subclonal complexity* confers an independent adverse impact for overall survival. Altogether, the integration of the subclonal architecture and mutational complexity in prognostic indexes may improve the stratification of CLL patients.

S116

FBXW7 MUTATIONS LEAD TO ACCUMULATION OF NOTCH1, HIF1-ALPHA AND C-MYC IN CLL CELLS

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Background: Chronic lymphocytic leukemia (CLL) is a heterogeneous disease with recurrent mutations that are of pathogenic and prognostic relevance. Mutations in *FBXW7* are among the most common mutations in CLL, yet their functional consequences are unknown. FBXW7 is an E3 ubiquitin ligase that ubiquitylates oncoproteins like NOTCH1, HIF1-α and c-MYC and thereby targets them for proteasomal degradation.
Aims: 2.5-4% of CLL patients harbor *FBXW7* mutations. Approximately 60% of *FBXW7* mutations alter arginine residues that are involved in substrate targeting. In T-cell acute lymphoblastic leukemia these arginine mutations are associated with chemotherapy resistance. In CLL however, the role of dysfunctional FBXW7 is unclear. We therefore aimed to delineate the prevalence of *FBXW7* mutations in CLL patient cohorts and characterize its functional role.
Methods: *FBXW7* mutations were analyzed via amplicon-based targeted next generation sequencing in primary CD19-sorted samples of previously untreated CLL patients (n=905) as well as in CLL (n=8), MCL (n=5), T-ALL (n=2), Burkitt lymphoma (n=1) and LCL cell lines (n=3). In silico modeling with PolyPhen-2 predicted a potential impact of the mutations on the structure and function of *FBXW7*. For functional analysis, *FBXW7* mutations were induced using CRISPR/Cas9 in the CLL cell line HG3, which does not harbor a *NOTCH1* mutation. Both in this CRISPR/Cas9 mutated cell line and in primary CLL cells with *FBXW7* mutations, the protein levels of FBXW7 substrates were examined. In addition, we quantified NOTCH1 and HIF1-α activity with Luciferase reporter assay in *FBXW7* mutated HG3 cell lines.
Results: Heterozygous mutations in *FBXW7* were found in 41/905 (4.5%) of CLL patients. The most common mutations of *FBXW7* were missense mutations (32/41) that target the substrate binding domain of the FBXW7 protein as well as non-sense mutations (4/41). Interestingly, 5 patients harbored two concurrent *FBXW7* mutations. By the use of the PolyPhen-2 software, all except one missense mutation in *FBXW7* were predicted to be most likely damaging. No mutations in *FBXW7* were found in the CLL, MCL and LCL cell lines analyzed. To determine the functional consequence of *FBXW7* mutations in CLL, we induced either a heterozygous or a homozygous truncation of *FBXW7* in the CLL cell line HG3, resulting in the loss of the substrate binding site of the WD40 domain. The homozygous truncation of *FBXW7* resulted in an increase of NOTCH1, HIF1-α and c-MYC protein levels, whereas no difference of Cyclin E protein amount was detectable. In addition, an elevation of NOTCH1 activity was found in both the heterozygously and homozygously truncated mutant cell lines in comparison to the wildtype HG3 cell line. To confirm this finding, protein levels of 5 CLL patients with *FBXW7* mutations were analyzed with a similar outcome.
Summary/Conclusions: Mutations in *FBXW7* are frequently found in CLL, especially missense and nonsense mutations affecting the WD40 domain. We hypothesize that this has functional consequences on FBXW7 substrate binding and hence leads to accumulation of oncogenes. In line, the induced truncation of the WD40 domain of FBXW7 in the HG3 cell line resulted in the accumulation of protein substrates and corresponding increase of their activity implicated in the pathogenesis of CLL. Taken together our data show that FBXW7 can target proteins for degradation that are commonly dysregulated in CLL and that drive disease progression.

S117

INTEGRATIVE ANALYSIS OF THE GENOME, EPIGENOME, TRANSCRIPTOME AND THREE-DIMENSIONAL CHROMATIN STRUCTURE IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Different omics studies have focused on the analysis of individual layers of information in chronic lymphocytic leukemia (CLL), such as the genome, transcriptome and DNA methylome. However, besides the DNA methylome, other layers of the epigenome, like histone modifications, remain relatively unexplored and an integrative molecular portrait of CLL is not available yet.
Aims: The aim of this study was to extensively map and analyse the epigenome

of CLL in relation to the mutational, transcriptional and three-dimensional (3D) chromatin landscape.
Methods: Seven CLL patients with distinct clinico-pathological features and five mature B-cell subpopulations were extensively analysed using (i) ChIP-seq of six different histone marks with non-overlapping features (H3K27ac, H3K4me1, H3K4me3, H3K9me3, H3K27me3 and H3K36me3); (ii) single stranded RNA-seq; iii) transposase-accessible chromatin assays (ATAC-seq) and iv) whole-genome bisulfite sequencing (WGBS), creating a unique reference epigenome for CLL. These data were complemented with the 3D chromatin landscape in one CLL case measured by high-throughput chromatin conformation capture (HiC-seq) and promoter capture Hi-C (PCHi-C). Furthermore, we mapped the active chromatin landscape of 100 CLL patients by H3K27ac ChIP-seq and ATAC-seq. Whole-genome sequencing data was available for 44 of these patients. We applied a broad range of bioinformatic tools to analyze the data in an integrative way.
Results: CLL is distinct from normal B cells for all layers of the reference epigenome (7 CLLs) and the active chromatin landscape (100 CLLs). CLL though is closer to naive and memory B cells than to germinal center B cells and plasma cells. Interestingly, in CLL we not only saw activation of regions that are active in naive and memory B cells, but also an unexpected activation of genomic regions that are specifically active in germinal center B cells and plasma cells. Changes in activation in these and other regions could furthermore distinguish the two major clinical subgroups of CLL with unmutated and mutated immunoglobulin heavy chains (IGVH). CLLs did not only differ from normal B cells regarding the separate layers of information, but also using combined patterns of histone marks, which for example can define regulatory elements as active promoters (H3K4me3 and H3K27ac) or active enhancers (H3K27ac and H3K4me1). More specifically, we detected 534 genomic regions with *de novo* gain (n=498) or loss (n=36) of active regulatory regions in CLL. Large regions (>10kb) showing *de novo* gain of regulatory elements in CLL (n=51), were located into, close to, or interacted in 3D space with genes important for CLL pathogenesis, *e.g.*, *LEF1*, *BCL2* and *FMOD*. Interestingly, non-coding somatic mutations in IGVH mutated CLLs accumulate in these and other active regulatory regions, likely being off-target effects of the somatic hypermutation machinery. Besides changes in regulatory elements, we observed that CLLs lose poised promoters, which are replaced by repressed/inactive regions. This change, mainly occurring in developmental genes, does not affect gene expression levels, as these genes are already silent in normal B cells. It may however represent loss of plasticity during CLL pathogenesis in which these genes become permanently inactive.
Summary/Conclusions: With this integrative study, we generated new conceptual avenues to understand the complex link among the epigenetic, mutational, transcriptional and 3D chromatin landscape in CLL. In addition we provide the community with an extensive resource of epigenetic information of this lymphoid neoplasm.

S118

THERAPEUTIC DISRUPTION OF THE BAFF- B-CELL RECEPTOR CROSS-TALK IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS
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Background: Although small molecule inhibitors of BCR-associated kinases (BCRi) revolutionized therapy in CLL, they provide incomplete responses. Tumor necrosis factor receptor superfamily ligands BAFF and APRIL induce NFκB, which in turn upregulates pro-survival Bcl-2 family proteins and thereby drives anti-apoptotic responses, potentially accounting for resistance to BCRi. The exact roles of the individual NFκB pathways, as well as the implications of targeting BCR in context of BAFF signaling in CLL remain understudied.
Aims: We explored the mechanistic underpinnings of CLL cell survival in response to BAFF signaling.
Methods: We established a novel BAFF-expressing stromal co-culture model and employed inhibitors of Bruton tyrosine kinase (BTK, ibrutinib), phosphoinositide-3 kinase (PI3K, idelalisib) and spleen tyrosine kinase (SYK, entospletinib). We quantified CLL cell apoptosis, migration, NFκB activity, protein and mRNA expression by flow cytometry, immunoblotting, ELISA, RT-PCR and immunocytochemistry.
Results: CLL cells co-cultured with BAFF-expressing stroma were resistant to spontaneous apoptosis (12.3±3.2% after 24 h, *vs* 34.8±6.2% off stroma) and chemotherapy agents (bendamustine, fludarabine). Gene expression profiling exposed the NFκB pathway gene targets as the most significantly upregulated upon BAFF stimulation ($p<0.0001$). We and others have shown that CD40L-expressing stroma induces canonical and non-canonical NFκB in CLL. By contrast, while BAFF led to strong activation of the non-canonical NFκB with processing of p100 (to p52) by 4 h and a 5-fold increase in p52 DNA-binding activity by 24 h, canonical NFκB (RelA) activation was less pronounced. BAFF predominantly induced Mcl-1, compared to CD40L which strongly upregulated Bcl-X. BCR is a major driver of canonical NFκB signaling in CLL. Thus, we studied whether BAFF co-opted BCR signaling in CLL. BAFF induced rapid (15 min) phosphorylation of the proximal BCR kinases SYKand LYN, sustained for up to 4 h, as well as ERK, in CLL cells. AKT activation occurred late (>2 h), suggesting that BAFF induced AKT independent of BCR. BAFF-mediated BCR activation did not correlate with *IGHV* mutational status. Like IgM, BAFF induced CLL cell chemotaxis. SYK inhibition effectively antagonized survival and chemotaxis of BAFF-stimulated CLL cells. By contrast, targeting BTK or PI3K was less effective. All BCRi's fully blocked canonical NFκB activation in BAFF-stimulated CLL cells (suggesting its dependence on BCR signaling), but none inhibited the non-canonical pathway. By contrast, pevonedistat, an inhibitor of Nedd8-activating enzyme which we have previously shown to abrogate TNFR-mediated NFκB activation, blocked both canonical and non-canonical NFκB activity in BAFF-simulated CLL cells. SYK inhibitor entospletinib, but not other BCRi's, decreased Mcl-1 expression in CLL cells co-cultured with BAFF-expressing stroma and abrogated BAFF-mediated upregulation of pSTAT3, a transcription factor which regulates Mcl-1. This was accompanied by a decrease in Mcl-1 transcript. BAFF receptor signals via the TRAF complex to induce non-canonical NFκB activation in neoplastic B-cells. We supposed that TRAF complex could be directly responsible for SYK activation by BAFF. Indeed, IP experiments demonstrated that SYK directly complexed with TRAF2/3 in BAFF-stimulated neoplastic B-cells.
Summary/Conclusions: Thus, BAFF-mediated induction of BCR-associated kinases and Mcl-1 contributes to CLL cell survival. SYK inhibition is a promising therapeutic strategy uniquely poised to antagonize crosstalk between BAFF and BCR, thereby disrupting the pro-survival microenvironment signaling in CLL.

Pathogenesis of MDS

S119

LOW MYBL2 EXPRESSION OBSERVED IN MYELODYSPLASTIC SYNDROME PATIENTS WITH WORSE PROGNOSIS IS ASSOCIATED WITH ALTERED DNA REPAIR MECHANISMS IN HAEMATOPOETIC STEM CELLS

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Background: MYBL2 is a transcription factor with roles in the cell cycle and genome integrity. *MYBL2* is located on chromosome 20, within a region commonly deleted in human blood disorders (del20q). Our published data shows that reduced levels of MYBL2 predispose to development of myelodysplastic syndrome (MDS)-like disease in mouse models during ageing, indicating that MYBL2 could be acting as a tumour suppressor gene within del20q abnormality. Moreover, our previous work demonstrated that regardless of del20q deletion, MYBL2 expression is reduced in $CD34^+$bone marrow cells from MDS patients with worse prognosis. Because it has been shown that the cell of origin of MDS is the haematopoietic stem cell (HSC) and given the role of MYBL2 in DNA replication fork progression and maintenance of genome integrity, we hypothesised that low MYBL2 levels in HSC could contribute to elevated somatic mutations through changes in DNA repair pathways and drive disease development.
Aims: The aim of this study was to determine if low MYBL2 levels affect the double strand break (DSB) DNA repair damage response in HSC.
Methods: In this study we used our mouse model in which animals express ~50% normal levels of MYBL2 ($Mybl2^{+/\Delta}$). We characterised the ability of HSCs from young (7 weeks) and old (70 weeks) animals to respond to *in vivo* ionising radiation (2Gy) in terms of proliferation, apoptosis and colony forming ability. We measured the activation of the two main DNA repair pathways operating in the cells to deal with DSB: the error prone non-homologous-end-joining (NHEJ) and the error-free homologous recombination (HR) by assessing 53BP1 and Rad51 recruitment by immunofluorescence, respectively. Finally, we analysed the frequency of chromosome abnormalities present in the progeny of $Mybl2^{+/\Delta}$ HSC that have previously been irradiated to determine the long term effects of changes in DNA repair.
Results: We observed that $Mybl2^{+/\Delta}$ HSCs had limited proliferative potential and displayed an increased sensitivity to ionizing radiation which increased during ageing. $Mybl2^{+/\Delta}$ HSCs also displayed altered kinetics of 53BP1 and Rad51 recruitment and clearance, including retention of 53BP1 foci at later time points following irradiation and decreased levels of Rad51 foci when compared to $Mybl2^{+/+}$HSCs. Using plasmid functional assays, we showed that $Mybl2^{+/\Delta}$ HSCs repair quite efficiently by NHEJ, but this efficiency is disrupted when cells are challenged with ionising radiation. Furthermore, $Mybl2^{+/\Delta}$ HSCs have increased sensitivity to inhibition of DNA-PKC (required for NHEJ) but not ATM (required by HR). We also observed that after ionizing irradiation $Mybl2^{+/\Delta}$ HSCs progeny displayed an increased percentage of chromatids with fragile telomeres. Moreover, by making use of publically available RNA-seq datasets from MDS patients, we have identified a clear association between low MYBL2 levels and low expression of DNA-repair genes in patients with worse prognosis.
Summary/Conclusions: In summary, we have shown that decreased expression of MYBL2 leads to an imbalance in the DSB DNA-repair pathway choice, ultimately resulting in increased genomic instability of the blood cell progeny. These findings are supported by a signature of deregulated DNA-repair genes which strongly associates with low MYBL2 levels in MDS patient samples, providing a mechanistic understanding for the progression of blood disorders occurring during ageing. This study demonstrates a novel role for MYBL2 in DSB repair in HSCs and suggests that low levels of MYBL2 in human MDS could contribute to the emergence of further genetic abnormalities by deregulation of DNA-repair pathways.

S120

A NOVEL GENETIC AND MORPHOLOGIC PHENOTYPE OF ARID2-MEDIATED MYELODYSPLASTIC SYNDROMES

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Background: Clinical heterogeneity of myelodysplastic syndromes (MDS) and related myeloid neoplasms reflects molecular diversity. Most common genetic associations with distinct clinical or pathomorphologic phenotypes have been already reported, but many other common somatic lesions exist and their clinical context still remains elusive. AT rich interactive domain 2 (*ARID2*), which is located on chromosome 12q, encodes a component of the SWI/SNF complex that is involved in chromatin remodeling. In recent years multiple groups detected *ARID2* mutations in a variety of solid tumors.
Aims: Here, we present whole exome sequencing-guided identification of novel *ARID2* mutations in myeloid neoplasms. Specifically, in addition to copy number analysis and deep targeted and exome sequencing, here we include RNA sequencing and splicing analyses of the roles of spliceosomal mutations in *ARID2* missplicing and gene expression.
Methods: Bone marrow aspirates or blood samples were collected from 1,473 patients with MDS (n=455), myelodysplastic/myeloproliferative neoplasms (MDS/MPN) (n=201), myeloproliferative neoplasms (MPN) (n=56), sAML (n=221), and primary acute myeloid leukemia (pAML) (n=540) at the Cleveland Clinic and The University of Tokyo; the registered data at The Cancer Genome Atlas were also included. Diagnoses were classified using World Health Organization criteria. Informed consent for sample collection was obtained according to a protocol approved by each Institutional Review Board in accordance with the Declaration of Helsinki.
Results: By comprehensive genetic investigation of these cases, we characterized here cases (10%) in which decreased expression of *ARID2* mediated their clinical effects in MDS and other myeloid neoplasms via multiple kinds of genetic lesions. We showed that insufficient *ARID2* expression mainly in MDS arose from *ARID2* mutations, deletions, and missplicing due to *U2AF1* mutations that yielded defective *ARID2* transcripts. Clonal architecture analyses showed that *ARID2* mutations and deletions occurred as initial events of MDS or myelodysplastic/myeloproliferative neoplasms, and not during progression to acute myeloid leukemia. Morphologically, progressive maturation in myeloid and erythroid lineages and hypolobated megakaryocytes (indicated by arrow heads in Figure 1) were common in cases with *ARID2* mutations and deletions, and were also found in cases with *U2AF1* mutations. Functionally, we utilized *in vitro* knockdown models of *ARID2* expression in hematopoietic cell lines and bone marrow mononuclear cells. Since no homozygous deletion or mutation of *ARID2* was identified, we transduced shRNA in neoplastic and healthy hematopoietic cells to obtain disease models with partial reduction of *ARID2* expression. Two myeloid cell lines (HL60 and K562) in which *ARID2* expression was knocked down showed significantly lower cell counts compared to those with normal *ARID2* expression, compatible with more apoptotic cells in knockdown experiments. Flow cytometric analysis of the cell lines with reduced *ARID2* expression revealed increased cell-surface maturation markers, CD11b and glycophorin A (GPA), suggesting that reduced expression of *ARID2* resulted in more differentiation in myeloid and erythroid lineages. Knockdown of *ARID2* failed to reduce colony formation in bone marrow mononuclear cells. These results indicate that reduced *ARID2* expression might induce more differentiation in myeloid/erythroid lineages and more apoptosis to reduce cell populations without reduction of proliferation capacity in hematopoietic progenitor cells. Finally, we examined morphological findings associated with knockdown *ARID2* expression. Compared to control cells, K562 cells with reduced *ARID2* expression formed more hypolobated megakaryocytes, which confirmed morphological findings seen in *ARID2* and *U2AF1* defects.

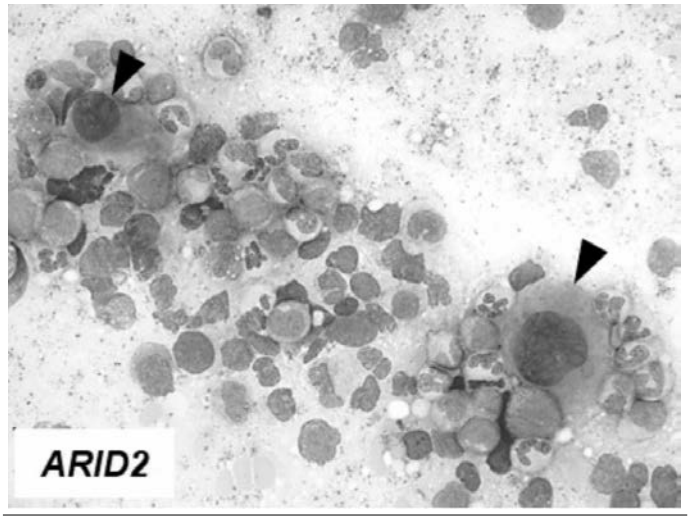

Figure 1.

Summary/Conclusions: *ARID2* is a MDS-suppressor gene whose expression is attenuated by multiple mechanisms as it shapes the distinct morphological phenotype of a subset of myelodysplasia.

S121

THE VALUE OF NGS PANEL SEQUENCING TO MOLECULARLY DEFINE MYELOID MALIGNANCIES AND CLARIFY BORDERLINE CASES: A STUDY ON 39 GENES IN 1143 PATIENTS

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Background: The 2016 revision of the WHO classification for myeloid malignancies includes numerous molecular markers for classification and prognostication. Next generation sequencing allows analyzing relevant genes in one panel.
Aims: Exploit clinical usefulness of panel sequencing in routine diagnostics in order to describe genetic changes and use respective patterns in cases with undefinitive morphology.
Methods: According to WHO 2016, 1143 patients were morphologically categorized as AML (n=261), MDS (n=176), MPN (n=19), CMML (n=51) or AML/MDS (n=21) and MDS/MPN overlap (n=28). Patients, who did not fulfill all characteristic criteria or had insufficient sample quality, were classified as "possible" AML (n=28), MDS (n=211), MPN (n=5), CMML (n=14) and as reactive (n=193) or unclear (n=136). DNA was isolated from BM (n=958) or PB (n=185) for NextSeq or MiSeq sequencing after TruSeq library preparation (all Illumina, San Diego, CA). Data was analyzed with SeqNext 4.3 (JSI Medical Systems, Kippenheim, Germany). *FLT3*-ITD and *KMT2A*-PTD data was obtained according to standard protocols.
Results: Analyzing 39 genes, we found ≥1 molecular change in 90% of patients (500/556) with a definite morphologic diagnosis (median: 2 genes; max: 7).
In de novo AML, 212/229 (93%) patients showed ≥1 molecular hit, of which 211 (92%) had aberrations that define WHO categories or have prognostic (according to ELN/MRC) or predictive value. More than one mutation was found in 166/229 patients (72%), including information of adverse impact (*e.g.* of 68 *NPM1* positive patients, 17 had *DNMT3A* mutations and 20 *FLT3*-ITD). Following *NPM1*, *RUNX1* was the second most frequently mutated gene (46/225; 20%) and mutations were significantly more common in patients with ≥3 aberrations (38/104; 37% *vs* 8/96; 8%; p<0.001). A similar *RUNX1* pattern was found in s-AML and t-AML. In the cohort of "possible AML" (including MDS overlap), 45/48 (94%) patients had ≥1 hit. Most frequently mutated were *ASXL1* (16/48; 33%), *TET2* (32%; 14/44) and *SRSF2* (29%; 14/48); 16% had all three mutated. This combination is also the most frequent three-way interaction in CMML (10/44; 23%). In MDS, 124/157 (79%) cases showed mutations, of which 108 had ≥1 prognostic change (according to Bejar, 2015). The prognostically favorable *SF3B1* mutation was present in 31/157 (20%) and significantly enriched among cases with ring sideroblasts (p<0.001). Overall, *TET2* showed the highest mutation rate (25%) and was also the most commonly mutated gene in cases with "possible" MDS (36/190; 19%), reactive morphologic changes (17/201; 8%) or even unclear morphology (19/116; 17%). Of these three subsets, five patients had only the *TET2* mutation with <10% burden, which is observed in clonal hematopoiesis of indeterminate potential (CHIP), too. However, using panel sequencing in cases with possible MDS, unclear or reactive morphology revealed at least one molecular marker for clonal disease in 47% (91/199), 36% (43/118) or 17% (36/211) of cases, respectively (excluding sole *ASXL1*, *DNMT3A*, *TET2* mutations with <10% burden).
Summary/Conclusions: WHO 2016 requires information on numerous genes for diagnosis, prognosis and therapeutic decisions. This challenges conventional laboratory approaches and suggests panel sequencing. We demonstrate the feasibility in routine settings for a broad spectrum of myeloid malignancies and identify 1) relevant patterns and mutation interactions; 2) genetic aberrations supporting diagnosis for samples with borderline morphology or poor quality and 3) patient-specific clonality useful for follow-up.

S122

IDENTIFICATION OF ABERRANTLY SPLICED GENES AND DEREGULATED PATHWAYS/GENE ONTOLOGY THEMES IN MYELODYSPLASTIC SYNDROME PATIENTS WITH SPLICING FACTOR GENE MUTATIONS

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Background: The myelodysplastic syndromes (MDS) are disorders of the hematopoietic stem cell (HSC) and patients suffer from anemia and other cytopenias and show increasing bone marrow blasts over time. Mutations in spliceosomal genes (including *SF3B1*, *SRSF2* and *U2AF1*) occur in >50% of MDS patients.
Aims: We aimed to identify the deregulated pathways and gene ontology (GO) categories associated with aberrantly spliced genes in CD34+cells and in differentiated cells of MDS-affected lineages isolated from the bone marrow of MDS patients harboring spliceosome mutations.
Methods: Transcriptome data were generated using RNA sequencing (RNA-seq) and splicing factor mutant cases were compared to wildtype cases and to healthy controls. Aberrant (including cryptic) splicing events were identified using rMATS. Deregulated pathways and GO themes were identified using Ingenuity Pathway Analysis and GOseq.
Results: RNA-Seq was performed on CD34+cells obtained from the bone marrow of 91 MDS patients (including 28, 8 and 6 cases with *SF3B1, SRSF2* or *U2AF1* mutations, respectively) and 8 healthy controls. The aberrant splicing events associated with each mutated splicing factor tended to affect different sets of genes, although some overlap was observed. GO analysis of the aberrantly spliced genes associated with *SF3B1*, *SRSF2* or *U2AF1* mutations showed a marked convergence of significantly enriched ontology themes: 26 of the top 30 most significant GO categories, including 'RNA splicing' and 'translation', in the comparison of mutant cases for each splicing factor gene to healthy controls (18 of 30 in the comparison to wildtype cases) were common to all three mutated splicing factor genes. Pathway analysis revealed deregulated pathways (*e.g.* 'oxidative phosphorylation' and 'mitochondrial dysfunction') that were common to more than one mutated gene (*i.e. SF3B1* and *SRSF2*), and pathways specific for one mutated splicing factor gene (*e.g.* 'protein ubiquitination' in *SF3B1* mutant cases). An analysis of upstream transcriptional regulators showed a significant overlap between the aberrantly spliced genes associated with each mutant splicing factor gene (in the comparison to both wildtype cases and to healthy controls) and genes regulated by several transcription factors, including E2F1. RNA-Seq was also performed on CD34+cells and on differentiated erythroid, granulocytic and monocytic cell populations isolated from the bone marrow of each of 7 *SF3B1* mutant MDS cases, 7 wildtype cases and 5 healthy controls, in order to explore similarities/differences between aberrantly spliced genes and deregulated pathways and GO themes in cells of different lineages. There were many aberrantly spliced genes in one cell population that did not overlap with aberrantly spliced genes in other populations. A small proportion (*i.e.* <5%) of aberrantly spliced genes were common to all four cell populations. GO analysis of the aberrantly spliced genes identified showed that 6 of the top 30 most significant categories (including 'RNA binding' and 'translation') in the comparison of *SF3B1* mutant cases to healthy controls (4 of 30 in the comparison to wildtype cases) were common to all four cell populations studied. Pathway analysis revealed that several pathways were deregulated in specific cell populations (*e.g.* 'mTOR signaling' in erythroid cells), and some pathways (*e.g.* 'EIF2 signaling', involved in protein synthesis initiation) were deregulated in all four cell populations studied.
Summary/Conclusions: Our study has identified aberrantly spliced genes and deregulated pathways associated with spliceosome mutations in the HSCs and the major cell lineages affected in MDS, providing new insights into how these mutations impact cellular processes in this disorder.

S123

TRANSCRIPTOME SEQUENCING REVEALS DISTINCT SUBTYPES OF MYELODYSPLASIA WITH PROGNOSTIC SIGNIFICANCE

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Background: Myelodysplastic syndromes (MDS) and related myeloid disorders ("myelodysplasia") are a heterogeneous group of clonal hematopoietic disorders with a highly variable clinical outcome.
Aims: The purpose of this study was to establish a novel gene expression-based classification of myelodysplasia for better prognostication.

Methods: We performed transcriptome sequencing of bone marrow mononuclear cells (BMMNCs) and/or CD34+cells obtained from patients with myelodysplasia. Consensus clustering was used to identify stable patient clusters. A classifier of the gene expression-based subgroups was constructed using the 100 CD34+cell samples as a training set, followed by validation in an independent cohort of 183 MDS patients. Another classifier was constructed using BMMNC samples from 51 patients, who had been assigned to the subgroups by the gene expression data of their CD34+cells. Prognostic significance of the model was tested in 114 patients of myelodysplasia.
Results: Unsupervised clustering of gene expression data of bone marrow CD34+cells from 100 patients identified two subgroups (Class-I and Class-II). The patients in the Class-II subgroup had higher percentages of bone marrow blasts compared to those in the Class-I subgroup (median 2% *vs* 11%, *P* <0.001). Pathway analysis revealed up-regulation of many signaling pathways in the Class-II subgroup. The Class-I subtype showed highly significant up-regulation of the genes related to erythroid lineages. The erythroid signature was rather suppressed in the Class-II subtype, which was characterized by increased expression of genes related to progenitor cells. Compared to the Class-I subtype, the Class-II subtype was associated with a significantly shorter survival in both univariate (hazard ratio [HR] 5.0 [95% CI, 1.8–14], *P* <0.001) and multivariate analysis (HR 6.8 [95% CI, 1.5–32], *P*=0.015). High frequency of leukemic transformation in the Class-II subgroup (38%) contrasted to no leukemic transformation in the Class-I subgroup. The prognostic significance of our classification was validated in an independent cohort of 183 patients. We also constructed a model to predict the subgroups using gene expression profiles of BMMNCs. The model was applied to 114 patients with BMMNC samples, of whom 47 (41%) were predicted to be the Class-II subgroup. Compared to the predicted Class-I subgroup, the Class-II subgroup was associated with a significantly shorter survival in univariate analysis (HR 7.2 [95% CI, 3.0–17], *P* <0.001). Again, association was more pronounced for leukemic transformation (HR 18 [95% CI, 4.2–80], *P* <0.001) than for overall survival. Multivariate analysis also demonstrated that the predicted Class-II subgroup was independently associated with leukemic transformation (HR 7.3 [95% CI, 1.3–41], *P*=0.024). Finally, we compared the prognostic value of our model with that of the LSC17 score, which has recently been proposed to predict a subset of poor risk acute myeloid leukemia based on the expression of 17 genes related to a leukemic stem cell signature. Our model outperformed the LSC17 score in predicting clinical outcomes of myelodysplasia, especially leukemia progression. The Class-II signature was shown to be more dramatically up-regulated during clonal evolution of myelodysplasia than the LSC17 score, which might be the basis of a better prediction of leukemia progression in our model.
Summary/Conclusions: Comprehensive transcriptomic analysis identified two subgroups of myelodysplasia with biological and clinical relevance, which could improve risk prediction and treatment stratification of myelodysplasia.

Lymphoma biology

S124

GENETIC ALTERATIONS INVOLVING PROGRAMMED DEATH LIGANDS IN EPSTEIN-BARR VIRUS-ASSOCIATED LYMPHOMAS

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Background: Checkpoint blockade using anti-PD-1/PD-L1 antibodies is a highly promising therapy for cancer, frequently showing dramatic anti-tumor responses in a wide variety of tumor types. Particularly, an exceptional response to anti-PD-1 antibodies has been demonstrated for classical Hodgkin lymphoma (HL), which is characterized by frequent copy number gains/amplifications involving *PD-L1* and/or *PD-L2*, suggesting a close link between *PD-L1/PD-L2* genetic alterations and the therapeutic response to these agents. Recently, we have reported frequent structural variations (SVs) in adult T-cell leukemia/lymphoma (ATL) caused by human T-cell leukemia virus type-1 (HTLV-1). These SVs invariably affect 3′-untranslated region (UTR) of *PD-L1*, leading to prominent *PD-L1* overexpression. Providing that viral antigens induce potent cellular immunity to virally infected cells, we hypothesized that a deregulated PD-1/PD-L1 axis might play a critical role in evasion from anti-viral immunity before these cells are clonally selected for neoplastic proliferation.
Aims: Epstein-Barr virus is a DNA tumor virus closely associated with various human cancers, including B- and natural killer (NK)/T-cell lymphomas, in which genetic alterations involving *PD-L1/PD-L2* may also be relevant to cancer evolution. In this study, to assess this hypothesis, we interrogated a variety of lymphomas for genetic abnormalities affecting *PD-L1* and *PD-L2*, especially focusing on EBV-associated lymphomas.
Methods: SVs and other genetic lesions affecting *PD-L1* and *PD-L2* were analyzed using targeted-capture sequencing with cRNA baits designed for capturing the entire sequences of *PD-L1* and *PD-L2* genes, including exons, introns, and 5′- and 3′-UTRs. More than 400 samples were analyzed obtained from different subtypes of non-Hodgkin lymphomas, including EBV-associated lymphomas, such as EBV-positive diffuse large B-cell lymphoma (DLBCL) and NK/T-cell malignancies.
Results: SVs and/or focal copy number gains involving PD ligands were successfully detected in various B-cell and T/NK-cell lymphomas, albeit at generally low frequencies (<10%). These lesions were the most frequently observed in PMBCL, accounting for more than 60% of the cases. Of note, high frequency (17−57%) of *PD-L1/PD-L2*-involving abnormalities were observed in mature NK/T-cell neoplasms, including extranodal NK/T-cell lymphoma, aggressive NK cell leukemia, and EBV-positive T-cell lymphoproliferative disorder, all of which were positive for EBV. Moreover, a substantial proportion (22%) of EBV-positive DLBCL cases possessed these lesions, whereas EBV-negative cases rarely exhibited these alterations (2%, P<0.01). For both *PD-L1* and *PD-L2* SVs, despite a large diversity of SV type (deletions, inversions, tandem duplications, and translocations), most of SVs resulted in 3'-UTR truncation, while the replacement of *PD-L1* or *PD-L2* promoter with an ectopic regulatory element was rarely observed. Interestingly, *PD-L1* SVs were detected in both B- and T-cell lymphomas, whereas *PD-L2* SVs were found exclusively in B-cell lymphomas.
Summary/Conclusions: We delineate the entire picture of genetic alterations involving PD ligands, and confirm the close association between these lesions and EBV-associated lymphomas. Our finding help to understand their pathogenesis and develop a new diagnostic strategy to identify patients who potentially benefit from PD-1/PD-L1 blockade therapy in non-Hodgkin lymphomas.

S125

FOXO1 CONTROL CD20 EXPRESSION AND INFLUENCE B-CELL LYMPHOMA RESPONSE TO RITUXIMAB-BASED IMMUNOTHERAPY

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Background: Recurrent somatic mutations of N-terminal region of FOXO1,

shown previously to increase FOXO1 nuclear localization and activity, have been linked to diminished survival in DLBCL patients uniformly treated with rituximab-based immunotherapy. Although the contribution of FOXO1 mutations to the therapeutic resistance of B-NHLs becomes apparent, the molecular mechanism underlying this phenomenon has not been explained so far. The diminished levels of CD20 on the cell surface of tumor cells are among several potential mechanisms underlying the resistance to treatment with anti-CD20 monoclonal antibodies.

Aims: We have recently reported that the tonic BCR signaling activates FOXO1, and that inhibitors of the downstream BCR signaling pathways downregulate CD20 expression. Therefore, here we sought to determine whether FOXO1 might regulate the abundance of CD20 on the surface of tumor cells thus influencing the response to rituximab-based therapies.

Methods: We used CRISPR/Cas9 genome editing technology and lentiviral transduction to study the role of FOXO1 protein in CD20 regulation. qRT-PCR and Dual Luciferase Assays was done to determine the influence of FoXO1 on CD20 transcription. To get insight into molecular interaction between FOXO1 and CD20 promoter we performed EMSA and ChIP experiments. For animal studies we used SCID Fox Chase mice model. All *in vivo* experiments were carried out at the animal facility of The Francis Crick Institute in accordance with the guidelines and were approved by the Ethics Committee.

Results: To determine the potential role of FOXO1 protein in CD20 regulation, we disrupted *FOXO1* locus using the CRISPR/Cas9 genome editing technology in Raji cells. In *in vitro* complement-dependent cytotoxicity assay we show that ablation of FOXO1 results in upregulation of CD20 levels and improvement of rituximab efficacy. To see whether FOXO1-dependent up-regulation of CD20 translates into improved antitumor efficacy of rituximab *in vivo* we have used SCID Fox Chase mice model. We found that mice treated with systemic rituximab survived longer when inoculated with sgFOXO1-transduced Raji cells as compared with mice inoculated with control Raji cells. Consistently, using clinically tested PI3K-AKT inhibitors - MK-2206 and GDC-0068 – in a set of CLL primary samples we show that also pharmaceutical inhibition of FOXO1 activity upregulated surface CD20 levels. Moreover, we demonstrated that FOXO1 regulate the CD20 promoter activity. In different B-cell lymphoma cell lines MK-2206 and GDC-0068 significantly downregulated the levels of *MS4A1* transcript (encoding CD20). Finally, using both EMSA and ChIP assays we detected specific binding of FOXO1 to the *MS4A1* promoter to the extent comparable to other known FOXO1 target genes.

Summary/Conclusions: Collectively, our results indicate that FOXO1 is strong negative regulator of CD20 expression and add new insights into the mechanisms underlying the contribution of *FOXO1* mutations to the resistance of B-NHLs to R-CHOP therapy. In light of current knowledge and our observations presented in this study, FOXO1 inhibition represents a novel strategy to increase the efficacy of anti-CD20 monoclonal antibodies.

Acknowledgements: Abstract supported by national grants: NCN, Poland, projects no: 2013/11/B/NZ5/03240 (BP) and 2015/18/E/NZ6/00702 (MW); MNiSW, Poland, project no: DI2014007344 (NM)) and European Comission (Horizon 2020, project no: 692180-STREAM-H2020-TWINN-2015, CSA action (JG).

S126

ALPHA-KETOGLUTURATE EXPOSES METABOLIC VULNERABILITIES IN B-CELL LYMPHOMAS

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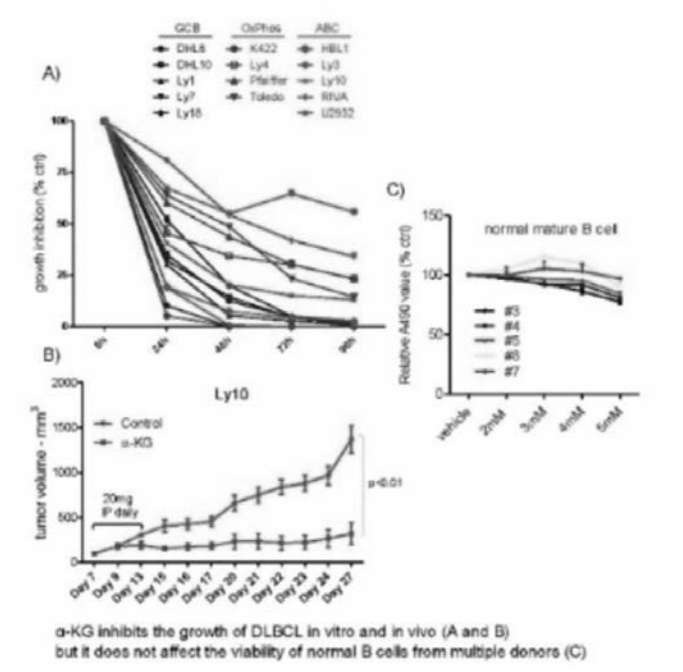
Background: Metabolic rewiring is a cancer hallmark. These metabolic changes can be secondary to a broader oncogenic-driven deregulation (*e.g.*, MYC), or they may be more specific and result from mutations in enzymes (*e.g.*, IDH1 and IDH2) that directly control energy flux in the cell. Mutant IDH acquires a neomorphic activity and aberrantly generates high levels of D2-hydroxyglutarate (D2-HG), a natural metabolite with marked structural similarity to alpha-ketoglutarate (α-KG). D2-HG functions as an oncometabolite by competitively inhibiting the activity of multiple α-KG-dependent dioxygenases, including TET DNA hydroxylases and JmJC HDMs. The role of a D2-HG/α-KG metabolic imbalance in cancer was expanded by our recent discovery of somatic loss-of-function mutations in D2HGDH in diffuse large B cell lymphomas (DLBCL) (Nat Commun. 2015 Jul 16; 6:7768). D2HGDH catalyzes the conversion of D2-HG into α-KG, and D2HGDH -mutant DLBCLs display deficiency of α-KG with attending impaired dioxygenase function. Together, these data suggest that while D2-HG is an oncometabolite, α-KG may function as tumor suppressive metabolite.

Aims: To explore the concept that α-KG has tumor suppressor activities, and to characterize the signaling nodes that mediate the anti-lymphoma activity of this intermediated metabolite.

Methods: We utilized a panel of 14 well-characterized DLBCL cell lines to test the growth suppressive activity of cell-permeable synthetic α-KG derivative, dimethyl-α-KG (DM-KG), using cell proliferation and apoptosis assays. The cell line data was expanded to primary mature B cell tumors as well as normal B cells. These *in vitro* assays were complemented by xenografts models of human DLBCL. Functional studies were performed in cell lines and primary tumors, and included the enzymatic quantification of ATP synthase activity, the effects of α-KG on cellular ATP levels, the measurement of AMPK activity and of the mTORC1 kinase.

Results: The cell-permeable DM-KG induced a marked dose-dependent growth suppression in a panel of DLBCL cell lines that encompasses the molecular heterogeneity of this disease (ABC, n= 5, GCB, n=5 and OxPhos, n=4). In most instances, the growth inhibition exceeded 80% of vehicle control exposed cells and it could be detected as early as 24h and reached its peak at 72-96h post-exposure (Figure 1A). In all cell lines examined, induction of apoptosis accounted for most of the anti-lymphoma effects of DM-KG. There was no clear segregation between the DLBLC molecular subtype and DM-KG-induced growth inhibition (*e.g.*, the ABC cell lines Ly10 and Ly3 were the most sensitive and most resistant, respectively, Figure 1A). Remarkably, we found that normal mature B cells (murine and human) were resistant to growth inhibition and apoptosis induced by DM-KG (Figure 1C). Contrary to that, exposing viable primary CLL, FL and DLBCL cells to DM-KG significantly induced apoptosis ($p<0.01$) in all tumors examined (n=17). In xenograft models of GCB- or ABC-DLBCLs (Ly7 and Ly10, respectively), DM-KG dosed intra-peritoneally significantly inhibited tumor growth in comparison to vehicle treated mice ($p<0.01$, n=16) (Figure 1B). To determine how DM-KG may induce growth suppression/apoptosis in mature B cell tumors, we first showed that in DLBCL cell lines α-KG inhibited the activity of ATP synthase, a key enzyme in the mitochondrial electron transport chain that generates most of the cellular ATP. Accordingly, short exposure (8h) to DM-KG suppressed ATP levels in all 14 DLBCL cell lines examined (mean=21%, range 3% to 50%). Next, we examined how the fuel stress generated by the α-KG-mediated ATP synthase inhibition influenced energy-saving cellular signals. We found that in cell lines and primary tumors, DM-KG consistently activated the kinase AMPK, with consequent marked inhibition of mTORC1. Importantly, these signals were also engaged in normal B cells, but they did not result in growth inhibition or apoptosis, thus highlighting the unique sensitivity of cancer cells to the modulation of energy metabolism.



α-KG inhibits the growth of DLBCL in vitro and in vivo (A and B) but it does not affect the viability of normal B cells from multiple donors (C)

Figure 1.

Summary/Conclusions: We showed that α-KG induces growth suppression and apoptosis in mature B cell tumors, *in vitro* and *in vivo*. We demonstrated that proximally α-KG exerts its tumor suppressive effects by inhibiting ATP synthase activity and ATP generation. This energy stress activates AMPK and suppresses mTORC1 resulting in growth inhibition and apoptosis in malignant cells, but not in their normal counterparts. These data highlight a metabolic cancer dependency and vulnerability that can be exploited therapeutically.

S127

DELETION OF THE F-BOX PROTEIN NIPA (NUCLEAR INTERACTION PARTNER OF ALK) IMPAIRS NPM-ALK DRIVEN TRANSFORMATION

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Background: ALCL is a high grade lymphoma characterized by anaplastic morphology, expression of CD30 (Ki-1) and T- or null cell phenotype. In 60% of systemic ALCL, the translocation t(2;5)(p23;q35) leads to expression of the oncogenic tyrosine kinase NPM-ALK. NIPA (Nuclear Interaction Partner of ALK) is an F-Box-Protein contributing to the timing of mitotic entry by defining an oscillating ubiquitin E3 ligase. NIPA deficient mice are viable but sterile due to impaired DNA double strand break repair. Co-expressed with NPM-ALK, NIPA is constitutively phosphorylated. However, the role of NIPA in NPM-ALK induced lymphomagenesis and the functional impact of this interaction remain unknown.
Aims: In this study, we aim to investigate the effect of *Nipa* deficiency on NPM-ALK driven cell proliferation and transformation in order to characterize the function of the protein in ALCL-induced lymphomagenesis.
Methods: Primary *Nipa*$^{-/-}$ MEFs infected with NPM-ALK were plated in softagar assays to evaluate their transformation ability. Moreover, NIPA was downregulated through targeted genetic approaches in Karpas299 and NPM-ALK infected Ba/F3 cells, which were analyzed regarding proliferation, signaling, and apoptosis. To assess the impact of *Nipa* deletion *in vivo*, we used a retroviral bone marrow transplantation model resembling human ALCL. Based on a Cre/loxP system under the LCK-Promotor, NPM-ALK expression and *Nipa*-deletion are restricted to early T cells. In wildtype background, mice die of systemic Thy1.2^{+}lymphoma with a latency of 4-6 months, developing neoplastic T-cell infiltration of bone marrow and lymphatic organs. Lymphomas were analyzed regarding immunphenotype and clinical presentation.
Results: Primary *Nipa*$^{-/-}$MEFs plated in softagar showed significantly reduced colony formation potential upon NPM-ALK expression (38 *vs* 79 CFUs; p<0.001). These results were substantiated in human and murine cell lines, where significantly reduced proliferation ability was observed in NIPA downregulating NPM-ALK expressing Ba/F3 cells (74% of ctrl; p<0.001) as well as in Karpas299 cells infected with NIPA miR (66% of wt growth; p<0.01). Moreover, treatment with the ALK inhibitor TAE-684 gave evidence of possible synergistic effects of ALK inhibition and NIPA knockdown. Mice transplanted with Lck-Cre$^{TG/wtNipaflox/flox}$MSNAIE infected bone marrow cells showed significantly prolonged disease development and progression (mean survival 143d *vs* 121d in wt). Morphologically, mice presented with enlarged thymi, splenomegaly, lymphadenopathy, and bone marrow infiltration. Immunphenotyping showed a pure T-cell phenotype in *Nipa*$^{-/-}$ lymphomas, thus resembling wildtype. In a long-latency model of NPM-ALK expression in enriched HSCs, a significantly prolonged survival (110 *vs* 80 days; p<0.01) and reduction of spleen colonies (10 *vs* 28 colonies/spleen; p<0.001) in mice transplanted with Mig$^{NPM\text{-}ALKNipa-/-}$ bone marrow compared to control animals were observed, thereby suggesting a crucial role of NIPA in NPM-ALK driven lymphomagenesis. To investigate the precise mechanism undelaying these results, we performed cell cycle analyses as well as cell viability assays. Indeed, we were able to detect significant differences in the cell viability in *Nipa* deficient NPM-ALK expressing cells, whereas cell cycle distribution seems not to be altered in knockout cells.
Summary/Conclusions: Taken together, we were able to show that NIPA is crucial for cell proliferation and transformation upon NPM-ALK expression. Investigations of the NIPA knockout mouse in a clinical relevant ALCL model highlight the importance of the NIPA/NPM-ALK axis in lymphoma development. Further analyses may thus elucidate NIPA as a novel molecular target for therapeutic intervention.

Thalassemia

S128

GENE THERAPY FOR BETA THALASSEMIA: INITIAL RESULTS FROM THE PHASE I/II TIGET-BTHAL TRIAL OF AUTOLOGOUS HEMATOPOIETIC STEM CELLS GENETICALLY MODIFIED WITH GLOBE LENTIVIRAL VECTOR

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Background: Gene therapy for transfusion dependent beta-thalassemia, as an alternative cure to allogeneic HSCT, is based on the autologous transplantation of hematopoietic stem cells (HSCs) engineered by lentiviral vectors expressing a transcriptionally regulated human beta-globin gene.
Aims: Our contribution to this field was devoted to the clinical development of a gene therapy protocol based on high-titer vector GLOBE, use of lenograstim and plerixafor as source of HSCs and a conditioning regimen based on myeloablative treosulfan and thiotepa favoring efficient engraftment of corrected cells with reduced toxicity (TIGET-BTHAL; EudraCT number 2014-004860-39).
Methods: On the basis of extensive efficacy and safety preclinical studies the clinical trial TIGET-BTHAL was approved and started in 2015 at Scientific Institute San Raffaele, Milan, Italy. The clinical study foresees treatment of 10 patients: 3 adults followed by 7 minors, with a staggered enrolment strategy based on evaluation of safety and preliminary efficacy in adult patients by an independent data safety monitoring board before inclusion of pediatric subjects. The chosen route of administration of gene modified HSCs is intraosseous in the posterior-superior iliac crests, bilaterally, with the aim of enhancing engraftment and minimizing first-pass intravenous filter.
Results: As of February 2017, seven patients (3 adults and 4 pediatric patients) with different genotypes (β^0/β^0, $\beta^+/\beta^+$and β^0/β^+) have been treated with GLOBE-transduced CD34^{+}cells at a dose of $16x10^6$-$19.5x10^6$ cells/kg and a vector copy number (VCN)/cell ranging from 0.7 to 1.5. The procedure was well tolerated by all patients, with no product-related adverse events. Multilineage engraftment of gene-marked cells was observed in all tested peripheral blood and bone marrow samples. Polyclonal vector integrations profiles have been detected in the first 3 patients tested.
Summary/Conclusions: So far, the clinical outcome indicates reduction in transfusion requirement in adult patients and greater clinical benefit in younger patients. Follow up analysis are ongoing and updated clinical outcome will be presented.

S129

LUSPATERCEPT INCREASES HEMOGLOBIN AND DECREASES TRANSFUSION BURDEN IN ADULTS WITH B-THALASSEMIA

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Background: Luspatercept (ACE-536), a fusion protein containing a modified activin receptor type IIB, is being developed for the treatment of β-thalassemia. Luspatercept binds to select TGF-β superfamily ligands (such as GDF11) reducing aberrant Smad2/3 signaling and promoting late-stage erythroid differentiation and increased hemoglobin (Hgb). Luspatercept corrected the effects of ineffective erythropoiesis in a mouse model of thalassemia (Suragani R, Blood, 2014) and increased Hgb and was well tolerated in a phase 1 study in healthy volunteers (Attie K, Am J Hematol, 2014).
Aims: This ongoing, phase 2, multicenter, open-label study followed by a long-term extension (ext) study evaluates the effects of luspatercept in patients (pts) with either transfusion-dependent (TD) or non-transfusion dependent (NTD) β-thalassemia with key endpoints of erythroid response (including Hgb increase) and pt-reported quality-of-life (QoL) in NTD patients, and reductions in RBC transfusion burden in TD patients.
Methods: Inclusion criteria: age ≥18 yr and either TD (≥4 RBC U/8 weeks prior

to first dose, confirmed over 6 months) or NTD (<4 RBC U/8 weeks prior to first dose with baseline Hgb <10 g/dL). Pts were treated every 3 weeks subcutaneously for up to 5 doses; 6 cohorts were treated at dose levels from 0.2-1.25 mg/kg. Pts in the expansion cohort and those who rolled over to the ext study were treated at ≥0.8 mg/kg with titration up to 1.25 mg/kg (base completed NCT01749540; ext ongoing NCT02268409).

Results: As of 02Sept2016, a total of 64 pts enrolled in the base study (31 TD, 33 NTD) and, of those, 51 enrolled in the ext study (24 TD, 27 NTD). Median (range) age (yr) was 38.5 (20-62); 67% had prior splenectomy. For TD pts, at baseline, median (range) transfusion burden was 8 U/12 weeks (4-18 U); mean (SD) liver iron concentration (LIC, mg/g dw) was 5.0 (5.3). For NTD pts, at baseline, median (range) Hgb (g/dL) 8.5 (6.5-9.8); mean (SD) LIC (mg/g dw) was 5.4 (3.8). In base and ext, respectively, 22/31 (71%) and 20/24 (83%) TD pts achieved ≥33% and 17/31 (55%) and 17/24 (71%) achieved ≥50% reduction in transfusion burden over any 12-week period compared to baseline. Median duration of ≥33% reduction was 6.3 months (treatment ongoing). In base and ext, respectively, in NTD pts treated with ≥0.6 mg/kg, 13/21 (62%) and 21/27 (78%) achieved ≥1.0 g/dL and 7/21 (33%) and 14/27 (52%) achieved ≥1.5 g/dL increases in mean Hgb over any 12-week period compared to baseline. Median duration of Hgb increase ≥1.0 g/dL over 12 weeks in responders was 13.5 months (treatment ongoing). Increases in mean Hgb over a 12-week period correlated with improvement in a pt-reported QoL questionnaire, FACIT-F. Luspatercept was generally well tolerated, with no related serious adverse events and few grade 3 related AEs: bone pain (n=3), asthenia (n=2), and headache (n=1). The most frequent related AEs (≥10%) were bone pain, myalgia, headache, musculoskeletal pain, arthralgia, and injection site pain.

Summary/Conclusions: Long-term luspatercept treatment in pts with β-thalassemia was generally safe and well tolerated. Efficacy was clinically relevant in both TD pts (decreased transfusion burden) and NTD pts (increased Hgb levels, improved QoL). A Phase 3, double-blind, placebo-controlled study of luspatercept in regularly transfused adults with β-thalassemia is ongoing (NCT02604433).

S130

DENOSUMAB INCREASES BONE MINERAL DENSITY IN PATIENTS WITH THALASSEMIA MAJOR AND OSTEOPOROSIS: RESULTS OF A RANDOMIZED, PLACEBO-CONTROLLED, DOUBLE BLIND, PHASE 2B CLINICAL TRIAL

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Background: Osteoporosis is a common complication of thalassemia major (TM) with a complex pathophysiology. We have previously shown that RANKL, the most potent osteoclast activator, is elevated in the serum of TM patients and correlates with reduced bone mineral density (BMD). Denosumab (DMB) is a human monoclonal antibody that targets and binds to RANKL and has been licensed for patients with different types of osteoporosis. However, there are no prospective data for the effects of DMB on TM-induced osteoporosis.

Aims: The primary objective of this study was to evaluate the results of DMB on lumbar spine (L1-L4) BMD in patients with TM and osteoporosis as compared to placebo at 12 months. Secondary endpoints included the evaluation of the effects of DMB on femoral neck (FN) and wrist (WR) BMD at 12 months, the safety profile of DMB as well as its effects on bone turnover.

Methods: This was a single-site, randomized, placebo-controlled, double blind phase 2b clinical trial. Main inclusion criteria included adult patients (>30 years of age) with TM and BMD T-score between -2.5 and - 4.0 in at least one of the examined sites (L1-L4, FN, WR). Main exclusion criteria included: impaired renal function (eGFR of ≤30 mL/min); elevated ALT and/or AST >2 fold the upper limit of normal (UNL), or elevated direct bilirubin >1.5xUNL; heart failure (NYHA above 2); administration of bisphosphonates within one year of study enrolment; presence of any other disorder that affects bone metabolism. Patients were assigned into two treatment groups: in group A, 60 mg DMB was administered sc, every 6 months for 12 months for a total of 2 doses (day 0 and day 180); in group B, placebo was administered sc, at the same time. All patients received calcium and vitamin D supplementation. Measurement of BMD with dual energy X-ray absorptiometry at three body sites (L1-L4, FN, WR) was performed during the screening period and at the end of the study.

Results: Sixty-three patients with TM and osteoporosis participated in the study (group A, n=31; group B, n=32). Patients of groups A and B showed no differences in BMD of all evaluated sites at baseline. Patients of group A (DMB arm) achieved an increase in both L1-L4 BMD (mean±SD: 0.811±0.105 g/cm2 *vs* 0.772±0.098, p<0.001) and FN BMD (0.653±0.121 g/cm2 *vs* 0.631±0.103, p=0.022), while there were no changes in WR BMD. Patients of group B (placebo arm) achieved a slight increase in their L1-L4 BMD (0.801±0.097 g/cm2 *vs* 0.775±0.080, p=0.004) and a significant decrease in their WR BMD (0.520±0.099 g/cm2 *vs* 0.549±0.098, p=0.008). The percentage increase of L1-L4 BMD was higher in DMB arm than in placebo arm (6.02±5.30% *vs* 3.11±5.46%, respectively; p=0.03), while the advantage of DMB regarding WR BMD was much higher compared to placebo (-0.22±5.40% *vs* -4.15±8.58%, respectively; p=0.02) as well as in FN BMD (p<0.001). No grade 3 or 4 toxicity was observed in this study.

Summary/Conclusions: This first analysis of our phase 2B study regarding the effects of DMB on BMD of different sites (the results of bone markers will be presented in the conference), suggests that DMB, given twice per year, increases the BMD of the L1-L4 more efficiently than placebo (in combination with vitamin D and calcium), after 12 months, in patients with TM and osteoporosis, with excellent safety profile. Furthermore, DMB increased the FN BMD, which was not increased in the placebo arm, while DMB has also a positive effect on WR BMD compared to placebo. These data support the use of DMB for the management of TM-induced osteoporosis.

S131

LONG-TERM HEALTH STATUS AFTER HSC TRANSPLANTATION FOR THALASSEMIA: THE FRENCH EXPERIENCE

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Background: In clinical practice**,** allogeneic hematopoietic stem cell transplantation (HSCT) is the only treatment offering a definitive cure for patients with beta-thalassemia. Its outcome has improved over the last 3 decades with currently a disease free survival rate of 90% when transplant is performed in childhood from an HLA-identical sibling. Few data are available on long-term toxicity and frequency of chronic complications after transplant.

Aims: The purpose of this study was to evaluate the long-term health status after a successful allogeneic HSCT for beta-thalassemia major in a national cohort of patients.

Methods: This French retrospective study included patients who successfully received allogeneic HSCT between 1985 -2012 and were alive at least 2 years after HSCT. Study was based on data collected in the national registry of patients with beta-thalassemia and conducted in collaboration with the French society of HSCT (SFGM-Tc). Late effect data were recorded by physicians through reference or transplant center visits. Collected data included medical examination results, long-term treatments administered and laboratory tests (serum ferritin, Hb, liver enzymes, creatinine level and thyroid evaluation). Linear-mixed model was used to analyze data evolution over time (for height and weight SDS, SF, Hb values).

Results: A total of 134 patients had received allogeneic HSCT for beta-thalassemia in France from 1985 to 2012. 107/134 patients experienced successful HSCT (6 after a second transplant) and were alive 2 years after transplantation. Six were not analyzed (back to their country or lost of follow-up) and two died of chronic graft-*versus*-host disease. 99 patients were analyzed for long-term effects. Median age at HSCT was 5.9 years (8 month-26 years). The source was bone marrow in 85% of cases and a matched sibling donor was used in 90% of cases. Conditioning mostly consisted (85%) of busulfan and cyclophosphamide (oral busulfan in 52%). Median age at the last visit was 19 years. Chronic complications, similar to those observed in patients treated with transfusion and chelation therapy occurred after transplant in 12% of patients: 7 hypothyroidism, 2 heart failure, 5 diabetes. 2 patients had chronic respiratory failure related to transplant. The height SDS improved after HSCT if performed at a young age. Weight

SDS values increased with time, especially in females. Although gonadal dysfunction was observed in 60% of women aged at least 13 years at the last evaluation, 12/27 aged more than 20 years experimented at least one successful pregnancy. 93 patients had stopped their immunosuppressive treatment two years after HSCT. 37 were treated with iron chelation therapy and/or phlebotomies. At least half of patients are receiving a long-term hormonal treatment or antibiotic prophylaxis at the last visit. Decrease in serum ferritin values after transplant was significantly influenced by age at transplant and pre-transplant serum ferritin value. The median hemoglobin value was 12.5 g/dL (86-165) at a mean age of 18 years and Hb values were significantly influenced by age, the sex of the donor and the presence of donor thalassemia trait.
Summary/Conclusions: A comprehensive and regular long-term follow-up should be established for all patients receiving allogeneic HSCT for beta-thalassemia major. In this national cohort, endocrinological complications were frequent after transplant. Fertility can be partly preserved, but this result has to be reevaluated with the more recent use of intravenous busulfan.

S132

CD34+AND HUMAN INDUCED PLURIPOTENT STEM CELL DIFFERENTIATION TO TRANSFUSION READY RED BLOOD CELLS

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Background: Donor-derived red blood cells (RBC) are the most common form of cellular therapy. However the source of cells is dependent on donor availability with a potential risk of allo-immunization and blood borne diseases.
Aims: We aim to produce unlimited numbers of cultured RBC with a defined `universal donor´ phenotype for transfusion purposes.
Methods: To this end we prepare for a clinical test using autologous cultured RBC to test their *in vivo* stability. In parallel we develop methods for unlimited production of cultured RBC. An immortal source to produce *in vitro* cultured RBCs (cRBC), such as iPSCs would allow selection of `universal donor` RBC, or provide an autologous end product with the absence of immune reactions.
Results: The *in vitro* production of RBC has proven to be successful, however there are barriers to overcome prior to clinical application. e.g: xeno-free culturing methods, scale up cultures to obtain transfusion units (1-2*10^{12} erythrocytes), and for iPSC we need virus- and transgene-free reprogramming protocols. To solve the above mentioned issues a customized humanized GMP-grade medium (Cellquin) was generated in order to control erythroid culture parameters and to reduce culture costs. This medium allowed 1*10^{8} times erythroid expansion from PBMCs to pure adult EBL cultures within 25 days, comparable to non-GMP commercial media. To generate iPSC, a non-integrative polycistronic episomal vector containing (OCT4-SOX2-KLF4-cMYC-LIN28) was used to reprogram PBMC-expanded EBLs to iPSC, displaying pluripotency potential and normal karyotype. iPSCs were adapted to single cell passage allowing directed colony differentiation using a feeder-free monolayer approach. From day 6 of differentiation Cellquin was applied with lineage-specific growth factors, resulted iPSC differentiation to EBLs which was initiated by the appearance of hemogenic endothelium following hematopoietic specification. Our differentiation method resulted in ~150*10^{6} CD41- CD34-CD71+CD235+CD36+expanded EBLs from 1200iPSCs within 21 days (12 days of iPSC diff. +9 days of expansion). Further maturation of iPSC-EBLs yielded CD71+CD235+CD36- pure orthochromatic normoblasts expressing mainly gamma globin chains (fetal) and small amount of beta globins (adult) in agreement with literature. Currently we are testing enucleation potential of matured iPSC-EBLs.
Summary/Conclusions: Here we showed that our monolayer approach is simple, highly controlled and compatible with upscaling. Avoiding virus-, integrative reprogramming, feeders and with our GMP-grade media we maintained a cost effective system moving toward clinical application.

AML Biology I: Towards molecular therapies

S133

FUNCTIONAL PROTEOMICS IDENTIFIES SETD2 AS A CRITICAL EFFECTOR OF MLL FUSION PROTEINS TO SAFEGUARD GENOMIC INTEGRITY

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Background: Acute Myeloid Leukemia (AML) frequently harbors chromosomal rearrangements involving the Mixed Lineage Leukemia *(MLL)* gene. More than 65 different MLL fusion genes exist and many of them have been described to act as strong cancer drivers. While critical effectors of several distinct MLL fusion proteins (MLL-FPs) were identified, it is not clear if transforming mechanisms are conserved across the entire family of MLL fusions.
Aims: We hypothesized that common oncogenic mechanisms are encoded in stable physical and genetic MLL-fusion-specific interaction networks. Thus, we aimed to identify common critical effectors of different MLL fusion proteins that are presumed to employ different mechanisms of oncogenic transformation.
Methods: Protein complexes of 7 molecularly distinct, affinity-tagged MLL-FPs (MLL-AF4, MLL-AF9, MLL-ENL, MLL-CBP, MLL-EEN, MLL-GAS7 and MLL-AF1p) were purified from stable cell lines allowing for inducible, single-copy transgene expression and characterized by mass spectrometry. Data analysis identified a comprehensive protein-protein interaction network, which was functionally interrogated by a subtractive shRNA screening approach. Validation experiments included detailed RNAi- and CRISPR/Cas9-mediated loss of function experiments in cell lines and primary cells *in vitro* and *in vivo*, using readouts for changes in proliferation, differentiation, apoptosis and DNA damage.
Results: Characterization of the protein complexes nucleated by 7 MLL fusion proteins by affinity purification coupled to mass spectrometry (AP-MS) revealed a densely interconnected protein-protein interaction network of 963 proteins, comprising previously known MLL-interacting protein complexes (such as PRC2 or SWI/SNF), as well as a high number of new interaction partners of MLL. 128 proteins were found to interact with ≥5 of all 7 MLL-fusions. This subset of conserved MLL-interaction partners was highly enriched for proteins with function in chromatin metabolism and transcriptional control. Systematic functional investigation of the conserved MLL-fusion interactome using subtractive shRNA screens identified the methyltransferase SETD2 as a critical effector of MLL fusion proteins. Both RNAi-based suppression and CRISPR/Cas9-mediated mutagenesis of SETD2 induced myeloid differentiation and apoptosis in human and mouse MLL-rearranged cell lines, while having only modest effects on the proliferation of MLL-wild-type leukemia cells. Depletion of *Setd2* in MLL-fusion-transformed mouse fetal liver cells resulted in loss of serial re-plating capacity *in vitro* and prolonged disease onset *in vivo*. Furthermore, knockdown of *SETD2* caused a proliferative disadvantage in primary cells from AML patients with different MLL-rearrangements without affecting MLL-wild-type AML cells. We found that SETD2 was essential for efficient repair of DNA breaks, as SETD2-deficient leukemia cells showed increased levels of DNA damage and activation of p53, leading to the accumulation of mutations.
Summary/Conclusions: In summary, our data highlight the functional relevance of combined proteomic-genomic cellular screening to identify critical effectors of MLL-FPs. In addition, our study identifies a novel role for SETD2 in the maintenance of genomic integrity during initiation and progression of MLL-rearranged AML and establishes SETD2 as a therapeutic target in leukemia with low genomic complexity.

S134

CEBPA-MUTANT ACUTE MYELOID LEUKEMIA IS SENSITIVE TO SMALL-MOLECULE-MEDIATED INHIBITION OF THE MENIN-MLL INTERACTION

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Background: The *CEBPA* gene - encoding for the transcription factor C/EBPα - is mutated in 9% of patients with acute myeloid leukemia (AML). *CEBPA* N-terminal mutations lead to selective loss of full length C/EBPα p42 expression without affecting translation of the shorter p30 isoform. As a balanced ratio of C/EBPα isoforms is crucial for hematopoietic homeostasis, depletion of p42 leads to increased cell growth and blocks myeloid differentiation, resulting in the development of AML. We have recently shown that the p30 variant of

C/EBPα can act as a gain-of-function allele with distinct molecular properties. However, the mechanistic basis of C/EBPα p30-induced leukemogenesis is incompletely understood.
Aims: We hypothesized that the interaction between the oncogenic C/EBPα p30 isoform and the MLL/SET histone methyltransferase complex is required for p30-dependent epigenetic and transcriptomic changes that contribute to leukemogenesis. Therefore, we aimed to investigate the sensitivity of *CEBPA*-mutant AML to perturbation of MLL/SET function.
Methods: We used CRISPR/Cas9-mediated mutagenesis to interfere with the MLL/SET complex in myeloid progenitor cells from a *Cebpa*$^{p30/p30}$ AML mouse model. Cellular competition assays were used to assess changes in proliferative capacity of mutant cells. Further, MLL activity was inhibited by the use of small molecules that block the Menin-MLL interaction. In both cases, proliferation, myeloid differentiation and apoptosis were used as readouts. Global changes in gene expression were measured by RNA-seq.
Results: We initially confirmed, via ChIP, that C/EBPα and MLL co-localize on the promoters of p30 target genes, indicating functional cooperativity in gene regulation. To investigate the importance of different, annotated functional domains within the MLL protein in the context of C/EBPα p30 expression, we introduced targeted mutations across the *Mll* gene in *Cebpa*$^{p30/p30}$ cells using the CRISPR/Cas9 system. This analysis revealed a strong dependence of *Cebpa*$^{p30/p30}$ cells on the expression of an intact MLL protein. Surprisingly, loss of the enzymatic activity of *Mll* by mutational targeting of the SET domain did not significantly affect cell survival. In contrast, cells were particularly sensitive to mutagenesis of the Menin-binding motif in MLL. *Mll* targeting strongly induced myeloid differentiation in *Cebpa*$^{p30/p30}$ cells as measured by increased levels of myeloid surface markers. To investigate functional consequences upon pharmacological perturbation of the MLL/SET complex, we used MI-463, a potent small-molecule inhibitor of the Menin-MLL interaction. Inhibitor treatment led to a time- and dose-dependent impairment of proliferation, induction of cell cycle arrest and increased apoptosis in *Cebpa*$^{p30/p30}$ cells. RNA-seq analysis showed that inhibitor treatment induced the expression of genes associated with myeloid differentiation, which could be confirmed by flow cytometry. Importantly, expression of C/EBPα p30 was associated with hypersensitivity to Menin-MLL inhibition, as *Cebpa*$^{p30/p30}$ cells were 2-6 fold more sensitive than other leukemia cell lines of mouse and human origin.
Summary/Conclusions: We show that *CEBPA*-mutated AML is highly sensitive to perturbation of the MLL/SET complex, either via genetic ablation of MLL or through pharmacological inhibition of the Menin-MLL interaction. Our data indicate that leukemic mutations of C/EBPα selectively cooperate with the SET/MLL complex to regulate gene expression. These findings expand our understanding of and may inform new therapeutic strategies for N-terminal *CEBPA* mutated AML.

S135

INHIBITION OF THE MYELOID MASTER REGULATOR PU.1 AS A THERAPEUTIC STRATEGY IN ACUTE MYELOID LEUKEMIA

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Background: Functionally critical decreases in PU.1 levels or activity are present across various different genetic and epigenetic subtypes of AML, and overall represent more than 50% of AML cases (Sive *et al.* Leukemia. 2016). However, approaches for the specific therapeutic targeting of these patients are thus far lacking.
Aims: Retroviral restoration of PU.1 expression has previously been explored but is difficult to achieve pharmacologically. Here, we tested the inverse strategy. As complete loss of PU.1 leads to stem cell failure, we hypothesized that AML cells harboring already low levels of PU.1 may be more vulnerable to further PU.1 inhibition.
Methods: We used two alternative approaches: RNA interference and newly developed small molecule PU.1 inhibitors.
Results: We found that inhibition of PU.1 with different shRNAs led to a significant decrease in proliferation and clonogenicity, and increased apoptosis of mouse and human leukemic cell lines with low PU.1 levels, as well as the majority of primary human AML cells tested. The pharmacologic targeting of transcription factor-DNA major groove interactions is challenging. However, specific PU.1 binding to chromatin critically depends on additional minor groove contacts upstream of the core ETS binding motif, which determine selectivity for PU.1. We used an integrated screening strategy utilizing biosensor surface plasmon resonance, DNA footprinting, and cell-based dual-color PU.1 reporter assays to develop novel small molecules of the heterocyclic diamidine family as first-in-class PU.1 inhibitors. Targeted occupancy by our compounds in the minor groove induces perturbations in DNA conformation that are transmitted to the PU.1 site in the major groove and thus inhibit PU.1 binding via an allosteric mechanism. Functionally, treatment with 3 different compounds decreased cell growth and colony forming capacity, increased apoptosis, and disrupted serial replating capacity of PU.1^{low} AML cell lines, and a majority of primary AML cell samples. ChIP and expression analysis showed that the compounds disrupt PU.1-promoter interaction and lead to downregulation of canonical PU.1 transcriptional targets in AML cells, confirming on-target activity. Genome-wide analysis showed highly significant enrichment of known transcriptional targets of PU.1, and selectivity over other ETS family members. Comparison with published transcriptomic and PU.1 ChIP-seq data sets, as well as ARACNe analysis of the PU.1 regulon in primary AML cells, demonstrated that the inhibitors antagonized PU.1-regulated pathways at a genome-wide level. Treatment of normal HSPC in colony forming assays led to decreased production of mature granulo-monocytic cells, consistent with PU.1's known role in this lineage. However, this effect was reversible upon drug removal, and serial replating capacity was not affected suggesting no significant effects on normal HSPC. Lastly, *in vivo* treatment with PU.1 inhibitors in mouse and human AML (xeno)transplantation models significantly decreased tumor burden and increased survival.
Summary/Conclusions: Our study describes for the first time a strategy inhibiting PU.1 in AML, establishing proof-of-concept for this approach. Furthermore, we report the development of first-in-class PU.1 inhibitors which interfere with PU.1-DNA interaction through an allosteric, minor groove-mediated mechanism. Our work shows that it is feasible to pharmacologically target PU.1, and raises intriguing possibilities for the potential targeting of other transcription factors through minor groove-directed approaches.

S136

METABOLIC ADAPTATIONS TO TARGETED THERAPY IN FLT3 MUTATED ACUTE MYELOID LEUKAEMIA

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Background: FLT3 tyrosine kinase (TK) activating mutations (FLT-3^{mut}) are amongst the most frequent in AML and are associated with a poor outcome. FLT-3^{mut}promote constitutive activation of survival/proliferation pathways and have also been shown to lead to changes in cellular metabolism, such as increased glycolysis. The FLT3 TK represents a valid therapeutic target and several FLT3 TK inhibitors (TKI) have been developed. However despite showing activity in the preclinical setting, FLT3 TKI have displayed limited efficacy in clinical trials. Resistance mechanisms to FLT3 TKI include receptor mutations and cell intrinsic adaptive mechanisms. Amongst the latter, metabolic adaptations might play a significant role although the exact mechanisms are still ill-defined.
Aims: We hypothesised that metabolic adaptations facilitate FLT3 TKI resistance and aimed to identify early metabolic changes in FLT-3^{mut} AML, following TKI treatment, in an attempt to unveil novel therapeutic vulnerabilities.
Methods: Liquid chromatography coupled to mass spectrometry (LC/MS), using stable isotope-based carbon flux tracing, and oxygen consumption rate/extracellular acidification rate as measured by an extracellular flux analyser (Seahorse, Agilent Technologies) were used to assess metabolic changes in FLT3^{mut} cells after FLT3 TKI treatment. Gene expression changes were measured in the same conditions by RNA sequencing. Changes in viability and reactive oxygen species (ROS) in various culture conditions were measured by FACS. Gene silencing was performed using CRISPR-Cas9 gene editing and inducible short hairpin RNA interference.
Results: Analysis of published gene expression datasets demonstrated that glycolytic, citric acid cycle (CAC), and oxidative phosphorylation genes are upregulated in FLT-3^{mut} compared to FLT3 wild-type (FLT3^{wt}) patient samples at diagnosis. We then confirmed that both human and murine FLT-3^{mut} cells display increased glycolytic and respiratory capacity compared to FLT-3^{wt} cells. Upon treatment with the highly selective FLT-3 TKI AC220 (quizartinib), currently used in a number of clinical trials, these metabolic phenotypes were partially reversed. However, whilst glucose uptake was reduced upon FLT3 TK inhibition, glutamine uptake was not affected. Metabolic flux analysis using [U-^{13}C]glutamine demonstrated that glutamine, while providing carbons for the CAC, was primarily used to support production of the major intracellular antioxidant glutathione upon AC220 treatment. This antioxidant function is necessary because, as expected, FLT-3^{mut} cells displayed a large increase in ROS levels following TKI treatment when grown in the absence of glutamine and these changes correlated with a significant reduction in viability in the same conditions. Glutamine dependency of FLT3^{mut} cells upon FLT3 TKI treatment was independently validated via a CRISPR-Cas9 drop-out genome-wide screen as glutaminase (GLS), the first enzyme in glutamine catabolism, and several CAC enzymes were shown to be synthetically lethal with AC220. We went on to show that the combination of AC220 with a specific clinical grade GLS inhibitor (CB-839) or GLS gene silencing resulted in a significant reduction in viability and increase in ROS levels which could be rescued by supplementation of the media with the antioxidant N-acetylcysteine or a cell-permeable form of the CAC intermediate α-ketoglutarate.

Summary/Conclusions: Our data suggest that upon AC220 treatment, glutamine metabolism becomes a critical metabolic dependency in FLT3^{mut} AML. Glutamine metabolism is mostly channelled towards glutathione production, while also supporting the CAC and both these fates contribute to its protective effects following FLT3 TK inhibition by respectively counteracting oxidative damage and sustaining macromolecule biosynthesis and cellular energetics. These data predict that a combined inhibition of glutamine metabolism and FLT3 TK activity may improve the eradication of FLT3^{mut} AML cells.

Hematopoiesis, stem cells and microenvironment

S137

STEP-WISE REPROGRAMMING OF ENDOTHELIAL CELLS INTO IMMUNE-COMPETENT HEMATOPOIETIC STEM CELLS

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Background: The molecular pathways and microenvironmental cues that choreograph the conversion of endothelial cells (ECs) into long-term repopulating hematopoietic stem cells (HSCs) remain poorly defined. This is due to lack of models that recreate the ephemeral transition of an endothelial cell to a hemogenic state to the emergence of HSCs.
Aims: To reprogram adult mouse ECs into long-term repopulating HSCs that give rise to all hematopoietic lineages, including functional T cells *in vivo*.
To provide a platform to deconvolute the process by which endothelial-to-hematopoietic transition is possible.
Methods: Here, we have developed a modular *in vitro* model in which—by precise, conditional expression of transcription factors: *FosB*, *Gfi1*, *Runx1*, and *Spi1* (FGRS), and reintroduction of a proper inductive niche—adult mouse ECs were reprogrammed into HSCs (rEC-HSCs) with multi-lineage engraftment potential (rEC-MPPs). Adult, non-lymphatic ECs isolated from various organs of Runx1-IRES-GFP reporter mice were transduced with *FGRS* and co-cultured in direct contact with vascular niche.
Results: Within 14 days, ECs initiated a hematopoietic program, turning on the endogenous expression of Runx1 and transitioning into hematopoietic cells. Expansion of these cells for another 14 days resulted in generation of rEC-HSCs and rEC-MPPs. Transplantation of rEC-HSCs and rEC-MPPs (CD45.2^{+}) into lethally irradiated mice (CD45.1^{+}) reconstituted both short-term (rEC-MPPs) and long-term hematopoiesis, with secondary engraftment potential (rEC-HSCs). rEC-HSCs gave rise to both functional myeloid and lymphoid cells with full complement of polarized T cell subsets. rEC-HSC-derived T cells undergo T-cell receptor (TCR) rearrangement and restore adaptive immune function in Rag1$^{-/-}$ mice.
Summary/Conclusions: This multi-phasic, step-wise approach provided an interrogable model to decipher pathways involved in EC transition into hematopoietic cells. This will provide cues to devise strategies to convert autologous ECs into large numbers of HSCs for genetic modification and subsequent treatment of both genetic and acquired hematological disorders.

S138

MARROW MESENCHYMAL STEM CELLS RESCUE BONE MARROW ENDOTHELIAL CELLS SUFFERING CHEMOTHERAPY STRESS BY TRANSFERRING MITOCHONDRIA THROUGH NANOTUBES

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Background: The tunneling nanotube (TNT) is a newly discovered,long and thin tubular structure between cells and can facilitate the intercellular exchange of diverse cellular signals and components ranging from electrical signalling to organelles .
Recent reports show that mesenchymal stem cells (MSC) rescue injured target cell and promote target cell recovery from a variety of stress including oxidative tress. ultraviolet radiation, ischemia/reperfusion (SI/R)etal .However, it is still unclear if bone marrow mesenchymal stem cells (BMMSC) can also form TNT to communicate and rescue injured bone marrow -derived endothelial cells (BMdEC)and promote it recovery from chemotherapy stress . In our study, we set out to test the hypothesis that BMMSC can rescure suffering endothelial cells by transferring mitochondria to endothelial cells through nanotubes.
Aims: To investigate the novel intercellular communication TNT between BMMSC and BMdECs or HUVEC, illuminating its constituent and investigating the significance of transport of mitochondrial through TNT between BMMSC and BMdECs or HUVEC suffering from chemotherapy stress of cytosine arabinoside.
Methods: We established two direct co-culture system for human primary bone marrow mesenchymal stem cells (BMMSCs) and bone marrow-derived endothelial cells (BMdECs) or Human umbilical cord vein endothelial cells (HUVECs) respectively.
Results: Firstly, We observed the TNTs formed between BMMSCs and endothelial cells including HUVECs and BMdECs. We identified the TNT structure between BMMSCs and HUVECs or BMdECs are composed with F-actin, microtubule in addition to membrane. Live cell imaging showed the two xenogeneic cells form TNTs by retaining a thin thread of membrane upon dislodge-

ment .Besides,we observed that TNT was more likely to occur between healthy bone marrow mesenchymal stem cell and endothelial cells after cytarabine (Ara-C) treatment. Single-cell analysis showed that stressed endothelial cells and cell lines in the early stages of apoptosis caused by cytarabine (Ara-C) treatment form TNT to interact with untreated BMMSCs and then mesenchymal stem cells transport mitochondria to injured endothelial cell or cell line.Notably,the rescue effect was inhibited when the formation of TNTs were impaired by incubating with an F-actin-depolymerizing drug and tubline -depolymerizing drug, indicated that these TNTs transferring mitochondria have a distinct cytoskeletal composition which composed with F-actin and microtubule. Our results also suggest that the delivery of functional mitochondria from untreated BMMNCs to HUVECs via TNTs can mediate the recovery of injured HUVECs from the apoptosis, contribute to proliferation and remodel the formation of capillary-like structures in Matrigel®-coated plates of HUVECs suffer from chemotherapy stress of Ara-C.

Summary/Conclusions: BMMSCs can transfer mitochondria via TNTs formed between endothelial cells and rescued endothelial cellssuffering chemotherapy stress,which can alleviate apoptosis of stressed endothelial cells, relieve its proliferation inhibition and alter its formation of capillary-like structures.Our study offers the clues to help know about cell-cell communication of niche components in the HSC niche in bone marrow.

S139

SHORT-TERM FEEDING OF A HIGH-FAT DIET DISTURBS LIPID RAFT/TGF-BETA SIGNALING-MEDIATED QUIESCENCE OF HEMATOPOIETIC STEM CELLS IN C57BL/6J MOUSE BONE MARROW

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Background: Some studies show that a high-fat diet (HFD) induces major perturbations in murine hematopoietic stem cells (HSC) and hematopoietic system homeostasis. However, it is currently difficult to say whether these alterations are related to direct effects such as changes in lipid metabolism in HSC or indirect "side effects" on HSC, such as pathophysiology related to obesity or inflammation observed after an extended diet over several months or a diet very rich in fat (>60 kJ% of fat). For example, HFD-induced obesity significantly alters hematopoiesis in bone marrow (BM), with a decreased proliferation of HSC, a general suppression of progenitors, an enhancement of lymphopoiesis, and an activation of myeloid cell production from BM progenitors. Inflammation also affects HSC homeostasis, as Interferon alpha is well-known to activate dormant HSC *in vivo*.

Aims: Our strategy is to characterize the impact of a short-term HFD on HSC and hematopoiesis in non-obese C57BL/6J mice.

Methods: In a prospective study, C57BL/6J mice were fed a control diet (4 kJ% of fat) or HFD (42 kJ% of fat), over a short period of 4 weeks, to investigate the direct-impact of such a diet on hematopoiesis.

Results: While fat intake leaded to an increase in plasma cholesterol levels, mice did not develop obesity, and no inflammatory monocytes and no modulation of pro-/anti-inflammatory cytokine levels were detected in blood and BM, respectively. No significant impact was observed on the total number of cells in blood and BM. However, we noted an increase in the number of progenitors and a loss of more than 50% of the most primitive HSC (SLAM). We validated this loss via transplantation of BM isolated from HFD-fed mice (Ly.1) in competition with control BM (Ly.2), in lethally irradiated recipient mice which only reconstitute 20% of the recipient hematopoiesis from HFD HSC. To further investigate lipid metabolism in HSC, we quantified the major lipid constituents in control and HFD HSC. Among altered lipids, cholesterol was the most affected in HFD HSC. These changes might alter the structure of the HSC plasma membrane such as lipid rafts (LR), which are important for signal transduction in hematopoiesis, driving the retention/dormancy of HSC in BM. To determine if fat intake may affect LR quantity in hematopoietic cell membrane, we stained Lineage-negative (Lin^-) cells with the cholera toxin subunit B (CTB) and analyzed the distribution of different populations of hematopoietic cells, expressing either high (CTB^{hi}) or low (CTB^{lo}) levels of LR. Fat intake disrupts CTB^{hi} cells in the Lin^- $Sca\text{-}1^+$ $c\text{-}Kit^+$(LSK) and LSK-$CD34^-$ compartments, while no variation was detectable among progenitors (Lin^- $c\text{-}Kit^+$and Lin^-). Importantly, we discovered that CTB^{hi} cells were enriched with SLAM HSC (46%, *versus* 2% for the CTB^{lo}). While we found ~50% of CTB^{hi} cells among LSK-$CD34^-$ HSC in control diet-fed mice, a HFD has led to a loss of the CTB^{hi} population. Using the proliferation marker Ki67, we observed a decrease in the proportion of LSK-$CD34^-$ primitive HSC in G0, meaning that HSC quiescence state was affected by a HFD. Transforming growth factor (TGF)-β signaling has long been known to be involved in modulating HSC quiescence, partly by preventing HSC re-entry into the cell cycle. As a HFD induced a loss of LR on HSC, we looked at the localization of TGF-β receptor 1 (TR1) on the LSK-$CD34^-$cell surface. While TR1 strongly colocalized with LR in macrodomains on HSC, we observed that LR were more organized in microdomains and the delocalization of TR1 among LR was furthermore detected when mice were fed a HFD. Moreover, reduced phospho-Smad2/3 indicated lower activation of the TGF-β pathway in HSC purified from HFD-fed mice. Finally, injection of recombinant TGF-β1 led to the rescue of the 4 week HFD-dependent SLAM HSC depletion, which clearly highlights that a HFD affects TGF-β signaling on HSC.

Summary/Conclusions: In conclusion, HFD markedly and rapidly affects primitive hematopoiesis and impairs the maintenance of primitive HSC in non-obese mice. Not only our results uncover the impact of HFD independently of obesity but they also identify the disturbance of LR/TGF-β signaling-mediated quiescence as its main molecular mechanism of action.

S140

A NOVEL MODEL OF HUMAN LYMPHO-MYELOID PROGENITOR HIERARCHY BASED ON SINGLE CELL FUNCTIONAL AND TRANSCRIPTIONAL ANALYSIS

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Background: Human hemopoiesis produces 10 billion new, terminally mature, blood cells daily; a production that is also rapidly responsive to external change. Dysregulation of this complex process can lead to hemopoietic and immune deficiencies and blood cancers. In humans the hemopoietic progenitor hierarchy producing lymphoid and granulocytic-monocytic (myeloid) lineages is unclear. Multiple progenitor populations give rise to lymphoid and myeloid cells but they remain incompletely characterized at the immunophenotypic, transcriptional and functional level.

Aims: Here, we aim to understand the clonal functional output and transcriptional programs of current primary human lympho-myeloid containing progenitor populations - the lymphoid-primed multi-potential progenitor (LMPP)[1], multilymphoid progenitor (MLP)[2] and granulocyte-macrophage progenitor (GMP).

Methods: We devised a FACS-staining and sorting strategy to prospectively purify eight human hematopoietic stem and progenitor cell (HSPC) populations. We compared function of LMPP, MLP and GMP *in vitro* by quantitative CFU assays, single cell liquid cultures or limit dilution analysis and *in vivo* by transplantation into humanized ossciles. We performed population RNA sequencing and single cell RT-PCR analysis to understand the relationship between functional and transcriptional heterogeneity.

Results: Our study comprehensively characterized the LMPP, MLP and GMP lympho-myeloid populations. Both LMPP and MLP are very rare within the mononuclear fraction (1 in 10^4 to 1 in 10^5). We cultured 3806 single LMPP, GMP and MLP cells (isolated from 21 cord blood units and equivalent to ~10^{11} mononuclear cells) under three different culture conditions. We observed marked functional heterogeneity in the three lympho-myeloid progenitor populations. Focusing on the wells that gave single cell initiated cultures the majority of cells from LMPP, MLP and GMP gave unilineage output (50-80%). Bi-lineage output was the next most common output, while multilineage output was rare and only seen from LMPP and GMP cells (9-15%). In vivo transplantation using a novel humanized ossicle assay increased the engraftment of lympho-myeloid progenitors 10-fold compared to previous reports. In vivo, the LMPP and GMP gave robust engraftment but the MLP substantially less engraftment. The LMPP gave rise to both GM and B cell engraftment, the GMP myeloid engraftment and the MLP mainly B cell output. RNA-Seq revealed distinct transcriptional signatures of these populations: MLP signature enriched for lymphoid-affiliated genes and transcription factors (TF), GMP gene signature enriched for myeloid-affiliated genes and TF and LMPP a hybrid lympho-myeloid signature. Analysis of the expression of 72 genes in 919 single LMPP, MLP and GMP shows that the 3 populations form a transcriptional continuum. Moreover, this analysis allowed us to further purify the myeloid potential of GMP population with the use of an alternative sorting strategy.

Summary/Conclusions: These data change our understanding of human hematopoiesis and propose a radically new model of lympho-myeloid progenitor specification. This model has important implications for human immune deficiencies and hemopoietic malignancies.

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DK and BS are equally contributing authors.

Gene therapy, cellular immunotherapy and vaccination 1

S141

WILMS' TUMOR 1 RNA-ELECTROPORATED DENDRITIC CELL VACCINATION AS POST-REMISSION TREATMENT TO PREVENT OR DELAY RELAPSE IN ACUTE MYELOID LEUKEMIA: FINAL RESULTS OF A PHASE II STUDY IN 30 PATIENTS

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Background: Relapse is a major problem in acute myeloid leukemia (AML) and adversely impacts survival.
Aims: The aim of this phase II study was to determine the clinical efficacy of dendritic cell (DC) vaccine therapy in AML, and, more specifically, whether this form of immunotherapy can be applied in the post-remission adjuvant setting to decrease the risk of relapse following chemotherapy and to improve survival.
Methods: We vaccinated 30 AML patients in remission following poly-chemotherapy, but at very high risk of relapse with autologous DCs loaded with the Wilms' tumor 1 (WT1) antigen by means of mRNA electroporation, a technique that allows for human leukocyte antigen haplotype-independent, multi-epitope antigen presentation to T-cells. The vaccines were administered intradermally. *WT1* mRNA levels in blood and marrow were followed as a measure of minimal residual disease. Circulating WT1-specific CD8+T-cells obtained before vaccination and after the 4th dose of DCs were stained with WT1 peptide-HLA-A*0201 tetramers.To assess cell-mediated immunity *in vivo*, delayed type hypersensitivity (DTH) skin testing was performed 2 weeks after the 4th DC vaccination by intradermal injection; DTH-infiltrating lymphocytes collected from skin biopsies were expanded for 2-3 weeks in medium with interleukin-2, harvested and tested for WT1 specificity and reactivity.
Results: There was a demonstrable anti-leukemic response in 13/30 patients (overall response rate 43%). Nine patients achieved molecular remission as demonstrated by normalization of *WT1* transcript levels, 5 of which are sustained after a median follow-up of 109.4 months, including 1 patient who went from partial remission to complete remission by DC vaccination only. In the remaining 4 responding patients, the clinical response was characterized by stable disease as demonstrated by elevated but stable *WT1* transcript levels in blood for 3-12 months and stable blood values without blasts. Five-year overall survival was 40%, as compared to 24.7% in the SEER data of the National Cancer Institute; it was significantly higher in responders than in non-responders (53.8% *vs* 25.0%; *P*=0.01). In patients receiving DCs in first complete remission (CR1), there was a vaccine-induced relapse reduction rate of 25% and the 5-year relapse-free survival was significantly higher in responders than in non-responders (50% *vs* 7.7%; *P*<0.0001). In patients ≤65 and >65 years who received DCs in CR1, 5-year overall survival was 69.2% and 30.8% respectively. Of the 30 patients, 11 are alive in CR, including 5 who relapsed after DC vaccination; 2 proceeded to allogeneic stem cell transplantation, while the 3 other patients were brought back into CR by chemotherapy alone, 2 of them surviving more than 7 and 4 years respectively after reachieving CR. Long-term clinical response was correlated with increased circulating frequencies of poly-epitope WT1-specific tetramer+CD8+T-cells. Long-term overall survival was correlated with interferon-γ+and tumor necrosis factor-α+ WT1-specific responses in DTH-infiltrating CD8+T-lymphocytes.
Summary/Conclusions: Vaccination of AML patients with *WT1* mRNA-electroporated DCs can be an effective and non-toxic strategy to prevent or delay leukemia relapse after standard chemotherapy, translating into improved overall survival rates, which are correlated with the induction of WT1-specific CD8+T-cell responses.

S142

FIRST-IN-HUMAN MULTICENTER STUDY OF BB2121 ANTI-BCMA CAR T CELL THERAPY FOR RELAPSED/REFRACTORY MULTIPLE MYELOMA: UPDATED RESULTS

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Background: To test the safety and efficacy of the CAR T cell modality in relapsed/refractory multiple myeloma (MM), we have designed a second-generation CAR construct targeting B cell maturation antigen (BCMA) to redirect T cells to MM. bb2121 consists of autologous T cells transduced with a lentiviral vector encoding a novel CAR incorporating an anti-BCMA scFv, a 4-1BB costimulatory motif and a CD3-zeta T cell activation domain. We will report updated safety and efficacy results following promising initial results (Berdeja *et al.*, ENA 2016).
Aims: The primary objective is to determine the maximally tolerated dose of bb2121 in subjects with MM whose tumors express BCMA, to determine and test a recommended phase 2 dose for future studies. The secondary objective is to provide preliminary efficacy data on the anti-tumor effects of treatment with bb2121 in subjects with MM whose tumors express BCMA.
Methods: CRB-401 (NCT02658929) is a multi-center phase 1 dose escalation trial of bb2121 in patients with relapsed and/or refractory MM who have received ≥3 prior regimens, including a proteasome inhibitor and an immunomodulatory agent, or are double-refractory, and have ≥50% BCMA expression on plasma cells. Peripheral blood mononuclear cells are collected via leukapheresis. Patients undergo lymphodepletion with Flu (30 mg/m^2) Cy (300 mg/m^2) daily for 3 days then receive 1 infusion of bb2121. The study follows a standard 3+3 design with planned dose levels of 5.0, 15.0, 45.0, 80.0 and 120 x 10^{7}CAR+T cells.
Results: Asof November 18, 2016, 11 patients had been infused with bb2121 in the first 4 dose cohorts, and 9 patients had reached at least 1 month of follow-up. As of data cut-off, no dose limiting toxicities, and no >Grade 2 neurotoxicities or cytokine release syndrome (CRS) had been observed. Grade 1-2 CRS has been reported in 8/11 (73%) treated patients. All patients treated with doses of 15.0 x 10^7 or higher remained on study and the overall response rate (ORR) in the 9 evaluable patients is 100%, including 2 sCRs and 2 MRD-negative responses (sCR and VGPR). CAR+T cell expansion has been demonstrated consistently. An additional 6 months of follow up on reported results and initial data from an additional ~10 patients will be presented.
Summary/Conclusions: bb2121 shows promising efficacy at dose levels above 5.0 x 10^{7}CAR+T cells, including 2 sCRs and ongoing clinical responses at 6 months with mild and manageable CRS to date. These initial data support the potential of CAR T therapy with bb2121 as a new treatment paradigm in MM.

S143

BASELINE AND EARLY POST-TREATMENT CLINICAL AND LABORATORY FACTORS ASSOCIATED WITH SEVERE NEUROTOXICITY FOLLOWING 19-28Z CAR T CELLS IN ADULT PATIENTS WITH RELAPSED B-ALL

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Background: CD19-specific chimeric antigen receptor (CAR) modified T cells produce high anti-tumor activity in relapsed or refractory (R/R) ALL, but can be associated with cytokine release syndrome (CRS) and neurotoxicity (NTX).
Aims: We examined baseline and post-treatment clinical and laboratory parameteres to identify factors associated with severe NTX (≥Grade 3) in our phase I clinical trial of CD19-specific 19-28z CAR T cells for adult patients (pts) with R/R B-ALL (NCT01044069).
Methods: 51 adult pts with R/R B-ALL were treated with 19-28z CAR T cells following conditioning chemotherapy at MSKCC. In order to identify clinical and serum biomarkers associated with severe NTX (sNTX), we examined demographic, treatment, and clinical blood parameters as well as *in vivo* CAR T expansion and serum cytokines, and performed univariate and multivariate analysis.
Results: In this cohort of ALL pts, 20, 8, 2, 18 and 3 pts experienced Gr 0, 1, 2, 3, and 4 NTX, respectively. No pt developed grade 5 NTX and no cerebral edema was seen. Disease burden (≥50% blasts) at the time of T cell infusion (p=0.0045) and post-treatment ≥Gr3 CRS (p=0.0010) were significantly associated with sNTX, but we found no association with age, weight, T cell dose, choice of conditioning chemotherapy (Flu/Cy s. Cy), and prior lines of treatment. Among the clinical and blood parameters, fever, low PLT, high ferritin and MCHC as well as elevated GM-CSF, IFNγ, IL-15, IL-5, IL-10, IL-2 at day 3 of T cell infusion at day 3 of T cell infusion were significantly associated with sNTX (all p<0.01). While some of these cytokines were also elevated in severe CRS cases, IL-5 and IL-2 at day 3 were unique to sNTX. Furthermore, *in vivo* peak CAR T expansion at day 7 (p=0.0001) significantly correlated with sNTX (p<0.01). Lastly, multivariate analysis revealed baseline PLT <60 or MCHC >33.2% and morphologic disease (>5% blasts) has 95% sensitivity and 70% specificity of identifying sNTX pts.
Summary/Conclusions: These data provide a characterization of early clinical and serum biomarkers of sNTX in adult pts receiving 19-28z CAR T cells and should help identify appropriate pts for early intervention strategy to mitigate NTX.

S144

FIRST EVIDENCE DEMONSTRATING ENGRAFTMENT AND REPOPULATION ADVANTAGE OF GENE-CORRECTED HEMATOPOIETIC REPOPULATING CELLS IN NON-CONDITIONED FANCONI ANEMIA PATIENTS

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Background: Fanconi anemia (FA), is a monogenic inherited syndrome associated with bone marrow failure (BMF), that has been considered a candidate disorder for hematopoietic stem cell (HSC) gene therapy. Up to date, three clinical trials have been performed, all of which failed to demonstrate engraftment of corrected HSCs.
Aims: To demonstrate engraftment of gene-corrected HSCs in non-conditioned Fanconi anemia patients.
Methods: To improve previous results, we proposed a new approach based on two clinical trials. First, to increase the HSC collection, we designed a trial employing a plerixafor plus G-CSF mobilization regimen. Second, to improve the quality of corrected HSCs, cells were pre-stimulated for only 8-10 hours and transduced with a new lentiviral vector (PGK-FANCA.Wpre*) for 12-14h, a substantially shorter duration than in previous trials. To avoid chemotherapy-induced damage, a conditioning regimen was not included in the trial, based on the expected proliferative advantage of autologous corrected HSCs.
Results: Eight patients have been included so far in the HSC collection trial. No severe adverse events (SAE) related to the procedure have been reported. The most relevant AE has been the transfusion of packed red blood cells and platelets. Six FA patients aged 3-6 years underwent collections after mobilization of significant numbers of CD34+cells (10 to 70 CD34+cells/μL) to peripheral blood. Two patients (15 and 16 years) failed to mobilize. On average, 5 million CD34+cells/Kg were collected, with 45% recovery after immunoselection. In the first patient included in the gene therapy trial, fresh immunoselected CD34+cells were transduced with the therapeutic vector. Subsequently, two patients, were infused with transduced CD34+cells that remained cryopreserved for almost 2 years. Infused cell products contained 0.5 to 1.4 million CD34+cells/kg, and vector copy numbers per cell (VCN/cell) that ranged between 0.17 to 0.45. To-date, there has been no SAE related to the procedure. Engraftment of gene corrected cells has been observed in the three patients. Notably, increased gene marking levels and significant phenotypic correction in the hematopoietic progenitor cells, deduced from the acquired resistance of the colony forming cells to mitomycin C (15% of BM CFCs survived to 10 nM MMC), have been demonstrated after 9 months of follow up in one of the patients.
Summary/Conclusions: Our preliminary results show that 1) HSCs collection is both safe and efficient in very young FA patients after mobilization with G-CSF and plerixafor, and 2) Engraftment and proliferation advantage of gene-corrected HSPCs has been demonstrated in FA patients even in the absence of conditioning regimens.
The long-term follow up of patients included in these clinical trials will demonstrate the feasibility of restoring the hematopoietic function of FA patients by means of a gene therapy approach in the absence of conditioning.

S145

TARGETING FLT3 WITH CHIMERIC ANTIGEN RECEPTOR T CELLS CONFERS POTENT REACTIVITY AGAINST ACUTE MYELOID LEUKEMIA

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Background: Adoptive immunotherapy with chimeric antigen receptor (CAR)-modified T cells has therapeutic potential in hematologic malignancies. We are pursuing FMS like tyrosine kinase 3 (FLT3) as a novel CAR target in acute myeloid leukemia (AML). FLT3 is a homodimeric transmembrane protein with uniform expression on AML, irrespective of cytogenetic and histomorphologic subtype. FLT3 provides survival signals to AML blasts and is a key driver of leukemia-genesis in AML cases with internal tandem duplication (FLT3-ITD). These attributes suggest FLT3 may be an 'Achilles heel', making AML blasts susceptible to CAR T-cell mediated recognition and elimination.
Aims: We therefore explored the anti-leukemia efficacy of FLT3-CAR modified T cells against FLT3-ITD+and FLT3 wild type AML in pre-clinical models *in vitro* and *in vivo*.
Methods: A FLT3-CAR comprising a single-chain variable fragment (4G8), fused to an IgG-Fc spacer, and signaling module with CD3 zeta and CD28 was encoded in a lentiviral vector (epHIV7) for gene-transfer into CD8+and CD4+T cells of healthy donors (n>4) and AML patients. CAR T-cell mediated cytolytic activity was evaluated in FACS-/luminescence-based assays, cytokine production analyzed by ELISA and proliferation assessed by CFSE dye dilution. Immunodeficient NSG mice were engrafted with AML cell line (Molm-13) or primary AML blasts and treated with $5x10^6$CAR-modified or control T cells (CD8:CD4 ratio=1:1).
Results: We confirmed specific recognition and high-level cytolytic activity of CD8+FLT3-CAR T cells against a panel of AML cell lines including THP-1 (FLT3 wild type), and Molm-13 (FLT3-ITD heterozygous). Both CD8+and CD4+FLT3-CAR T cells produced IFN-γ and IL-2, and underwent proliferation after antigen stimulation. FLT3-CAR T cells that we prepared from AML patients exerted specific anti-leukemia reactivity against autologous primary AML blasts, with near-complete cytolysis within 24 hours of co-culture. Further, FLT3-CAR T cells conferred a potent anti-leukemia effect in *in vivo* models of systemic leukemia, both with AML cell lines (Molm-13) and primary AML blasts. A single dose of FLT3-CAR T cells conferred complete eradication of leukemia from peripheral blood, bone marrow and spleen, as confirmed by bioluminescence imaging and flow cytometry. FLT3 is not expressed in any normal solid tissues and mature hematopoietic cells, but shows limited expression in hematopoietic progenitors and hematopoietic stem cells (HSCs). Preliminary data show that FLT3-CAR T cells recognize FLT3+/high normal HSCs and interfere with normal hematopoiesis, but preserve a proportion of HSCs capable of reconstituting hematopoietic lineages. Studies to assess recognition of normal HSCs *in vivo* are ongoing.
Summary/Conclusions: Collectively, our data demonstrate that T cells expressing a FLT3-specific CAR mediate potent reactivity against FLT3 wild type and FLT3-ITD+AML *in vitro* and *in vivo*, and establish FLT3 as a novel CAR target in AML. FLT3-ITD positivity identifies a high-risk AML subgroup that may particularly benefit from adoptive therapy with FLT3-CAR T cells, *e.g.* in order to achieve 'minimal residual disease' (MRD) negativity prior to allogeneic HSC transplantation. Our data further suggest that in contrast to CD33 and CD123, which are pursued as alternative CAR targets in AML, targeting of FLT3 may preserve a fraction of normal HSC and enable the implementation of CAR therapy outside the transplant setting.

Best abstracts

S146

BPX-501 DONOR T CELL INFUSION (WITH INDUCIBLE CASPASE 9 SUICIDE GENE) FACILITATES HLA-HAPLOIDENTICAL STEM CELL TRANSPLANT IN CHILDREN WITH BOTH HEMATOLOGICAL MALIGNANCIES AND NON-MALIGNANT CONDITIONS

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Background: Allogeneic haploidentical hematopoietic stem cell transplant (HSCT) offers curative therapy for children who lack an available HLA-identical donor with hematopoietic disorders such as Primary Immune Disorders (PIDs), hemoglobinopathies, erythroid disorders and acute leukemias. αβ T-cell depletion mitigates the risk of GVHD after haplo-HSCT, but is associated with extended immunodeficiency, leading to complications due to infections . We have performed αβ TCR-depleted haplo-HSCT with post-transplant infusion of BPX-501 gene modified T cells to allow for more rapid immune reconstitution. Upon occurrence of GVHD, administration of rimiducid (AP1903) dimerizes the Caspase 9 suicide switch and rapidly induces apoptosis of the transduced BPX-501 cells and mitigates the GVHD.
Aims: This study was performed to determine the impact of BPX-501 T cell infusion on outcomes (treatment related mortality (TRM), disease recurrence, GvHD incidence and immune reconstitution) after HSCT.
Methods: We report on a large multicenter, prospective Phase I-II study enrolling children receiving αβ T-cell depleted Haplo-HSCT. Patients were infused with BPX-501 T cells 2 weeks post-transplant. 104 patients have >100 day follow-up, 81 patients have follow up ≥180 days and 51 with >1 year follow-up. All patients received myeloablative therapy and low dose ATG prior to transplant. No pharmacologic GvHD prophylaxis was given (Table 1).

Table 1. Diagnoses of Patients with >100 day follow-up.

Non-Malignant	N=66	Malignant	N=38
SCID	11	ALL(3 CR1, 21 CR2)	24
WAS	6	AML	14
CGD	4		
Thalassemia Major	8		
Sickle Cell Disease	4		
Fanconi Anemia	8		
HLH	5		
Others	20		

Results: Cumulative incidence of TRM remains very low at 100 days (0%), 180 days (1.6%) and 1 year (2.8%). Of the 81 patients with >180 day follow-up, 20 patients had acute GvHD 1-3 (24.7%) (Figure 1A); 10 with Grade 1, 8 with Grade 2, 2 with Grade 3 and one Grade 4 skin. Mild cGvHD was seen in 2 patients, moderate cGvHD in 2 patients and one case of severe cGvHD in a malignant patient, attributed to the allograft, not BPX-501. Rimiducid was used in 4 patients with Grade 2 GvHD with rapid resolution of symptoms, as it did in the severe cGvHD patient. In both malignant and non-malignant patients. CD3, CD4, CD8 (Figure 2B) and B cells (Figure 3C) immune reconstitution was brisk. CD3+/CD19+ T-cells were detectable at one year via flow cytometry analysis of peripheral blood. In Wiskott-Aldrich patients, platelet recovery remains in the normal range at 180 days with mean platelet counts of $246.3x10^3$/uL. At 180 days and 1 year, the patients with hemoglobinopathies remain transfusion-free with a normal mean HgB value of 11.4 g/dL.
Summary/Conclusions: These data suggest that infusion of BPX-501 modified T cells may facilitate T cell depleted Haplo-HSCT in children who would benefit from HSCT for either malignant or non-malignant conditions. The availability of a suicide gene mechanism in donor T cells infused after T depleted Haplo-HSCT, results in low rates of infection and rapidly reversible GvHD when the dimerizer is infused to activate the suicide switch. Rapid cellular and humoral immune reconstitution makes BPX-501 after T depletion a safe and viable option for children who do not have a matched donor transplant and in whom transplantation has been deemed curative.

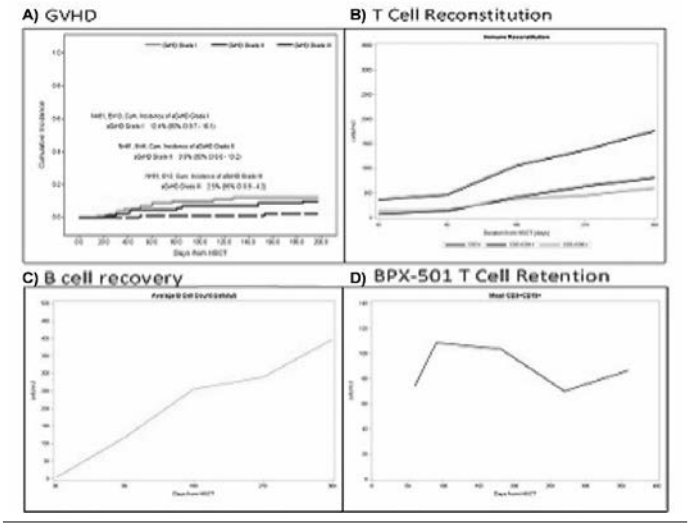

Figure 1.

S147

RE-CREATING HEREDITARY PERSISTENCE OF FETAL HEMOGLOBIN WITH CRISPR/CAS9 TO TREAT SICKLE CELL DISEASE AND BETA-THALASSEMIA

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Background: Extensive human genetic and epidemiological data demonstrate that the genetic condition Hereditary Persistence of Fetal Hemoglobin (HPFH) substantially ameliorates the pathology of Sickle Cell Disease (SCD) and β-thalassemia (β-Thal). This condition is associated with several genetic variants at the β-globin locus that lead to transcriptional reactivation of γ-globin genes, resulting in upregulation of fetal hemoglobin (HbF).
Aims: CRISPR/Cas9 is a revolutionary technology that allows for precise, directed changes to genomic DNA. Our strategy is to use CRISPR/Cas9 in human primary $CD34^+$ hematopoietic stem and progenitor cells (HSPCs) to re-create specific HPFH genetic variants as well as other variants associated with elevated HbF and demonstrate their causal relationship to elevated HbF as a potential therapeutic strategy to treat SCD and β-Thal.
Methods: Using CRISPR/Cas9 gene editing, we have successfully re-created genetic variants linked to high HbF levels in HSPCs from healthy donors and SCD and β-Thal patient samples, and determined the relationship of different genetic variants to upregulation of γ-globin in bulk and clonal populations of differentiated erythrocytes. Off-target editing was assessed, and on-target editing in long-term repopulating subsets of HSPCs was measured *in vitro* and by engraftment in immunocompromised mice. Finally, editing rates at clinical scale in a GMP-capable manufacturing facility were demonstrated.
Results: We first optimized cell culture and electroporation conditions that led to high rates of genomic editing across multiple loci, achieving 84.9±6.2% (Mean±SD) editing efficiency at key regions of interest in $CD34^+$ HSPCs from mobilized peripheral blood of healthy donors (n=16). Similar rates of editing were attained using $CD34^+$ HSPCs derived from healthy-donor bone marrow (n=6). Cas9 delivery as recombinant protein improved cell viability when compared to mRNA-based delivery (88.8±3.7% compared to 75.5±9.3%, Mean±SD, n=56 for each) with no observed reduction in editing efficiencies. To investigate gene editing impact on HbF, edited cells were erythroid differentiated from healthy donors as well as from SCD and β-Thal patients. Specific gene edits significantly increased γ-globin mRNA expression to therapeutically-relevant levels (increased expression to 29-37% as a ratio of γ/α in one β-Thal patient sample and to 25-45% as a ratio of γ/(γ+β) in six SCD patient samples). We demonstrated similarly high rates of editing in the $CD34^+CD38^-CD90^+CD45RA^-$ long-term repopulating HSPCs and bulk $CD34^+$ HSPCs (87.9±6.4% compared to 89.7±3.6%, Mean±SD, n=4). We confirmed that engraftment levels of edited cells in immunocompromised mice were similar to control cells (% human CD45 in peripheral blood = 28.6±6.9% in controls *versus* 27.1±6.6% and 26.3±7.9% for two guide targets, Mean±SD, n=48 for each). In-depth off-target analysis

for a selected guide RNA confirmed no detectable genomic cleavage at over 5000 predicted off-target sites with a detection sensitivity of 0.2%, supporting its safety for clinical use. Finally, we have demonstrated editing rates of >85% at clinical scale in a GMP-capable manufacturing facility to enable clinical development for SCD and β-Thal. Required safety toxicology studies are ongoing.
Summary/Conclusions: Using CRISPR/Cas9 we successfully created gene edits that upregulate HbF in both healthy donor and patient samples. We have also dissected the genotype-phenotype relationship for specific genetic modifications, identifying the editing strategies which are most promising for re-expressing HbF. We have optimized the conditions for modifying HSPCs, including at clinical scale in a GMP-compliant setting, and demonstrated potential safety with no detectable off-target editing. These experiments support the further development of specific CRISPR/Cas9 editing strategies of HSPCs to treat SCD and β-Thal patients.

S148

EXPOSURE TO INFECTION TRIGGERS PAX5 AND ETV6-RUNX1 CHILDHOOD BCP-ALL

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Background: B-cell precursor acute lymphoblastic leukemia (BCP-ALL) of childhood remains a major cause of death in high-income countries. It has a yet unexplained peak incidence between 2-6 years of age and a potential trigger was theorized a century ago with several possibilities of exposure to infection in infancy. Recently *in vitro* and *in vivo* evidence strengthened the causal role of exposure to infection in BCP-ALL (1, 2). However, it remains unknown which molecular BCP-ALL subtype can be triggered by exposure to infection and how the pre-leukemic clone evolves to BCP-ALL.
Aims: Aiming to understand the role of infection exposure in the etiology of childhood BCP-ALL.
Methods: We have developed and characterized two independent GEMMs, in addition to the *Pax5*+/- infection model (1), which were exposed to a common infection environment. These represent childhood *BCR-ABL1^{p190}* BCP-ALL and the most common subtype *ETV6-RUNX1* BCP-ALL. Both model systems ensure Sca1-directed expression of *BCR-ABL1^{p190}* or *ETV6-RUNX1* in HSC/PC in C57BL/6 x CBA mice and were characterized extensively with respect to clinical, immunephenotypic and molecular genetic characteristics. Whole exome (WES) and whole genome sequencing (WGS) was performed of murine BCP-ALL on a HiSeq 2500 (Illumina) platform.
Results: *Pax5*+/- and *Sca1-ETV6-RUNX1* mice develop BCP-ALL only after exposure to common pathogens whereas *BCR-ABL1^{p190}*micedevelop BCP-ALL independent of exposure to common infection. The molecular mechanism leading to BCP-ALL identified in the infection dependent GEMMs is determined by the genetic predisposition (*Pax5*+/- or *ETV6-RUNX1*). Pax5+/- mice acquire constitutive activating *Jak3* mutations (6/9) in a susceptible B-cell precursor population ((pro+pre)-B and immature B cells) (1). On the other hand *Sca1-ETV6-RUNX1* mice develop BCP-ALL at a low penetrance (10.75%; 10 out of 93) with a CD19^{+}B220^{+}IgM^{-} cell surface phenotype and manifested with blast cells in the peripheral blood (PB) and clonal immature BCR rearrangement. High expression of Recombination Activating Gene 1 (*Rag1*) and loss of function mutations in *Ebf1* were identified in murine BCP-ALL and are well known in the context of human *ETV6-RUNX1* leukemia. Additionally we identified a high proportion of mutations in genes implicated in histone modification, *i.e. Kdm5c* (no. J408) causing a premature stop. CRISPR-Cas9 knock down studies of KDM5C in a precursor B-cell line revealed facilitated Rag1/2 binding to the histone complex (H3K4me3) as a potential molecular mechanism inducing Rag1 off target activity in pre-leukemic *ETV6-RUNX1* HSC/PC after exposure to infection. In contrast to *Pax5*+/- and *Sca1-ETV6-RUNX1* mice, *Sca1-BCR-ABL1^{p190}* mice develop BCP-ALL independent of exposure to common pathogens by reprogramming of a HSC/PC and subsequent loss of *Pax5*.
Summary/Conclusions: In summary, exposure to common pathogens can trigger childhood BCP-ALL based on Pax5 loss of function or the common *ETV6-RUNX1* rearrangement. However the underlying molecular mechanism (Jak-Stat signaling in *Pax5*+/- mice and histone modification in *ETV6-RUNX1* mice) triggered by exposure to common infection is determined by the genetic predisposition. On the other hand BCP-ALL, which emerge on the basis of a potent oncogene (such as *BCR-ABL1^{p190}*) can develop independent of exposure to infection. These findings are important for encouraging the prospect of novel interventions that might help to prevent or treat a significant proportion of childhood BCP-ALLs.

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S149

REVERSIBLE PHARMACOLOGICAL TARGETING OF RHOA ALLOWS IMPROVED STORAGE, SURVIVAL AND HEMOSTATIC ACTIVITY OF PLATELETS *IN VITRO* AND *IN VIVO*, IN MICE AND IN PRIMATES

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Background: The use of platelets in transfusion has increased dramatically in the last three decades. Cold temperature induces changes in glycosylation and clustering of platelet glycoprotein (GP) Ib and cytoskeletal rearrangements, which are recognized by host receptors resulting in lectin-mediated platelet phagocytosis and clearance from circulation. As a result, current practice of platelet storage for transfusion uses room temperature and associates with a relatively high risk of bacterial growth and infection in susceptible patients.
Aims: Due to the cytoskeletal nature of the platelet changes upon refrigeration, we hypothesized that the RHO family GTPase activity is pivotal in the cold platelet lesion. Targeted intervention may benefit refrigerated storage.
Methods: Analysis of RhoA, Rac1 and Cdc42 activity was performed using GST-Rhotekin and GST-PAK effector domain pulldown assays. Platelets were obtained from anticoagulated (CPD or EDTA) human, Rhesus monkey and murine whole blood. G04, NSC23766 and Casin, specific inhibitors for RhoA, Rac and Cdc42, respectively, were titered and used at concentrations of 75 mcM, 50 mcM and 10 mcM, respectively. RhoA deficient murine platelets were obtained from polyI:C-treated *Mx1-Cre;RhoA $^{f/f}$* mice. Aspirin was administered at a dose of 5 mg/Kg b.w. to mice and monkeys. Bleeding time was performed using standard animal protocols. Transfused human/monkey platelets were stored in plasma or PAS-III (67%)/plasma(33%) at RT or 1-9C for 7 days or 1-4 hours for murine platelets.
Results: We found that either short- or long-term refrigeration activates RHOA and RAC1, but not CDC42. Genetic deletion of RhoA or RHOA inhibition with the small molecule inhibitor G04 suffices to completely prevent cold-induced platelet clearance after long-term cold storage of murine or human platelets. The effect of G04 is on-target since it mimics but does not modify the response of RhoA-deficient platelets. The effect of G04 is reversible since removal of G04 after 7-day storage restores RHOA activity to normal levels and allows normal extent of shape change and spreading on fibrinogen. To analyze the kinetics and hemostatic activity of cold stored inhibitor treated human platelets after xenotransfusion, we analyzed the survival of xenotransfused human platelets after long term (7-day) refrigeration in the presence and absence of inhibitors cocktail or individual inhibitors in macrophage depleted, sub-lethally irradiated NSG mice (N=20/group) as well as autologously transfused platelets in a crossover trial in Rhesus monkeys (n=5). Our results show that reversible inhibition of RHOA in refrigerated platelets suffices to survival levels similar to the unrefrigerated control in 100% of mice and 80% of monkeys ($p<0.001$). Our data further show that washing of platelets stored for 7 days in G04/plasma maintains collagen–induced shape change as well as normal aggregation of human platelets and restores bleeding time correction after congenic or autologous transfusion in all aspirinated mice and 80% of aspirinated Rhesus monkeys, respectively. RHOA inhibition blocks the process of intracellular traffic of GP through lipid rafts and endocytotic intermediates as assessed by confocal microscopy of GpIb and the vacuolar sorting protein VPS33b, as well as biochemical fractionation of detergent-insoluble membrane lipid rafts, resulting in reduced blebbing and formation of microparticles upon storage in G04/plasma.
Summary/Conclusions: We demonstrate that activation of RHOA is a pivotal mechanism of refrigerated platelet storage lesion and phagocytosis. Reversible inhibition of RHOA allows the extended cold storage of platelets which are effective *in vitro* and *in vivo*, suitable for use in clinical safety and efficacy trials. Our study also provides the mechanism and a stringent proof-of-principle for the translational application of a novel approach to refrigerated platelet storage.

S150

TREATMENT REDUCTION IN PATIENTS WITH ADVANCED-STAGE HODGKIN LYMPHOMA AND NEGATIVE INTERIM PET: FINAL RESULTS OF THE INTERNATIONAL, RANDOMIZED PHASE 3 TRIAL HD18 BY THE GERMAN HODGKIN STUDY GROUP

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Background: The German Hodgkin Study Group (GHSG) applies the intensive eBEACOPP regimen (dose-escalated bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone) to all newly diagnosed advanced-stage HL patients regardless of their individual risk-profile. However, some patients might not be in need of such an intensive treatment to achieve cure. Unfortunately, baseline risk factors as defined in the international prognostic score cannot identify these patients reliably. Recent clinical research suggests that early metabolic response assessment after 2 cycles of therapy using FDG-PET (PET-2) can better predict the individual outcome. In particular, a rapid response as determined by PET-2 negativity might allow reducing the overall treatment intensity.

Aims: To assess the feasibility of decreasing the number of eBEACOPP cycles in patients with negative PET-2 without loss of efficacy as determined by progression-free survival (PFS).

Methods: Between 05/2008 and 07/2014, we recruited patients with newly diagnosed, advanced-stage HL aged 18–60 years. All patients gave written consent before study entry. PET-2 was centrally assessed with FDG uptake not higher than the mediastinal blood pool defined as negative. Patients with negative PET-2 were randomly assigned to receive 6 or 2 additional cycles (*i.e.* 8 or 4 cycles of eBEACOPP in total, respectively). PET-positive residues after chemotherapy were irradiated. Based on the results of our previous HD15 trial, the protocol was amended in June 2011 and the standard therapy was reduced from 8 to 6 cycles of eBEACOPP in total. The trial was designed to exclude inferiority of 6% or more of the experimental treatment (4 cycles of eBEACOPP) compared with the pooled standard treatment (8 or 6x cycles of eBEACOPP) at 5 years.

Results: We enrolled 2,101 patients. 1,005 patients with negative PET-2 were randomly assigned to either 8/6 cycles of eBEACOPP (n=504) or 4 cycles of eBEACOPP (n=501). With a median follow-up of 55 months, estimated 5-year PFS in the per-protocol set was 90.8% (87.9–93.7) with 8/6 cycles of eBEACOPP and 92.2% (89.4–95.0) with 4 cycles eBEACOPP (difference +1.4%, 95% CI -2.7–5.4, excluding the non-inferiority margin of -6%). In the standard arm, 95% of patients had at least one acute hematological toxicity of CTCAE grade 3-4 compared with 90% in the experimental arm, including severe infections in 75 (15%) and 40 (8%) patients, respectively. Acute severe organ toxicities were documented for 91 (18%) and 38 (8%), respectively. 25 patients (5%) in the standard group (8/6 cycles of eBEACOPP and 9 (2%) in the experimental group (4 cycles of eBEACOPP) died; most frequent cause of death was second malignancy (11 and 1 patient, respectively). No patient in the experimental group died from treatment-related toxicities. Estimated 5-year overall survival (OS) in the per-protocol set was 95.4% (93.4–97.4) with standard eBEACOPP, and 97.7% (96.2–99.3) with 4 cycles of eBEACOPP (log-rank p=0.004).

Summary/Conclusions: Metabolic response assessment using FDG-PET after 2 cycles of eBEACOPP allows the reduction from therapy with 8/6 to only 4 cycles without loss of efficacy as determined by PFS in advanced-stage HL patients. Furthermore, the abbreviated treatment with 4 cycles of eBEACOPP is associated with improved tolerability and consequently leads to a significant OS benefit over standard therapy. PET-guided reduced therapy with eBEACOPP combines outstanding efficacy with high safety. We therefore recommend this treatment strategy for advanced-stage HL patients.

POSTER SESSIONS I

Acute lymphoblastic leukemia - Biology 1

P151

TARGETED SINGLE CELL SEQUENCING TO IDENTIFY MUTATIONAL HIERARCHY IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Acute lymphoblastic leukemia (ALL) is a common childhood malignancy caused by clonal proliferation of immature B or T lymphoid cells. ALL patients are primarily young children who respond well to chemotherapy, with survival rates above 85%. However, if relapse develops, survival rates drop to 15-50%. Recent studies have shown that at diagnosis, different ALL subclones are present that are likely the result of clonal branched evolution. Understanding this clonal evolution and the order at which mutations are acquired can provide improved insights into the origins of leukemia relapse.
Aims: To use single-cell sequencing to investigate (i) the heterogeneity of leukemic T-ALL cells present at diagnosis and (ii) unravel the order in which mutations were acquired during leukemia evolution.
Methods: Bone marrow samples taken at diagnosis and remission from 4 T-ALL patients underwent whole genome and RNA sequencing. Somatic mutations, indels and chromosomal translocations were confirmed using Sanger sequencing. Primers were designed to specifically target these genetic alterations, and included 40 primers against heterozygous SNPs for quality control assessment. A total of 1517 single cells (average of 379 cells per patient), were sorted using flow cytometry or a microfluidic device and analyzed with targeted sequencing. Cells were discarded from further analysis if locus and allelic drop-out exceeded 33,3%. Jaccard hierarchical clustering was applied to identify subclones and a new graph-based algorithm was developed to determine the order of mutation acquisition. Single $CD34^+CD38^-$ hematopoietic stem/progenitor cells (HSPCs) from the same samples were also isolated to test for the presence of mutations in early progenitors.
Results: We detected between 2 and 4 separate clones in each T-ALL patient sample. Every patient harboured one dominant clone comprising 46 to 98% of all single cells that was highly mutated, accompanied by a number of smaller subclones carrying fewer mutations. No mutually exclusive mutations, fusion genes or deletions were observed between the clones arguing against independent leukemic clonal initiation events. Instead, a more stepwise clonal hierarchy became likely, with each clone harbouring more mutations than the last. Using our newly developed graph-based algorithm, we found that early mutations mostly occurred in genes of unknown significance and may represent a pre-leukemic state. Three out of four patients also had an early mutation event in a known oncogene (MED12, STAT5B or NOTCH1). Intermediate events included loss of 9p and appearance of fusion genes, while alterations in NOTCH1 were usually late events. Analysis of 185 single $CD34^+CD38^-$ HSPCs from the diagnostic and remission samples found that most early mutations were detected in HSPCs at diagnosis and remained detectable at remission, further confirming the pre-leukemic nature of these variants. Interestingly, 2 of 4 T-ALL cases had HSPCs at diagnosis in which all mutations could be detected, indicating that the accumulation of mutations can occur in progenitor cells or in committed T-cells. During remission, we could no longer detect HSPCs with all leukemic mutations.
Summary/Conclusions: We demonstrate that T-ALL patients have limited heterogeneity at diagnosis and that targeted single cell sequencing can be used to determine the cell of origin and the order of mutation acquisition. These data also illustrate that HSPCs at remission carry a few early, pre-leukemic events, while highly mutated HSPCs are eradicated during treatment, which is in line with long term remission in T-ALL.

P152

BCL-2 INHIBITION AS NEW THERAPEUTIC OPPORTUNITY FOR RPL10 R98S MUTANT PEDIATRIC T-ALL

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Background: The ribosomal protein L10 (RPL10) R98S mutation occurs in 8% of pediatric T-cell acute lymphoblastic leukemia (T-ALL) cases. RPL10 R98S leads to a proliferation defect in lymphoid cells but its oncogenic contribution in pediatric T-ALL remains unclear. Treatment intensification and risk stratification has reduced the relapse rate of T-ALL to ~15% but further improvements will require strategies that focus on specific subtypes as RPL10 R98S; if the long-term sequalae of toxic therapy are to be avoided.
Aims: 1) Explore the oncogenic contribution of the RPL10 R98S mutation in pediatric T-ALL. 2) Define new therapeutic opportunities for RPL10 R98S defective T-ALL. 3) Identify a biomarker indicative of the RPL10 R98S mutation in T-ALL.
Methods: Quantitative label-free proteomics was used to screen for protein differences between RPL10 WT and R98S expressing Ba/F3 cells. Hits were confirmed by western blot in lineage negative (lin-) bone marrow (BM) cells extracted from RPL10 WT and R98S knock-in mice and in RPL10 WT and R98S pediatric T-ALL samples. Serial re-plating was established by plating 2000 cells/ml in Methocult. Oxidative stress and mitochondrial activity was determined by Dihydroethidium and mitotracker. Viable cell counts were determined by Annexin V exclusion. Chromatin immunoprecipitation was performed using the Imprint Ch-IP kit followed by qRT-PCR. Human pediatric T-ALL samples were transplanted into NOD-SCID/$IL2\gamma^{-/-}$(NSG) mice for *in vitro* and *in vivo* inhibitor studies.
Results: The RPL10 R98S mutation provided a cell survival advantage in Ba/F3 cells and in serial re-plating assays of lin- BM cells derived from RPL10 R98S knock-in mice. Proteomic profiling revealed metabolic reprogramming in RPL10 R98S cells through enhanced expression of peroxisomal enzymes Acox1, Acox3 and Paox. This expression facilitated peroxisomal β-oxidation of long chain fatty acids which are substrates for PPARγ and which were consequently upregulated together with CPT1A. Peroxisomal hyperactivation causes high intracellular H_2O_2 levels, explaining the observed elevated levels of reactive oxygen species (ROS) in RPL10 R98S cells that could not be scavenged by the increased catalase expression. High ROS levels and enhanced PPARγ binding drives the constitutive overexpression of anti-apoptotic protein B-cell lymphoma 2 (Bcl-2), responsible for the leukemia cell survival benefit of RPL10 R98S cells. Bcl-2 targeted therapy using venetoclax (ABT-199) reduced the expansion of RPL10 R98S knock-in BM cells by 50%, while RPL10 WT BM cells were not inhibited by ABT-199. *In vivo,* DMSO or ABT-199 50mg/kg therapy was started after the engraftment of >2% human cells in the blood of mice xenografted with T-ALL samples and was maintained 1/wk till disease end stage. RPL10 R98S xenografted mice that received ABT-199 therapy presented a complete inhibition of human CD45+ leukemia progression in the blood, which was characterized by a 70-85% reduction in spleen weights, and 20-50% reduction of bone marrow engraftment. Spleen weights of ABT-199 treated RPL10 R98S xenografted mice were only slightly increased as compared to spleen weights of healthy NSG mice. In contrast, mice xenografted with RPL10 WT T-ALL samples showed poor *in vivo* responses to ABT-199 treatment and all animals showed progressive disease. Bcl-2 overexpression induced by peroxisomal hyperactivation was defined as new target in RPL10 R98S defective T-ALL. Additionally, due to peroxisomal hyperactivation, a peroxisomal oxidase involved in purine degradation may have contributed to the observation that the waste product of purine degradation, uric acid, was elevated above reference levels in the blood of RPL10 R98S mutant pediatric T-ALL patients at diagnosis (Figure 1).

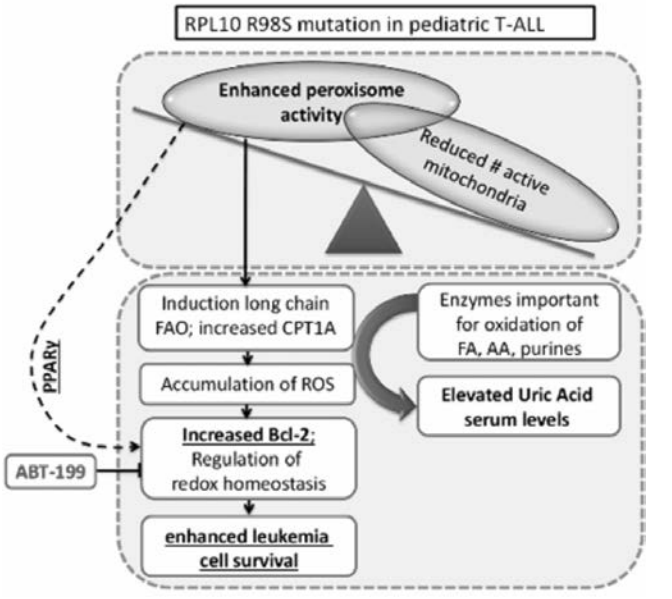

Figure 1.

Summary/Conclusions: Uric acid provides an indicative biomarker of RPL10 R98S mutations in pediatric T-ALL patients, which may be used for screening, providing early diagnosis and appropriate selection of patients in whom a Bcl-2 targeted therapeutic approach could be considered.

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TRANSLATOME ANALYSIS OF THE T-ALL ASSOCIATED RIBOSOMAL PROTEIN L10 R98S MUTATION REVEALS ALTERED SERINE METABOLISM

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Background: We previously described a recurrent arginine-to-serine mutation on residue 98 (R98S) in ribosomal protein L10 (RPL10), with a frequency of 8.6% in pediatric T-ALL cases. The R98 mutated residue contacts the catalytic core (peptidyltransferase center, PTC) of the ribosome and causes ribosome biogenesis and translational fidelity defects in yeast and lymphoid cells. These observations suggest that the RPL10-R98S mutation may contribute to T-ALL pathogenesis by inducing translational changes.
Aims: The spectrum of translated proteins (translatome) of RPL10 R98S mutants was investigated in order to identify translational changes caused by the mutation and potentially driving oncogenesis.
Methods: We performed ribosome footprinting (RNA sequencing of ribosome bound RNA), polysomal RNA sequencing, total RNA sequencing and mass spectrometry based quantitative proteomics on engineered RPL10-R98S or RPL10-WT mouse lymphoid Ba/F3 cells.
Results: RPL10 R98S cells showed significant upregulation for 3% (n=178) of the measured proteins and a downregulation of 1% (n=68). Moreover, polysomal RNA sequencing and ribosome footprinting showed respectively 57 and 22 genes with significantly higher translational efficiency in RPL10 R98S, and 22 and 29 genes, with reduced translational efficiency. Among them, we also found genes involved in T cell differentiation and proliferation. In particular, Mapk6 presented reduced translational efficiency in the ribosome footprinting, potentially due to differences in ribosome occupancy of an upstream ORF, whereas the transcription factor Ikzf2, a master regulator of the upregulated transcripts, was overexpressed at the transcriptional and protein level. Interestingly, the results from the mass spectrometry and the polysomal RNA sequencing datasets showed a significant enrichment and upregulation of members of the JAK-STAT signaling pathway with Csf2rb/2, Jak1 and several Stats being 1-3-fold elevated at the protein level and higher translation efficiency for Lif, Socs1, Pim1, Osm, Il10ra, Cish and Il21r. Another interesting candidate showing 5-fold upregulated protein levels was phosphoserine phosphatase (Psph), a key enzyme in serine biosynthesis. Ribosome footprinting revealed that this upregulation originates from a combination of higher transcription and translational efficiency of the encoding gene. Elevated Psph protein levels were confirmed by immunoblots in the RPL10 R98S Ba/F3 cells and in hematopoietic cell cultures derived from Rpl10 R98S knock-in mice. Interestingly, exhausted medium from RPL10 R98S Ba/F3 cells contained higher residual serine levels as compared to RPL10 wild type expressing cells and this medium could support the survival of wild type Ba/F3 cells. Our data suggest that RPL10 R98S expressing cells enhance their endogenous serine production, leaving more serine that can support survival of neighboring cells.
Summary/Conclusions: Analysis of the translational changes associated with the RPL10 R98S mutation reveals alterations for genes involved T cell differentiation and proliferation: the atypical MAP kinase Mapk6, whose reduced translational efficiency still needs to be validated at the protein level, and the transcription factor Ikzf2. Alterations were also found in the JAK-STAT signaling, an established oncogenic cascade in T-ALL. Moreover, this is the first description of a mutation in T-ALL that is linked to alterations in cellular serine biosynthesis.

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REPOSITIONING EXISTING DRUGS AS NOVEL THERAPEUTICS: OXIDATIVE STRESS AS A TARGET FOR HIGH-RISK LEUKAEMIA IN CHILDREN

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Background: Remarkable improvements made in the treatment of childhood acute lymphoblastic leukaemia (ALL) in past decades have resulted in 5-year survival rates approaching 90%. However, prognosis remains dismal for certain subgroups of high-risk patients, including poor responders to induction therapy, infants with ALL that harbor rearrangement of the *Mixed Lineage Leukaemia* (*MLL/KMT2A*) gene, and children with Philadelphia chromosome positive ALL. In particular, infant ALL patients with *MLL* disease have survival rates below 50% despite the use of intensified treatments, necessitating the development of more effective, less toxic therapeutics for them.
Aims: The aim of this study is to identify candidates that target *MLL*-rearranged leukaemia cells using drug-repurposing, whereby an approved drug is applied to treat a disease other than the one for which it was originally intended. This drug discovery strategy is gaining popularity as it potentially avoids the lengthy process of drug development and FDA approval.
Methods: 3707 approved drugs and pharmacologically active compounds were initially screened against an infant ALL cell line with *MLL*-rearrangement, PER-485 and a paediatric leukaemia cell line wild-type for *MLL*, CEM, using a resazurin-based cell viability assay. Hit compounds were further tested in a panel of 16 leukaemia cell lines, seven solid tumour lines and two normal cell lines. Compounds were subsequently evaluated *in vitro* for cytotoxic activity against a panel of 20 paediatric high-risk ALL patient-derived xenograft (PDX) cells. Apoptosis was measured by Annexin V positivity and PARP cleavage. Reactive oxygen species (ROS) levels were assessed by DCF-DA staining and detection by flow cytometry. Nrf2 protein expression levels were measured by Western blotting.
Results: The screen resulted in the identification of two FDA-approved drugs that were preferentially cytotoxic against *MLL*-rearranged ALL and other leukaemia cell lines, compared to solid tumours and normal cells. Auranofin was originally developed for rheumatoid arthritis and was later fast-tracked into Phase II clinical trial for adult chronic lymphocytic leukaemia, while Disulfiram, which was developed for treatment of chronic alcoholism, is currently in several clinical trials for cancers including metastatic melanoma and glioblastoma. These drugs also showed potent activity in high-risk paediatric leukaemia PDX cells cultured *in vitro*, including *MLL*-rearranged ALL and Philadelphia-positive ALL with IC_{50} values between 100-400 nM for Auranofin and 30-60 nM for Disulfiram. Induction of apoptosis was evident at 6 hours post Auranofin treatment, or after 12 hours Disulfiram treatment. Each drug significantly increased intracellular ROS as early as one hour post-treatment ($p<0.01$), which was accompanied by induction of Nrf2, a master regulator of the antioxidant response. Incubation with ROS scavenger N-acetyl cysteine prior to treatment with either drug prevented the increase in cellular ROS levels ($p<0.05$) and rescued cells from apoptosis ($p<0.0001$), indicating involvement of reduction-oxidation and increased ROS generation as mechanisms of leukaemia cell death induced by these drugs.
Summary/Conclusions: In summary, we have identified two FDA-approved drugs that demonstrated potent anti-leukaemia activity through induction of ROS, potentially opening up new avenues for clinical treatment of high-risk paediatric leukaemia. We will now be testing these potential therapies *in vivo* using relevant PDX models of high-risk paediatric ALL.

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TP53 MUTATIONS DISRUPTING DNA BINDING LEAD TO CHEMOTHERAPY RESISTANCE IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Polychemotherapy resistance is a major challenge in the treatment of children with relapsed acute lymphoblastic leukemia (ALL). Mutation of *TP53* is tightly associated with poor response to treatment in ALL relapse patients.
Aims: We studied mutations of *TP53* in ALL relapses and in six ALL cell lines to shed light on mechanisms and pathways mediating *TP53* dependent drug resistance in relapsed ALL. First, we analyzed the spectrum of *TP53* mutations in ALL relapses and correlated it to treatment response of patients. Second, we studied drug sensitivity in *TP53* wild type (wt) *versus TP53* mutant ALL cell lines.
Methods: *TP53* was sequenced by the method of Sanger. Drug sensitivity was determined by IC50 in ALL cell lines. Drugs included in the study were DNA damage inducing agents as topoisomerase II inhibitors, alkylating agents, nucleotide analoga, and other agents, most of which are used in ALL relapse treatment protocols.
Results: We identified 20 different *TP53* mutations in 34 patients. We classified *TP53* mutations into 'hot spot' (R175, G245, R248, R273 and R282), non-hot spot and frameshift, respectively. We found that hot spot *TP53* mutations were enriched in ALL relapse patients with non-response to treatment compared to good responding patients (64 *versus* 27%). In ALL cell lines, we confirmed *TP53* mutations in Jurkat (R196*) and Loucy (V272M) and identified R248P in MHH. Three ALL cell lines were *TP53* wt (SUP-B15, UOC-B6, NALM-6) and used as controls. Topoisomerase II inhibitors upregulated expression of wt p53. In contrast, nucleotide analoga showed no p53 induc-

tion. IC50 measurements showed that *TP53* mutations lead to resistance against topoisomerase II inhibitors and alkylating agents, but not against other drugs. The upstream pathway of p53 (CHK1, CHK2) and DNA damage recognition (γH2AX) were not impaired in the six ALL cell lines. To study the effect of *TP53* mutation on resistance to treatment in more detail, we focused on the R248P mutation, located in hot spot codon 248, that we found in a relapse patient with non-response to treatment and in the MHH cell line. Using a CRISPR/Cas9 knockout (KO) of endogenous p53 and lentiviral based re-expression in NALM-6, we generated p53 KO, and KO+wt p53, KO+R248P and KO+GFP cell lines. The KO cells showed a similar resistance to DNA damage inducing drugs as KO+R248P cells. Overexpression of wt p53 in KO cells restored the sensitivity to DNA damage inducing drugs. In contrast to wt p53, R248 did not inhibit cell proliferation under drug treatment. We found that this mutant was unable to induce downstream targets of p53 (p21, BAX). Moreover, ChIP-seq showed that R248P cannot bind the promotor and induce expression of typical p53 targets MDM2, p21, BAX, BCC3/PUMA, FAS or RRM2B/p53R2. This result indicates that R248P is deficient in binding the consensus element of p53. However, the binding motif analysis showed that the R248P mutant still binds DNA at a different and purine-rich sequence. In summary, R248P leads to loss of wt p53 function and mediates resistance to topoisomerase II inhibitors and alkylating agents.

Summary/Conclusions: Overall, our results show that mutations affecting *TP53* hot spots, in particular codon 248, are associated with resistance of ALL cells to chemotherapy and reveal first insights into underlying mechanisms and pathways.

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GENETIC ACTIVATION AND THERAPEUTIC TARGETING OF PIM1 IN T-CELL ACUTE LYMPHOBLASTIC LYMPHOMA

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Background: T-cell acute lymphoblastic leukemia (T-ALL) and T-cell acute lymphoblastic lymphoma (T-LBL) are aggressive immature T-cell malignancies that are considered one disease entity according to the World Health Organization (WHO). Both T-ALL and T-LBL are often characterized by improper T-cell receptor (TCR) recombinations leading to aberrant activation of proto-oncogenes.

Aims: Despite some genetic and phenotypic similarities between T-ALL and T-LBL, T-ALL risk group stratification cannot be extrapolated to T-LBL patients. Therefore, it is our goal to find new T-LBL markers and develop new targeted therapies based on those T-LBL specific markers.

Methods: Here, we used Targeted Locus Amplification (TLA, de Vree *et al.*, *Nat Biotechnol, 2014*) to identify a novel translocation (leading to PIM1 kinase overexpression) in a human T-LBL patient. Unraveling the importance of PIM1 activation in T-LBL disease biology prompted us to do both RNA sequencing and phosphoproteomic studies to identify its downstream targets. T-LBL patient engraftment in NSG mice enabled us to study the therapeutic potential of PIM1 inhibition.

Results: Applying the TLA technique lead to the identification of a novel TCRβ driven t(6;7)(p21;q34) translocation in a human T-LBL patient resulting in aberrant activation of the PIM1 proto-oncogene. PIM1 is a constitutively active serine/threonine kinase involved in cell cycle progression, apoptosis, transcription and drug resistance and is overexpressed in a variety of human cancers. Further characterization of this *PIM1* rearranged patient sample revealed cooperative genetic alterations that target known T-ALL/T-LBL onco- and tumor suppressor genes, including NOTCH1, IKZF1, EP300 and CDKN2A. Comparing *PIM1* expression between normal T-cell subsets, T-ALL and T-LBL patient samples showed that T-LBL patients express significantly higher *PIM1* levels, confirming PIM1 activation is implicated in T-LBL disease biology. Next, we looked at allelic expression ratios of PIM1 and interestingly, we found skewed allelic expression in T-LBL, but not in T-ALL patients. To study the oncogenic properties of PIM1 in the context of malignant T-cell transformation, we did RNA sequencing and phosphoproteomics on the T-ALL/T-LBL tumor line HSB-2 (high PIM1) after PIM inhibition with TP-3654 (Foulks *et al.*, *Neoplasia*, 2014). These data revealed that PIM1 inhibition has broad effects on transcription and phosphorylation substrates involved in cell cycle, translation and apoptosis. Finally, we evaluated the therapeutic potential of PIM1 inhibition. Daily TP-3654 treatment (4 weeks) of T-LBL engrafted NSG mice resulted in strong anti-leukemic effects. Currently, we are evaluating if combination of PIM1 inhibition with chemotherapeutics triggers a more profound anti-leukemic response (Figure 1).

Summary/Conclusions: All together, our study identifies PIM1 as a putative oncogene in T-LBL and suggests that inhibition of this serine/threonine kinase could serve as a novel therapeutic strategy in this aggressive T-cell neoplasm.

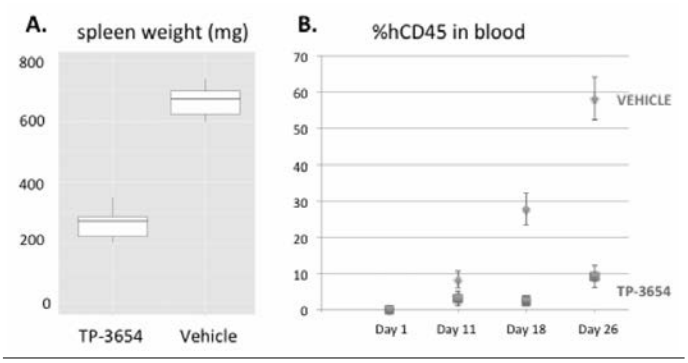

Figure 1.

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IL-7 FLEXIBLY REGULATES AUTOPHAGY-DEPENDENT VIABILITY OF T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA CELLS

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Background: T-cell acute lymphoblastic leukemia (T-ALL) constitutes an aggressive subset of ALL, the most frequent childhood malignancy. T-ALL cases are high risk and a significant fraction of the patients still relapse despite intensive chemotherapy, prompting the need for a deeper understanding of T-ALL biology in order to develop novel therapies. Autophagy is a homeostatic intracellular process characterized by the sequestration of cytoplasmic compartments within double-membrane vesicles (autophagosomes) to promote their degradation. Importantly, autophagy is upregulated during starvation, cellular stress or in rapidly dividing cells, such as cancer cells, as a compensatory mechanism to provide nutrients and stress relief. By mitigating stress and allowing cell survival, autophagy may serve as a pro-tumoral mechanism. On the other hand, persistent autophagy can lead to cell death and thereby prevent tumor growth. Interleukin-7 (IL-7) is essential for normal T-cell development but there is considerable evidence that IL-7-mediated signaling can also contribute to leukemogenesis. A majority of T-ALL patients expresses the IL-7 receptor and IL-7 accelerates T-ALL disease progression *in vivo* and promotes T-ALL cell proliferation, survival and metabolic activation *in vitro* via PI3K/Akt/mTOR pathway (a master negative regulator of autophagy). IL-7 can also activate MEK/Erk pathway (which has been implicated in promotion of autophagy).

Aims: Since IL-7 has the ability to activate signaling pathways with potentially opposing roles in autophagy regulation, our goal was to explore the actual impact of IL-7 on the autophagic process in T-ALL cells and elucidate its molecular mechanisms and functional consequences.

Methods: We used an IL-7-dependent leukemia T-cell line (TAIL7) and "primary" cells from patient-derived xenografts (PDX). We used inhibitors of PI3K (LY294002), mTOR (rapamycin), MEK1/2 (UO126) and ULK1/2 (MRT68921). Analysis of viability and cell size was performed by flow cytometry. Signaling pathway activation and LC3-I/-II conversion was performed by western blot analysis. LC3 puncta formation was assessed by confocal microscopy. Autophagosome/autolysosome formation was analyzed by electron microscopy.

Results: We show that in optimal culture conditions (medium with serum) IL-7 *inhibits* autophagy in T-ALL, albeit in a complex manner that involves triggering both pro- (via MEK/Erk) and anti- (via PI3K/Akt/mTOR) autophagic signaling. In this scenario, IL-7-mediated viability relies on the latter pathway, as we previously described. In contrast, under stress conditions (serum starvation) IL-7 *promotes* autophagy in leukemia cells. In this situation, IL-7-mediated survival partially relies on autophagy activation and strictly requires MEK/Erk activation. Mechanistically, we provide evidence that depending on culture conditions, IL-7 can balance the relative activation of PI3K/Akt/mTOR and MEK/Erk pathways towards or against autophagy in order to consistently promote T-ALL cell viability.

Summary/Conclusions: Our results suggest that IL-7 makes use of a 'flexible strategy' to promote T-ALL cell viability by recruiting both pro- and anti-autophagic pathways, which are differentially recruited to prevent tumor cell death depending on the microenvironmental conditions. Our data strengthen the notion that combination therapies against PI3K/Akt/mTOR and MEK/Erk pathways may be of particular relevance in the context of T-ALL.

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PRECLINICAL ACTIVITY OF ENTOSPLETINIB IN CHILDHOOD B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: B-cell acute lymphoblastic leukemia (B-ALL) is the most common malignancy of childhood and is highly curable with modern risk-adapted chemotherapy. However, 15-20% of children and >60% of adults with B-ALL develop chemoresistance and relapse, indicating need for new therapies. Addition of kinase inhibitors to chemotherapy for patients with *BCR-ABL1*-rearranged (Ph+) B-ALL has dramatically improved event-free and overall survival, and similar approaches are now under active clinical investigation in patients with *BCR-ABL1*-like (Philadelphia chromosome-like or Ph-like) B-ALL. Recent studies have demonstrated activated spleen tyrosine kinase (SYK) signaling in various genetic subtypes of B-ALL and preclinical activity of the SYK/FLT3/JAK inhibitor fostamatinib. However, SYK activation in B-ALL and potential correlation with specific leukemia-associated mutations remains incompletely characterized. We hypothesized that constitutive activation of SYK signaling occurs across a genetic spectrum of infant and high-risk childhood B-ALL and can be therapeutically targeted *in vivo* with the selective SYK inhibitor entospletinib (ento).
Aims: (1) Assess basal SYK signaling activation in childhood B-ALL specimens. (2) Quantify treatment efficacy, pharmacokinetics (PK), and pharmacodynamic (PD) effects of ento in childhood B-ALL patient-derived xenograft (PDX) models.
Methods: Total and phosphorylated (p) SYK levels were assessed by Simple Western analysis of splenic lysates from NSG mice well-engrafted with primary pediatric B-ALL specimens (n=19 Ph-like, n=4 infant *KMT2A*-rearranged (R), and n=4 infant non-*KMT2A*-R PDX models) to identify leukemias with constitutive SYK signaling activation. To assess *in vivo* activity of SYK inhibition, selected B-ALL PDX models with high basal pSYK (n=2) were treated with continuously provided control, ento 0.03%, or ento 0.07% chow. Cohorts of mice were sacrificed after 21-28 days, and peripheral blood and spleens were harvested for downstream studies. Flow cytometric analyses of murine tissues were performed to assess initial human ALL engraftment and to measure ento treatment responses. PK and PD assessments were performed in terminal peripheral blood and spleens, respectively.
Results: Constitutive pSYK signaling was observed in 10/19 Ph-like, 4/4 *KMT2A*-R, and 1/4 non-*KMT2A*-R B-ALL specimens. Ento treatment of *KMT2A-MLLT3* (ALL3103) and Ph-like *NUP214-ABL1* (NH011) PDX models significantly inhibited ALL proliferation *in vivo versus* control animals at both 0.03% and 0.07% chow formulations (representative data in Figure 1; $p<0.05$). Steady state concentrations were maintained throughout the study duration with terminal PK values of 3.3 (± 0.5) and 7.9 (± 1.0) μM (0.03% and 0.07% ento arms, respectively). PD studies demonstrated dose-dependent *in vivo* inhibition of pERK measured in human leukemia cells within spleens of ento-treated mice without alterations in total SYK protein levels. In general, PD inhibition of SYK target phosphoproteins was more pronounced in 0.07% ento-treated animals.

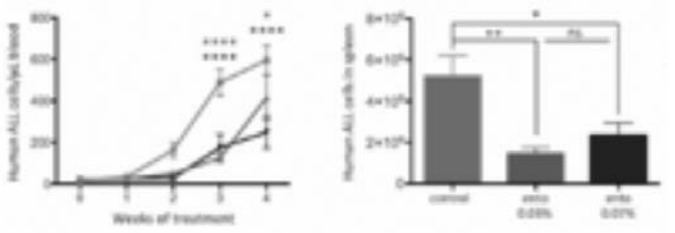

Figure 1.

Summary/Conclusions: Constitutive activation of SYK signaling occurs frequently in childhood Ph-like and infant *KMT2A*-R childhood B-ALL. Ento treatment of B-ALL PDX models potently inhibited SYK pathway signaling proteins and significantly inhibited leukemia proliferation *in vivo*.

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PHARMACOLOGICAL ACTIVITY OF CB-103 – AN ORAL PAN-NOTCH INHIBITOR WITH A NOVEL MODE OF ACTION

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Background: NOTCH signalling is a developmental pathway known to play critical roles during embryonic development as well as for the regulation of self-renewing tissues. Aberrant activation of NOTCH signalling leads to deregulation of the self-renewal process resulting in sustained proliferation, evasion of cell death, loss of differentiation capacity, invasion and metastasis, all of which are hallmarks of cancer. When the NOTCH pathway is inappropriately activated by genetic lesions (over expression of NOTCH ligands/receptors, GOF mutations in NOTCH receptors as well as chromosomal translocations), it becomes a major driver for NOTCH-dependent cancers and resistance to standard of care treatment. Over 250´000 patients are annually diagnosed with NOTCH dependent cancers, with no specific therapy available to date.
Aims: Given the importance of NOTCH signalling in human cancers, several therapeutic approaches have been utilized to block NOTCH signalling. Two of these strategies are; a) the use of monoclonal blocking antibodies (mAbs) against NOTCH ligands and receptors and b) the use of small molecule gamma-secretase inhibitors (GSIs). However, these approaches can only be effective if tumor cells express full-length ligand or receptor molecules. As validation of NOTCH as a therapeutic target, clinical activity of these in clinical studies were was observed in various trials for some of these inhibitors (mAbs, GSIs), but treatment and exposure were usually limited due to toxicities, mainly related to gastro-intestinal adverse events. On the contrary, in human cancers harbouring NOTCH gene fusion due to chromosomal translocations or specific NOTCH mutations, the use of mAbs and GSIs will have very limited clinical benefits. Cellestia has decided to follow a disruptive approach, by blocking NOTCH signalling in the most downstream part of the NOTCH cascade, at the level of the NOTCH transcriptional activation complex, using small molecule inhibitors.
Methods: Here we report the pharmacological characterization of CB-103, a first-in-class orally-active small molecule inhibitor of the NOTCH transcriptional activation complex.
Results: We demonstrate that *in vitro* CB-103 potently inhibits NOTCH signalling in various leukemic and lymphoma cell lines, and T-ALL blasts derived from relapse/refractory patients. In addition, CB-103 exhibited anti-tumor efficacy in multiple *in vivo* models of NOTCH-driven T-ALL using T-ALL cell lines and patients derived xenograft models.
Summary/Conclusions: Toxicology studies have been completed and clinical development of CB-103 with a first-in-human Phase I/IIA clinical study in advanced solid tumors and haematological malignancies is under preparation.

Acute lymphoblastic leukemia - Clinical 1

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IKZF1Δ4-7 CAN BE EASILY SCREENED BY PCR BUT DOES NOT PREDICT OUTCOME IN ADULTS WITH ACUTE LYMPHOBLASTIC LEUKAEMIA; DATA FROM 490 PATIENTS ENROLLED ON THE UKALL14 TRIAL

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Background: The *IKZF1* gene encodes the IKAROS zinc finger transcription factor and master regulator of lymphocyte differentiation. *IKZF1* lesions are common in acute lymphoblastic leukaemia (ALL) and have been reported as independent prognostic factors for poor outcome. *IKZF1*Δ4-7, resulting in the dominant negative *IK6* isoform is the most common single *IKZF1* deletion.
Aims: We aimed to generate and validate a simple, PCR-based screening assay for *IKZF1*Δ4-7 and to determine its utility as a prognostic marker in B precursor ALL using data from UKALL14 (ISRCTN 66541317)- a multicentre phase 3 randomised trial for adults aged 25-65 years.
Methods: Diagnostic DNA from 490 bone marrow samples was screened for *IKZF1*Δ4-7 using an endpoint PCR assay using primers located in introns 3 and 7. The lower limit of detection was determined by serial dilution of DNA from the *IK6*-expressing cell line SUP-B15 and calculated to be 0.001%. A total of 95 samples were also tested using the MLPA P335 kit to detect the full spectrum of IKZF1 deletion. Sanger sequencing confirmed the breakpoints in 27 cases.
Results: The median age of the patients tested was 46 years (range 25-65). Overall *IKZF1*Δ4-7 was detected in 97/490 (20%) patients but the frequency varied by genetic subtype. Patients with *BCR-ABL1* fusion had the highest *IKZF1*Δ4-7 frequency (46/150, 31%) followed by patients with B-other ALL (29/154, 19%). Patients with other classic cytogenetic abnormalities harboured significantly fewer *IKZF1*Δ4-7 – low hypodiploidy (3/26), *MLL* gene fusions (3/31), t(1;19), (1/11), high hyperdiploidy (2/9) and iAMP21 (0/3). MLPA did not detect any *IKZF1*Δ4-7 deletions that were not detected by PCR but did identify several samples with alternative *IKZF1* deletions affecting different exons (see Table 1). By contrast, the PCR assay did detect six *IKZF1*Δ4-7 deletions undetected by MLPA, consistent with the higher sensitivity of this approach. Interestingly, three of these samples harboured alternative *IKZF1* deletions in addition to *IKZF1*Δ4-7. In 70 (14%) cases, we observed a "faint" PCR band. Since the biological relevance of this was not clear, the 'faint' bands were not included in the main analysis. Interestingly the frequency of these "faint" bands was similar across all genetic subtypes: *BCR-ABL1* (14%), B-other (15%), MLL (21%), low hypodiploidy (19%). We examined the impact of *IKZF1*Δ4-7 on achievement of CR, persistence of minimal residual disease (MRD) at >1 x 10^{-4} (Ig/TCR quantitation by EuroMRD criteria) after courses 1 and 2 of therapy, EFS, OS and time to relapse, at a median follow-up of 23.1 months. Two thirds of patients (44/66) with *IKZF1*Δ4-7 were MRD positive at the end of phase 1 compared with 147/273 (54%) patients without the deletion (p=0.059). However, this relationship between *IKZF1*Δ4-7 and MRD did not persist after phase 2. We did not identify any association between *IKZF1*Δ4-7 and any of the other outcome parameters tested.

Table 1.

PCR	MLPA	Concordance	Total
Neg	Neg	56 concordant	73
	Del Ex 1-8 N=1 Del Ex 2-7 N=3 Del Ex 2-8 N=2 Del Ex 4-8 N=3 Del Whole gene N=6	15 'expectedly' discordant due to del >exons 4-7	
	Failed	2 concordance not assessable	
Pos	Pos	12 concordant	22
	Failed	4 concordance not assessable	
	Del exons 2-3, N=1 Del whole gene N=2	3 discordant probably due detection limit of 20% for MLPA	
	No deletion detected	3 discordant – probably due detection limit of 20% for MLPA	

Summary/Conclusions: *IKZF1*Δ4-7 can be detected by a simple and cheap PCR assay, which is more sensitive than MLPA. The frequency of *IKZF1*Δ4-7 was broadly comparable with previous studies. However, we did not find an association between *IKZF1*Δ4-7 and clinical outcome in this large clinical trial sample set. We are in the process of evaluating the impact of other *IKZF1* lesions.

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PROGNOSTIC SIGNIFICANCE OF MINIMAL RESIDUAL DISEASE DETECTED BY MLL FUSION GENE TRANSCRIPTS IN INFANT ACUTE LYMPHOBLASTIC LEUKEMIA. UPDATED RESULTS OF 76 PATIENTS ENROLLED INTO MLL-BABY STUDY

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Background: Fusion gene transcripts (FGTs) are rarely used for minimal residual disease (MRD) monitoring in acute lymphoblastic leukemia (ALL) cases, except of Ph-positive ALL. However in infant ALL, where *MLL* gene rearrangements are found the majority of cases, *MLL* FGTs are attractive targets for MRD detection.
Aims: To estimate prognostic significance of MRD by qualitative detection of different *MLL* FGTs in infant ALL treated by MLL-Baby protocol.
Methods: Seventy six infants (27 boys and 49 girls) with median age of 5.8 months (range 0.03-11.83) were included in the current study. Among them there were 39 (51.3%) *MLL-AF4*-positive cases, 14 (18.4%) *MLL-MLLT1*-positive, 12 (15.8%) *MLL-MLLT3*-positive, 6 (7.9%) *MLL-MLLT10*-positive, 4 (5.3%) *MLL-EPS15*-positive ones. MRD detection was performed in BM samples by real-time quantitative PCR and nested RT-PCR with sensitivity non-less than 1E-04. MRD-negativity was defined as absence of FGTs in the both assays. Median of follow-up period in the observed group was 6.4 years. Informed consent was obtained in all cases.
Results: We confirmed our earlier finding that the most informative TP for the MRD detection was TP4 (G. Tsaur at al, EHA 2012 abs O1096). MRD-positivity at TP4 led to unfavorable outcome in both *MLL-AF4*-positive patients stratified to high-risk arm of MLL-Baby protocol (EFS 0.05±0.04 *vs* 0.78±0.07 p<0.0001; cumulative incidence of relapse 0.78±0.10 *vs* 0.11±0.07 p<0.0001, respectively) and for all others *MLL*-rearranged patients treated by intermediate risk (ImR) arm (EFS 0.00 *vs* 0.71±0.11 p<0.0001; cumulative incidence of relapse 1.0 *vs* 0.29±0.10 p<0.0001, respectively). There were no significant differences in initial patients' characteristics and treatment response criteria (on days 8, 15, 36) among 38 MRD-positive and 38 MRD-negative patients. Multivariate analysis revealed that initial CNS disease (hazard ratio (HR) 2.703, 95% CI 1.255-5.284, p=0.011), non-M1 status of BM on day 15 (HR 3.090, 95% CI 1.465-6.515, p=0.003) and MRD-positivity at TP4 (HR 6.950 95% CI 2.617-18.456) were significant covariates with negative impact on hazard of unfavorable event. Based on dismal outcome of MRD-positive ImR patients we tried to augment their therapy and relocated 5 of them from ImR group to HR arm after TP4. Although all of them subsequently relapsed. We also wanted to find out which characteristics might predict relapse in ImR patients who were MRD-negative at TP4 (n=5). Of note, all these 5 relapsed patients (100%) had initial CNS disease while CNS disease was detected only in 2 out of 19 ImR patients (10.5%) who stayed in complete hematological and molecular remission (p=0.003). Also all 5 relapsed ImR patients who were MRD-negative at TP4 had breakpoint positions within intron 11 of *MLL* gene and they were MRD-positive by flow cytometry (MRD ≥0.01%) on day 15. None of MRD-negative patients by flow cytometry (MRD <0.01%) on day 15 relapsed later on (p<0.001).
Summary/Conclusions: MRD monitoring by detection of *MLL* FGTs was feasible and had significant prognostic impact. MRD-positivity at TP4 was an independent factor of unfavorable outcome in infants with *MLL*-rearranged ALL enrolled into MLL-Baby protocol irrespective of treatment arm. Treatment intensification for MRD-positive at TP4 ImR patients did not improve their outcome. MRD-positivity at TP4 in ImR group was associated with MRD-positivity by flow cytometry on day 15, *MLL* breakpoint positions within intron 11 gene and initial CNS disease.

P162

PRO-T CELL ALL/LBL: AN ULTRA-HIGH RISK CD2-NEGATIVE DISEASE SUBTYPE IN ADULTS DEFINED BY FLOW CYTOMETRY

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Background: Risk fators for T-LBL have not been systematically evaluated, in contrast to T-ALL
Aims: Our aim was to define immunophenotype of T-LBL/ALL in 71 consecutive patients by use of the flow cytometry (FCM) of tissue aspirates if peripheral blood (PB) and bone marrow (BM) were uninvolved. We also evaluated prognostic value of immunophenotype according to WHO 2008 subtype and ETP (Early T-cell Phenotype) definition in adult patients with T-ALL/LBL treated on uniform ALL protocol
Methods: Between 1997 and 2015, 71 adult patients with T-LBL/ALL were treated according to the GMALL 05/93 and T-LBL/2004 protocols. Immunophenotype was determined by immunohistochemical staining and by FCM of cellular suspension obtained from lymph nodes (n=31), mediastinal mass (n=12) or nasopharyngeal/perimandibular infiltration (n=2) by fine needle aspiration biopsy (FNAB), as well as of BM (n=10), PB (n=7) and pleural fluid (n=9). Disease subtype was defined according to WHO 2008 classification: pro-T (CD2-), pre-T (CD2+), cortical (CD1a+), medullary/mature (sCD3+). Recognition of pan-T cell CD antigens (pTAg) expression included: CD1a, CD2, sCD3, CD4, CD5, CD7, CD8. ETP-ALL/LBL immunophenotype was defined as follows: absent (up to 5%positive cells) CD1a and CD8 expression, absent or dim (75% positive cells) CD5 expression, expression(25% positive cells) of 1 or more myeloid (CD13,CD33, CD15) or stem cell (CD34, HLA-DR) markers.
Results: Patient characteristics: ALL (BM+ >20%): n=26(37%), LBL: n=45(63%), BM+<20% involvement (LBL): 27%, age<35ys: 72%, males: 67%, mediastinal mass (MM): 92%, primary CNS+: 8%.Immunophenotype: pro-T: 21%, pre-T: 17%, cortical: 55%, medullary: 7%. Number of pTAg present: 0-3: n=25(36%) or 4-7: n=45(64%) of pts. Most frequently expressed pTAg were: CD7: 97%, CD5: 87%, CD2: 74%, CD1a: 58%. Myeloid markers: CD13/33/15 were expressed in 13%/26%/10% and stem cell markers: CD34% in 42% of pts. Overall, 19% (13/67) of the study population had ETP-ALL/LBL: CD1a-/CD8-, but CD5 negative: 46% and CD5 weaker (20-71%): 54%, CD34/HLA-DR/13/33/15 expressed in 100%/50%/50%/75%/14% of ETP pts. 4 pts(31%) with ETP were categorized as pre-T and 9 pts(69%) as pro-T. With a median (95%CI) follow up of 137 (0.99, 1.733)months, 5-yr OS and DFS (95%C.I.) was 53% (0.42, 0.65) and 48% (0.36, 0.59),respectively. 5-yr OS (95%C.I.) for pts with CD2, CD1a and more than 3 pTAg present was 64% (0.511, 0.776), 66% (0.512, 0.803) and 64% (0.5, 0.782) compared to 11% (0.034, 0.256), 32% (0.152, 0.494) and 27% (0.097, 0.452) for pts without CD2, CD1a and 3 or less pTAg, (P<0.001, 0.009 and 0.002),respectively. OS and DFS was dependent on WHO subtype (p<0.001/p=0.002). 5-yr OS (95%C.I.):cortical: 69% (0.547, 0.837), pre-T 48% (0.196, 0.776),mature 40% (0.029, 0.829) and pro-T 10% (0.072, 0.272). There was no significant difference in OS and DFS in pts with ETP *vs* non-ETP (P=0.186, 0.159), 5-yr OS (95%C.I.): 31% (0.57, 0.559) and 55% (0.418, 0.685) for ETP and non-ETP pts, respectively. Among ETP pts, 4/13(31%) are alive, 3/4(75%) pts with pre-T and only 1/9((11%) with pro-T phenotype.3/7 pts were rescued with allo-HCT (Figure 1).

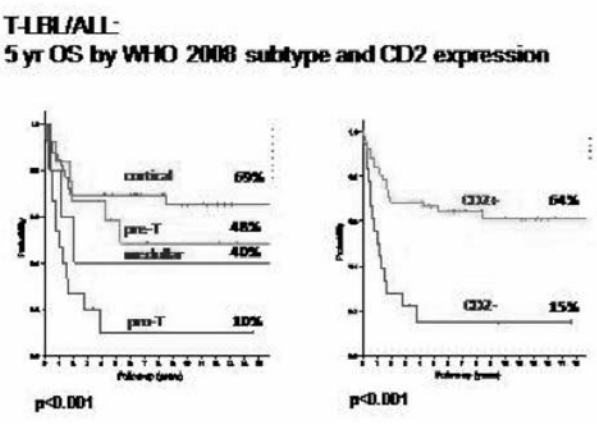

Figure 1.

Summary/Conclusions: Survival of T-LBL/ALL pts depends on CD1a and CD2 expression as well as on WHO subtype. ETP is a non-uniform category by pro-T/pre-T-cell origin. ETP phenotype was non-significant factor for OS/DFS (p=0.186/p=0.159) unless consistent with pro-T subtype (CD2-), only 1/9 pts alive. Pro-T (CD2-) is an ultra-high risk subtype of T-ALL/LBL and novel treatments are needed to improve pts outcomes.

P163

CLINICAL SIGNIFICANCE OF END OF INDUCTION MINIMAL RESIDUAL DISEASE IN ADULT PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA IN COMPLETE REMISSION AFTER A SINGLE CHEMOTHERAPY COURSE

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Background: In pediatric ALL end of induction minimal residual disease (EOI MRD) evaluated at day 29-33 after the first chemotherapy course is a primary determinant of outcome. The significance of EOI MRD in adult ALL is less clear.
Aims: To assess EOI MRD and its impact on survival and relapse risk in adult patients with Philadelphia-negative (Ph-) ALL in complete remission (CR) with a single chemotherapy course.
Methods: Induction chemotherapy for patients in the Northern Italy Leukemia Group 10/07 trial (ClinicalTrials.gov NCT-00795756; *Blood* 2016;128:176 [abstract]) consisted of CY/PDN pre-phase on days -5 to -1, VCR on days 1,8,15,22; Idarubicin on days 2,3; Dexa on days 1-5 and 15-18; L-Asp on days 9,11,13,16,18,20; intrathecal MTX, Ara-C, Dexa on days 1 and 15; and G-CSF from day 4. CR was assessed on day 28 by means of bone marrow morphology and MRD evaluation (RQ-PCR with case-specific probes, sensitivity $\geq 10^{-4}$). EOI MRD was correlated with risk subset, week 10 MRD, systemic relapse incidence (RI) and relapse-free survival (RFS), defining as favorable (*i.e.* negative) a response $<10^{-4}$. In this risk-oriented protocol MRD-based risk stratification was deferred until after early consolidation (week 10), at which point standard- and high-risk (SR, HR) patients with MRD $\geq 10^{-4}$ and very HR patients irrespective of MRD study results were eligible to allogeneic stem cell transplantation.
Results: Of 163 study patients, 139 were in CR after cycle 1 (85.2%), 95 had a sensitive molecular marker (68.3%) and 90 were successfully studied for EOI MRD. Median patient age was 39 years (range 17-67 years), 58.9% were male, 64.4% and 35.6% had B- and T-ALL, respectively, 44.4% were SR, 10% HR and 45.6% very HR (leukocytes >100, highly adverse cytogenetics [18.8%], early/mature-T phenotype). EOI MRD was undetectable in 34 (37.7%) and $<10^{-4}$ in 14 (15.1%), for an overall molecular response of 53.3%, while it was $\geq 10^{-4}$ in 42 ($\geq 10^{-3}$ in 28, 31.1%). EOI MRD did not correlate with patient age and leukocyte count but showed a more favorable course in patients with SR (n=40: $<10^{-4}$ 62.5%, $\geq 10^{-3}$22.5%) rather than HR/very HR features such as pro-B phenotype (n=11: 27.2%, 54.5%), early-T phenotype (n=12: 41.6%, 50%) and highly adverse cytogenetics (n=17: 47%, 41.1%). Notably most EOI MRD responders (42/44, 95.4%) and many of those in the 10^{-4}-10^{-3} range (10/14, 71.4%) were confirmed negative at week 10, contrary to only 7/28 (25%) with EOI MRD $\geq 10^{-3}$(P<0.0001). Five-year RI and RFS rates of EOI MRD negative *vs* positive groups (n=48 *vs* 42) were 15% *vs* 44% (P=0.01) and 68% *vs* 41% (P=0.03), respectively. The correlation between EOI and week 10 MRD identified three distinct prognostic groups: both timepoints negative (n=42), RI 14%, RFS 74%; any one positive (n=19), 43%, 49%; both positive (n=23); 44%, 31% (P=0.04 for RI; P=0.005 for RFS). In a multivariable prognostic model an EOI MRD $\geq 10^{-3}$increased greatly the risk of relapse (hazard ratio 2.67, P=0.01), followed by leukocytosis (>30 in B-ALL and >100 in T-LL; hazard ratio 2.35, P=0.04), while patient age and adverse phenotype or cytogenetics were not significant.
Summary/Conclusions: EOI MRD allows to differentiate early on the patients with the more favorable or most unfavorable treatment outcome, with cutoffs at $<10^{-4}$ (confirmed at week 10) or $\geq 10^{-3}$, respectively. This information can be incorporated into treatment algorithms for adult ALL and prompts the use of new agents/immunotherapeutics in induction regimens to optimize the rate of MRD negative CR.

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RESULTS FROM UKALL60+, A UK/HOVON COLLABORATIVE PHASE 2 STUDY IN ELDERLY PATIENTS WITH UNTREATED ACUTE LYMPHOBLASTIC LEUKAEMIA

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Background: The outcome for older adults with acute lymphoblastic leukaemia (ALL) is unsatisfactory. The UKALL12/ECOG2993 study showed that high risk cytogenetic abnormalities, were common, as well as lower rates of complete remission (CR) and 5 year overall survival (OS) in those aged 55- 65 years of age as compared to younger persons. There are few studies which focus on older patients with ALL, despite an increasing incidence with age.
Aims: A trial to establish age-appropriate baseline chemotherapy from which to design widely-applicable studies of novel agents in older people with ALL.
Methods: UKALL60+ offers five 'Arms' to be decided by investigator and patient choice; Arm A= Philadelphia chromosome positive (Ph+), Arm B= Non-intensive (designed to be delivered primarily out of hospital), Arm C= Intensive, Arm D= Intensive+, and Arm E= Registration only (in which treatment is at investigators discretion, including no active therapy). Any elderly patient with newly diagnosed ALL is eligible. There are no exclusions for co-morbidities, including prior malignancies. Baseline characteristics of each group including Charlson index, ECOG, Karnofsky and CRASH scores are being collected. The primary endpoint is the rate of complete remission (CR) after 2 phases of induction. Secondary objectives include determination of MRD status at 3 time points, EFS and OS at 1 year, treatment related mortality and quality of life.
Results: Since December 2012 85 patients have been recruited (4 excluded due to misdiagnosis) with a median age of 67 years (Range 55 – 83). Median follow up is 18.1 months. ECOG performance status was 0 in 33 (41%), 1 in 37 (46%), 2 in 8 (10%) and ≥3 in 3 (4%). Treatment allocation has been Ph+ n=18, Intensive n=34, non-Intensive n=11, Intensive+ n=7, and Registration only n=11 patients. It is too early to perform a full analysis of the reasons given for choosing each regimen but age appears to be a major factor for Ph-ve patients, with a median age of 74 years (Range 64-82) in the non-Intensive arm compared with 66 years (Range 56 -76) in the Intensive and Intensive+ arms. A total of 36/61 (57%) patients had high risk cytogenetics including *BCR-ABL1* (n=21), low hypodiploidy (n=10), complex karyotype (n=1) and *KMT2A-AFF1* (aka *MLL-AF4*) (n=4). Charlson index and CRASH score data is awaited. At the end of 2 phases of treatment on Arm A (Ph+ve) 17/18 (94%) patients achieved CR. On Arms B-D 27/52 (52%) patients achieved CR. Grade 3/4 AEs were seen in the majority of patients. The most common toxicities were haematological and infections. So far 30 relapses have been reported. 25 are isolated medullary relapses, 4 isolated CNS and combined in 1 patient. To date, 41 deaths have been reported; 32 patients died of ALL, 7 of infection, 1 cardiac and 1 multi-organ failure. Fifty one patients have had a PFS event. The median PFS is 13.2 months in Arm A (Philadelphia +ve) and 11.3 months Arm B-D. The median OS is 19.5 months in Arm A (Philadelphia +ve) and 15.5 months in Arms B-D (Figure 1).

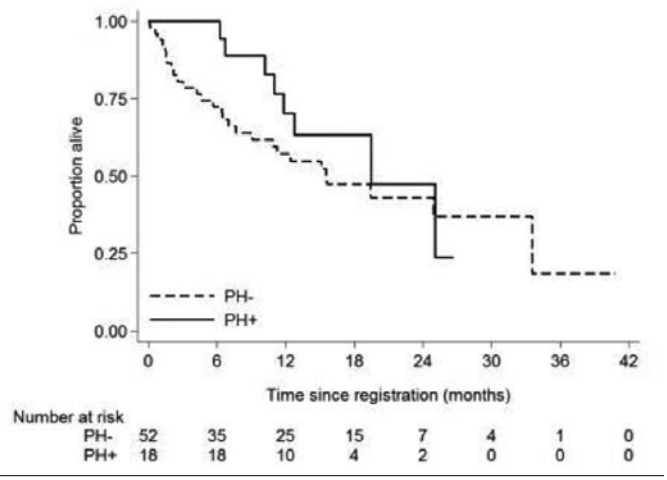

Figure 1.

Summary/Conclusions: ALL in older patients is challenging to treat, with a difficult balance between efficacy and toxicity. We observed a high rate of high risk cytogenetics, especially notable being the rate of low hypodiploidy. Initial high CR rates are seen in those with Ph+ve disease, this does not appear to translate into improved PFS and OS when compared with Philadelphia negative disease. The commonest cause of death in this group is ALL. We will use our baseline data to develop appropriate regimens for future studies of novel agents.

P165

CLINICAL OUTCOMES OF ELDERLY ACUTE LYMPHOBLASTIC LEUKEMIA/LYMPHOMA – A SINGLE INSTITUTION EXPERIENCE

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Background: Elderly acute lymphoblastic leukemia/lymphoma (ALL) is a rare disease with a poor prognosis and is underrepresented in clinical trials. This thought to be due to comorbidities, early death during induction, lower rates of complete remission, and higher risk of relapse with poor biological features (Gokbuget, Blood, 2013).
Aims: Describe clinical outcomes and prognostic factors of elderly ALL.
Methods: After IRB approval, we performed a retrospective study of patients (pts) age ≥60 diagnosed with ALL from 2000 to 2016 at Mayo Clinic Rochester. Statistical analysis was performed using JMP 10.0 software.
Results: Out of 210 adult ALL pts, we identified 63 (30%) consecutive pts with elderly ALL. The average age at time of diagnosis was 67 (60-82), & 38 (60%) were males. Median follow up was 16.1 months (0.23-126), during which time 40 (63%) deaths occurred; 25 (63%) related to the disease, & 15 (37%) secondary to infection or other causes. *Baseline characteristics at time of diagnosis:* 54 (86%) pts had B-cell phenotype, 19 (35%) were Ph+. Only 9 (14%) pts had T-cell phenotype. 20 (31%) pts had a Charlson Comorbidity Index ≥2 & 17 (27%) presented with ECOG PS ≥2. Median Hgb was 10.6 g/dl (4.9-18.5), WBC 6.2 x 10^9/l(0.5-160.8), PLT 51 x 10^9/l(4-750), peripheral blast 30% (0-95), marrow blast 87.5% (0-100), & LDH 381.5 U/L (141-8440). Lymphoblastic lymphoma was only evident in 3 (5%) pts. Among pts with available data, 34/58 (59%) had B symptoms, 16/57 (28%) lymphadenopathy, 7/57 (12%) splenomegaly, 6/60 (10%) pleural effusions & 10/45 (22%) of pts had CNS leukemia. *Cytogenetics at time of diagnosis:* Of 48 pts with available data, 20 (41%) had complex cytogenetics (≥5 abnormalities), 18 (38%) had a monosomal karyotype, 8 (17%) were hypodiploid, 4 (8%) were hyperdiploid, & 2 (4%) were a mix of hypo- & hyper-diploid. FISH studies were available for 50 pts: 10 (20%) had *CDKN2A* del, 3 (6%) t(4;11) *MLL-AF4*, 2 (4%) t(1;19) *E2A-PBX1*, 1 (2%) *IKZF1* deletion. *Treatment and Outcomes:* 10 (16%) pts received palliative therapy only, which included TKIs, chemotherapy, or hospice. The other 53 (84%) received induction chemotherapy. Only 12 (23%) had an up-front dose reduction due to comorbidities. 32 (60%) received Hyper-CVAD, concomitantly with rituximab in 11 (34%) pts, & TKIs in 9 (28%) pts. 21 (40%) pts received other regimens, of which 14 (67%) had asparaginase-based chemotherapy. Only 2 (4%) pts who received induction chemotherapy died within the first 60 days; both received Hyper-CVAD. Median number of cycles to achieve CR was 1 (1-8) with CR/CRi rate of 93%, & median time to CR1 was 34 days (19-459). 3 pts who underwent palliative chemotherapy achieved CR (all had Ph+ disease & received TKIs). 7 pts (13%) had primary induction failure. 50% of pts relapsed within a median time of 12.6 (3.6-72.8) months. Only 10 pts underwent allogeneic hematopoietic stem cell transplantation (HSCT), of which 2 (20%) relapsed in less than 180 days. Median survival after HSCT was not reached. *Predictors of survival*: Elderly ALL has worse mOS compared to our adult ALL cohort, 17.2 (IQR; 11.7-32.9) *vs* 52.1 (IQR; 27.6-169.9) mon (p=0.0016). In a univariate analysis model which included multiple variables, only ECOG PS ≥2, WBC>30,000, *CDKN2A* del, & CNS leukemia were statistically significant, however only CNS leukemia (p=.0009) & WBC (p=0.0168) retained statistical significance in multivariate mode, with a trend in *CDKN2A* del (p=0.06) (Figure 1).

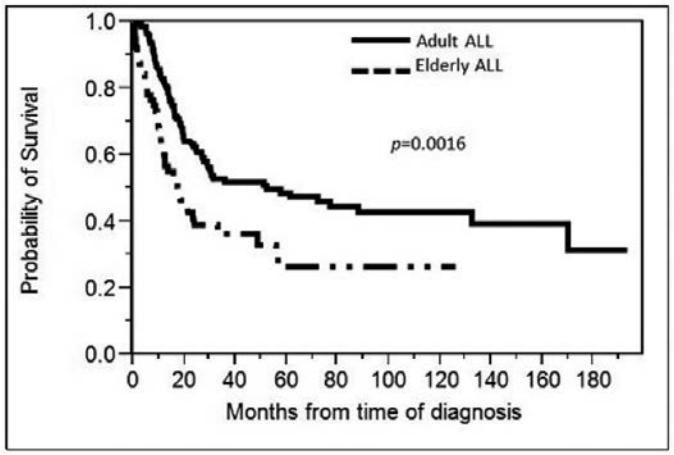

Figure 1.

Summary/Conclusions: Elderly pts with ALL have worse survival compared to younger adults. However, this was not reflected by a low CR rate, or a high rate of mortality during induction, but by grim disease overall. We report for the first time the incidence of 20% for *CDKN2A* del in this disease group. Further studies are needed to confirm this finding, as it could be a target for novel therapies.

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MANAGEMENT AND OUTCOME OF ADULT PH+ ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS TREATED AT THE "SAPIENZA" UNIVERSITY BETWEEN 1996 AND 2016

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Background: The outcome of adults and elderly (>60 years) patients with Ph+ ALL has improved since the introduction of tyrosine kinase inhibitors (TKI), used alone or in combination with chemotherapy during induction. Before 2005, all these patients were treated with chemotherapy; from 2005, a TKI-based "chemo-free" induction strategy was applied.
Aims: To evaluate the outcome of patients followed from 1996 at a single Center, and to correlate the short- and long-term responses with: a) induction treatment (chemotherapy or TKI); b) age; c) TKI used (imatinib or dasatinib); d) fusion protein; e) allogeneic stem cell transplant (SCT).
Methods: Sixty-eight patients (29M/39F) were treated; median age was 50 years (20-88) and 16 were elderly patients; 43 cases had the p190 protein, 19 the p210 and 6 had both; the latter 2 groups were merged together for further analyses. Fifty-two patients were enrolled in clinical trials. Median follow-up is 105 months (13-224).
Results: As induction, 28 patients received chemotherapy, 2 chemotherapy+TKI (considered as "chemotherapy±TKI group") and 38 TKI alone (24 imatinib and 14 dasatinib). All cases received TKI during consolidation/maintenance when it became available. All elderly patients but 1 received a TKI alone (plus steroids). Upon induction, 44 patients received consolidation chemotherapy, including 5 elderly. A SCT - carried out virtually only in adults - was performed in first complete remission (CR) in 13 cases (5 in the chemotherapy±TKI and 8 in the TKI groups). Overall, 91% patients achieved a CR; OS and DFS at 100 months are 42% and 45.5%, respectively. Among the 30 patients in the chemotherapy±TKI group, 25 (83%) achieved a CR, 4 were refractory and 1 died in induction; in the TKI group (n=38), 37 (97%) achieved a CR and 1 was refractory. Differences are statistically significant (p=0.03). Refractoriness was more frequent in p210+ than in p190+ cases (12% *vs* 5%): this finding did not translate into significantly different OS and DFS (30% *vs* 48% and 32% *vs* 51%, respectively). When patients were stratified by age, adults had a significantly better OS and DFS at 100 months than elderly (53% *vs* 19%, p= 0.04, and 57% *vs* 20%, p= 0.03, respectively), even more when considering only cases treated with TKI alone (75% *vs* 20%, p=0.01 and 73% *vs* 21.4%, p=0.017, respectively). The TKI used (imatinib or dasatinib) did not impact in adults, while a significant advantage in OS and DFS was observed for elderly patients receiving dasatinib (Figure 1): this might be due to the greater activity of dasatinib and also highlights the importance of consolidation chemotherapy, performed almost exclusively in adults. Considering adults only, within the chemotherapy±TKI group, 5 patients were transplanted and 19 were not: all transplanted cases are in CR, while in the non-transplanted cases 6 are in CR, 11 have relapsed and 2 have died in CR (p=0.01); within the TKI group, 8 patients underwent a SCT and 15 did not: of the transplanted cases, 6 are in CR and 2 have died due to complications, while 11 of the non-transplanted patients are in first CR, 3 have relapsed and 1 has died in first CR (p=n.s.). Of the 5 patients transplanted in second CR, only 1 is alive.

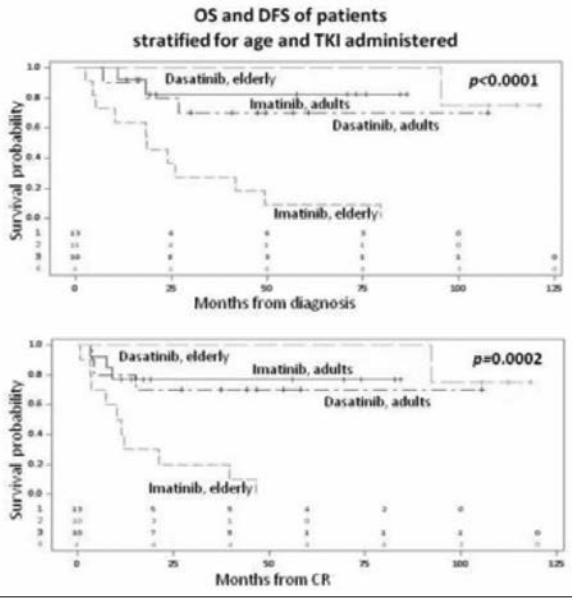

Figure 1.

Summary/Conclusions: This study further underlines the benefit of an induction based on a TKI alone. Since age holds strong prognostic significance, our results suggest that while imatinib followed by consolidation chemotherapy is the optimal choice for adults, in elderly cases dasatinib is more appropriate, since patients are often unfit to receive further chemotherapy. Finally, the advantage of SCT needs to be carefully redefined in the TKI era.

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THE TETRASPANIN CD9 IS A PROGNOSTIC MARKER FOR PREDICTING SURVIVAL OUTCOMES OF PEDIATRIC B-PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: B-precursor acute lymphoblastic leukemia (B-ALL) is the most common childhood malignancy, accounting for approximately 30% of pediatric cancers. With advances in risk-adapted chemotherapy, the overall cure rate of newly diagnosed B-ALL is approaching 85% in most developed countries. However, relapse still occurs in ~20% of patients and a significant portion of them are not initially classified in the high-risk disease entity, underscoring the need for development of additional informative prognostic biomarkers.
Aims: CD9, a tetraspanin family protein, regulates multiple physiologic processes including cell migration and adhesion, and has been associated with metastasis and progression of various types of cancers. In this study, we aim to evaluate its expression pattern and prognostic significance in pediatric B-ALL.
Methods: Cell surface CD9 expression on leukemic blasts at disease presentation was characterized by multicolor flow cytometry in a cohort of pediatric B-ALL patients. The CD9 expression status was correlated with clinical parameters, including age, sex, white cell count, cytogenetics and prednisone response. Kaplan-Meier survival analysis was performed to investigate the possible association of CD9 expression with clinical outcomes. The potential role of CD9 expression as a predictor of 5-year survival outcomes was evaluated using Cox regression models.
Results: Among 118 cases tested, blasts of 92 patients (78.0%) were CD9+ (≥20% of CD9-expressing blasts). There were no significant differences in age, sex and white cell count between CD9+ and CD9- patients. Major cytogenetics subgroups were similarly distributed except for hyperdiploidy (all patients were CD9+; P=0.022) and ETV6-RUNX1 translocation (higher prevalence in CD9- patients; P=0.001). Significantly more CD9+ patients were stratified into the intermediate-risk group (P=0.044) and a higher proportion of CD9- patients was stratified into the high-risk group (P=0.025). Besides, CD9- patients had poorer prednisone response (P=0.014). The 5-year overall survival (OS) and relapse-free survival (RFS) rates of CD9+ patients were significantly lower than those in CD9- patients (P ≤0.029). Subgroup analysis revealed remarkably poorer outcomes in CD9+ patients of the high-risk group (P ≤0.045). A similar trend was also observed in patients of the intermediate-risk group but not in the standard-risk group. In univariate analysis, CD9 positivity, age <1 year, white cell count ≥100 × 10^9/L and poor prednisone response were associated with lower RFS rate (P ≤0.050). In multivariate analysis, CD9 positivity (HR=6.0; P=0.019) and poor prednisone response (HR=3.9; P=0.015) remained as independent prognostic factors for lower RFS rates.
Summary/Conclusions: Our data indicate that expression of CD9 was significantly associated with inferior survival outcomes in pediatric B-ALL. The observed difference was most prominent for patients in the high-risk group, suggesting that CD9 expression could potentially be used in conjunction with other known prognostic factors for refinement of risk group stratification. Our study also lays the foundation for future development of CD9-targeted therapy for high-risk and relapsed/refractory pediatric B-ALL.

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PEDIATRIC MLL ACUTE LEUKEMIA PATIENTS SHOW DIFFERENTIAL HDAC EXPRESSION

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Background: Overexpression of histone deacetylases (HDACs) is a common feature in acute leukemias. Consequently, HDAC inhibitors (HDACi) have emerged as promising targeted therapy. However, non-specific HDACi may lead to accumulation of double stranded DNA lesions, so more selective isoform specific HDACi are needed. Expression patterns of HDACs in childhood leukemia have been scarcely studied.
Aims: To analyze the expression of HDAC isoforms in different subtypes of pediatric leukemia and correlate them with prognosis and clinico-biological features.
Methods: We evaluated the mRNA gene expression profile of class I, II and IV HDAC genes (HDAC 1-11) by quantitative PCR in 126 leukemic pediatric samples and a pool of non-neoplastic samples as calibrator. Patients were treated according to the Spanish Hemato-Oncology Cooperative Group protocols in a single center. The HDAC expression levels in different groups were compared by the Mann-Whitney test. The level of significance was set up at p<0.05. The analyses were performed with SPSS 24.0.

Results: Our series included 69 boys and 57 girls diagnosed with acute leukemia, with a median age of 5.1 years (range 0-17.4 years). We included 12 infant patients (<1 year old). Eighty-two (65%) patients had B-cell precursor acute lymphoblastic leukemia (BCP-ALL), 24 patients T-cell ALL and 20 patients had acute myeloblastic leukemia (AML). Globally, we found higher expression levels of class I HDAC isozymes (HDAC 1, 2, 3 & 8) in leukemic samples as compared to non-neoplastic samples, as previously reported. Interestingly, some HDAC isoforms associated with specific genetic aberrations. Those patients with rearrangement of MLL (KMT2A) gene (n=18, including 9 BCP-ALL and 9 AML; 7 infants and 11 pediatric) showed a significantly higher expression of HDAC9 (p<0.0001) and a statistically significant underexpression of HDAC1 and HDAC3 (p=0.003 & p=0.02, respectively, see Figure 1). Infants (n=12) had also a significantly lower expression of HDAC7 (p=0.043). In the same line, all pediatric patients with pro-B phenotype (CD10 negative) had low levels of HDAC7, but differences did not reach a statistical significance. After a median follow-up of 5.9 years, 15 patients died, with an overall survival (OS) of 96±2% for BCP-ALL, 82±8% for T-ALL and 55±13% for AML patients (p<0.0001). In the BCP-ALL subgroup, the expression of HDACs did not predict outcome, and only CNS infiltration and leukocytosis were unfavorable risk factors for OS. Again, CNS+, high WBC count and presence of minimal residual disease (MRD) post-induction were predictive for worse event free survival (EFS). Although the number of cases is low and these results must be taken with caution, T-ALL patients with the highest expression of HDAC3 (upper quartile) significantly correlated with worse OS (94% *vs* 25%, p=0.001) and a trend towards worse EFS (89% *vs* 53%, p=0.06). The only significant risk factor for EFS in this subgroup was the presence of MRD after induction (p=0.003).

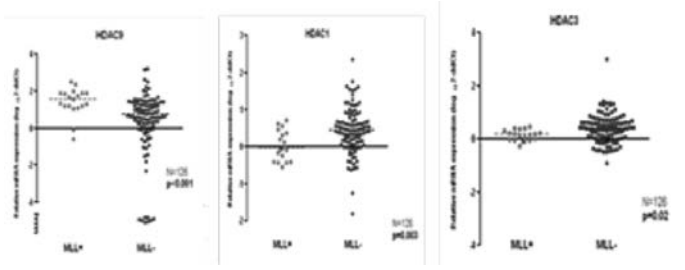

Figure 1.

Summary/Conclusions: We have observed a specific pattern of HDACs expression in pediatric patients with MLL rearrangement. Our study, if further confirmed, suggests that specific HDACi would potentially be a useful targeted treatment for pediatric patients with MLL rearranged leukemia.

P169

MINIMAL DISSEMINATED DISEASE DETECTION BY FLOWCYTOMETRIC IMMUNOPHENOTYPING IN T-CELL ACUTE LYMPHOBLASTIC LYMPHOMA

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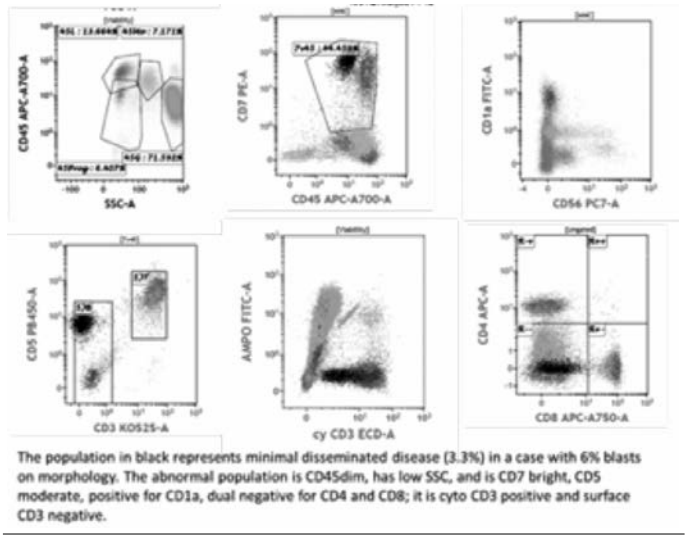
Background: T-cell acute lymphoblastic lymphoma (T-LBL) with minimal disseminated disease (MDD) is defined as the presence of T-LBL with <25% blasts in the peripheral blood (PB) and/or bone marrow (BM) by morphology and the presence of immunophenotypically abnormal T-lymphoblasts in bone marrow by flowcytometry. Published literature regarding the prevalence and clinical significance of this rare subgroup is sparse. In this study we analysed the presence of minimal disseminated disease is cases of T-LBL with <25% blasts using 8-10 colour flowcytometric immunophenotyping.
Aims: To evaluate the prevalence of minimal disseminated disease in bone marrow in cases of T-cell acute lymphoblastic lymphoma with <25% blasts in PB and BM using 8-10 colour flowcytometric immunophenotyping and evaluate the clinical and immunophenotypic features.
Methods: This study was a retrospective analysis of 42 patients of T-LBL with predominantly lymphomatous presentation with <25% blasts in peripheral and bone marrow. The following parameters were taken into account including complete hemogram, peripheral blood examination, bone marrow morphology and immunophenotyping, CSF analysis, pleural fluid morphology and immunophenotyping, tissue biopsy (lymph node or mediastinal mass), PET-CT findings and LDH levels. Flowcytometric immunophenotyping on bone marrow was performed on a 3 laser 10 color Beckman-Coulter Navios® platform and analysed using Kaluza® software. A minimum of 1,00,000 events were acquired and the presence of minimal disseminated disease was noted.
Results: A retrospective analysis of 42 cases of T-LBL with <25% blasts in peripheral and bone marrow was done. The mean age was 12.2 years (Range:2-48 years). M:F ratio was 1:1.7. Nearly all patients had normal haemoglobin, total leukocyte count and platelet counts. LDH was raised in majority of the patients (Mean 674U/L; N<190U/L). CSF examination was negative in all cases indicating that it is unlikely to have CNS involvement in patients with <25% blasts in PB and BM. Minimal disseminated disease was seen in 12 cases (12/42=28.6%) of cases. Of the 12 cases with minimal disseminated disease two cases were near early T-cell precursor acute lymphoblastic leukemia (near ETP-ALL) type and none were of ETP-ALL type. None of the cases showed circulating blasts in PB. The mean (range) bone marrow blast count in the group without MDD was 2.4% (0-4%) and in the group with MDD was 5.1% (0-15%). In the group with MDD (12 cases), only 5 cases showed >5%blasts/hematogones identifiable by morphology. This indicates flowcytometry is necessary in cases with <5% blasts to pick up cases of MDD. PET-CT was not sensitive to pick-up MDD as increased FDG uptake was seen in only a single case of MDD; it was negative in all cases without MDD. MDD by flowcytometry ranged from 0.007% to 18.5% (mean: 3.6%; median: 4%) (Figure 1).



The population in black represents minimal disseminated disease (3.3%) in a case with 6% blasts on morphology. The abnormal population is CD45dim, has low SSC, and is CD7 bright, CD5 moderate, positive for CD1a, dual negative for CD4 and CD8; it is cyto CD3 positive and surface CD3 negative.

Figure 1.

Summary/Conclusions: Our study shows that minimal disseminated disease is seen in more than one-fourth of cases (28.6%) of T-LBL with <25% blasts in PB and BM. This underlines the importance of flowcytometric evaluation of bone marrow in cases with <25% blasts identified by morphology. The identification of minimal disseminated disease in T-LBL is important as studies have shown inferior event free survival in T-LBL with minimal disseminated disease as compared to patients without minimal disseminated disease.

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INOTUZUMAB OZOGAMICIN IN COMBINATION WITH LOW-INTENSITY CHEMOTHERAPY (MINI-HYPER-CVD) AS FRONTLINE THERAPY FOR OLDER PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA: UPDATED RESULTS FROM A PHASE I/II TRIAL

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Background: Older patients (pts) with acute lymphoblastic leukemia (ALL) have poor tolerance of intensive chemotherapy, and novel strategies are needed in this population. In pts with relapsed/refractory ALL, inotuzumab ozogamicin (InO), an anti-CD22 antibody-drug conjugate, has been shown to improve survival compared to salvage chemotherapy.
Aims: We designed a phase I/II trial to evaluate the safety and efficacy of low-intensity chemotherapy (mini-hyper-CVD) plus InO as frontline treatment for older pts with newly diagnosed ALL.
Methods: Pts ≥60 years of age with newly diagnosed Philadelphia chromosome-negative pre-B received mini-hyper-CVD (compared to hyper-CVAD: no anthracycline, 50% dose reductions of cyclophosphamide and dexamethasone, 75% dose reduction of methotrexate, 83% dose reduction of cytarabine). InO was given on day 3 of the first 4 cycles. The first 6 pts received InO at a dose of 1.3 mg/m2 for cycle 1 followed by 0.8 mg/m2 for cycles 2-4; pts 7-34 received 1.8 mg/m2 for cycle 1 followed by 1.3 mg/m2 for cycles 2-4. Due to concern for veno-occlusive disease (VOD), the protocol was amended so that pts 35+ received InO at a dose of 1.3 mg/m2 for cycle 1 followed by 1.0 mg/m2 for cycles 2-4. Rituximab was given during the first 4 cycles in pts with CD20 expression ≥20%; all pts received IT chemotherapy prophylaxis with the first 4 cycles. Pts in CR after 8 cycles then received POMP maintenance for up to 3 years.

Results: Between 4/2012 and 12/2016, 47 pts have been treated, 4 of whom had received 1 cycle of prior therapy and were in CR at the time of enrollment. The median age was 68 years (range, 60-81), and median CD22 expression was 97% (range, 72-100%). Of 43 pts evaluable for response, 42 responded (ORR=98%). Best response was CR in 36 pts (84%), CRp in 5 (12%) and CRi in 1 (2%). MRD negativity by 6-color multiparameter flow cytometry was achieved in 31 of 41 evaluable pts (76%) on day 21 and in 44 of 46 evaluable pts (96%) within 12 weeks of treatment.
The median follow-up was 24 months (range, 1-55 months). 3 pts (6%) underwent allogeneic stem cell transplantation (ASCT) in first remission. Of the 46 responders, 6 pts (13%) have relapsed. 16 pts have died, 1 due to resistant disease, 4 after relapse, 1 after ASCT and 10 in CR/CRp. 21 pts remain on treatment (consolidation, n=3; POMP maintenance, n=19), and 5 pts have completed all therapy. The 3-year continuous CR and OS rates were 72% and 54%, respectively. Compared to a historical cohort of 79 older pts treated at our institution with hyper-CVAD ± rituximab, mini-hyper-CVD+InO resulted in significantly improved OS (3-year OS rate: 54% *vs* 31%; median OS not reached *versus* 16 months; P=0.007).
Treatment was overall well-tolerated. The median times to platelet and ANC recovery in cycle 1 were 22 days (range, 11-91 days) and 16 days (range, 0-49 days), respectively. In cycles 2-8, the median times to platelet and ANC recovery were 22 days and 17 days, respectively. Prolonged thrombocytopenia (*i.e.* lasting >6 weeks) occurred in 37 pts (79%) at some point during therapy; 8 pts (17%) experienced prolonged thrombocytopenia during induction and 36 (77%) during 1 or more subsequent courses. Grade ≥3 transaminase elevation occurred in 9 pts (19%), hyperbilirubinemia in 8 (17%) and hemorrhage in 7 (15%). 4 pts (9%) developed VOD (1 after ASCT, 3 unrelated to ASCT).
Summary/Conclusions: The combination of InO with mini-hyper-CVD is safe and effective in older pts with newly diagnosed ALL, resulting in a promising 3-year OS rate of 54%. These results appear superior to the outcomes of older pts treated with hyper-CVAD.

Acute myeloid leukemia - Biology 1

P171

RECURRENT MYB REARRANGEMENT IN BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM

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Background: Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare hematological malignancy that is derived from plasmacytoid dendritic cell precursors. BPDCN tends to occur in elderly people with frequent skin involvement and is associated with an aggressive clinical course and a poor prognosis. Although optimized diagnostics and therapies should improve patient outcomes, the pathobiological and genetic aspects of BPDCN remain unclear.
Aims: We planned this study to identify a critical genetic event in BPDCN, which could provide better understanding of BPDCN pathogenesis.
Methods: We enrolled fourteen patients (five children and nine adults) with BPDCN who were treated in our institutions. We primarily performed RNA sequencing-based comprehensive transcriptome analysis with their samples at the onset to detect gene fusions. These results were then used as the basis for genetic validation studies and functional analyses with an exogenous expression model.
Results: We identified a recurring gene rearrangement that involved the *MYB* proto-oncogene in all five pediatric patients (100%) and four of nine adult patients (44%) with BPDCN. The resulting fusion genes included *MYB-ZFAT* (four patients), *MYB-PLEKHO1* (three patients), *MYB-DCPS* (one patient), and *MYB-MIR3134* (one patient), none of which have been previously reported to our knowledge. The translocations corresponding to these fusions were not detected by the metaphase analysis except in one patient with t(1;6), who harbored *MYB-PLEKHO1*. These fusion genes were detectable at diagnosis and relapse but not at remission. Fluorescence *in situ* hybridization (FISH) analysis efficiently detected the breaking apart of *MYB* in formalin-fixed, paraffin-embedded sections. Consequent to the rearrangement, the negative regulatory domain of *MYB* was truncated, leading to constitutive MYB transcriptional activation, as described in other malignancies. Exogenous *MYB-PLEKHO1* expression in HEK 293T cells led to the upregulation of several known downstream MYB targets. Gene set enrichment analysis also confirmed the activation of MYB target gene sets. The identified significantly upregulated genes included cell surface molecule-encoding genes such as *NCAM1* (also termed *CD56*), *CD68*, *S1PR1*, and *CXCR4*, possibly providing targets for antibody-mediated anticancer therapies. We performed whole-exome sequencing of paired tumor–germline samples at diagnosis for four pediatric patients, which revealed a total of 91 (6–45 per patient) somatic mutations, a relatively large number compared with other pediatric cancers. However, no driver mutations were identified from the existing literature and database entries; only one missense mutation, *KMT2D* p.Cys1403Gly, was present on a driver gene, although this exact mutation had not been previously reported. Furthermore, we performed targeted sequencing covering genes associated with hematological malignancies in the remaining 10 patients. Consequently, children were not found to carry any identifiable driver mutations, whereas all adult patients harbored at least one point mutation in genes such as *TET2*, *ASXL1*, *IKZF1*, *ZRSR2*, *NRAS*, and *EZH2*, most of which were reported to be mutated in BPDCN and myeloid malignancies.
Summary/Conclusions: We identified a high frequency of *MYB* rearrangements that promoted the MYB transcriptional activity in BPDCN. *MYB* split FISH analysis can constitute a valuable diagnostic tool for detecting *MYB* rearrangements. We expect that our findings provide critical insights regarding BPDCN pathogenesis and contribute to molecular biology-oriented diagnostic techniques and molecular-targeted therapies for this intractable malignancy.

P172

BRANCHED CHAIN AMINO ACID METABOLISM REGULATES ALPHA-KETOGLUTARATE HOMEOSTASIS RESEMBLING MUTANT-IDH DRIVEN DNA HYPERMETHYLATION IN AML

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Background: The branched chain amino acids (BCAAs) valine, leucine, and isoleucine are essential AAs for the human body. The activity of BCAA metabolism and high levels of the enzyme BCAA Transaminase 1 (BCAT1) have recently been associated with aggressiveness in several cancer entities. However, the mechanistic role of BCAT1 in this process remains uncertain.
Aims: To elucidate the mechanistic link between BCAT1 function and epigenetic deregulation in leukaemia stem cells (LSCs) and consequences on clinical outcome.
Methods: High-resolution proteomics of LSCs, Knockdown and overexpression of BCAT1 in AML patient samples and AML cell lines, Gene set enrichment analysis, BCAA tracing experiments, Xenotransplantations, Metabolomics, DNA methylation arrays, correlative and mechanistic link to clinical data sets.
Results: We performed high-resolution proteomic analysis of human acute myeloid leukaemia (AML) stem cell (LSC) and non-LSC populations, which have been functionally validated by xenotransplantation into NSG mice, and we found the BCAA pathway enriched and BCAT1 overexpressed in LSCs. We show that BCAT1, which transfers α-amino groups from BCAAs to α-ketoglutarate (αKG), is a critical regulator of intracellular αKG homeostasis. Next to its role in the tricarboxylic acid (TCA) cycle αKG is an essential co-factor for αKG-dependent dioxygenases such as EGLN1 and the TET family of DNA demethylases. Knockdown (KD) of BCAT1 in leukaemia cells caused accumulation of αKG resulting in HIF1 protein degradation mediated by EGLN1. This resulted in a growth and survival defect and abrogated leukaemia-initiating potential. In contrast, overexpression (OE) of BCAT1 in leukaemia cells decreased intracellular αKG levels and caused DNA hypermethylation. BCAT1^{high} AML samples displayed a DNA hypermethylation phenotype similar to IDH^{mut} cases, in which TET2 is inhibited by the oncometabolite 2-hydroxyglutarate. High levels of BCAT1 were strongly correlated with shorter overall survival in IDH^{wt}TET2^{wt}, but not IDH^{mut}or TET2^{mut}AMLs. Gene sets characteristic for IDH^{mut} AMLs were enriched both in IDH^{wt}TET^{wt}BCAT1^{high} patient samples and in BCAT1-OE leukaemia cells. BCAT1^{high} samples showed robust enrichment for LSC signatures and paired sample analysis revealed a significant increase of BCAT1 levels upon relapse of the disease.
Summary/Conclusions: In summary, BCAT1 reduces dioxygenase activity by limiting intracellular αKG, thus linking BCAA catabolism to HIF1a stability and DNA hypermethylation. Our results suggest the BCAA-BCAT1-αKG pathway as a therapeutic target to compromise LSC function in IDH^{wt}TET2^{wt} AML patients.

P173

NUCLEAR RE-LOCALIZATION OF NPM1C+ INDUCES DIFFERENTIATION AND CELL GROWTH ARREST

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Background: *NPM1* mutated (*NPM1*c+) acute myeloid leukemia (AML) is a distinct entity of the 2016 WHO classification of hematopoietic tumors. NPM1 is a multifunctional nucleolar chaperone. All the mutations in NPM1 described so far result in cytoplasmic protein localization (NPM1c) through the acquisition of a nuclear export signal (NES) at the C-terminus, indicating that the cytoplasmic localization is critical for the leukemic phenotype. The most frequent NPM1 mutation is a heterozygous 4bp insertion in exon 12 (mutA).
Aims: Use gene editing and targeted drug treatment to determine whether NPM1 mutated AML cells are dependent on the cytoplasmic localization of NPM1c.
Methods: We sought to introduce indels adjacent to the mutation to disrupt the C-terminal NES in the *NPM1* mutated AML cell line OCI-AML3 and create novel edited alleles encoding for a mutant NPM1 with nuclear localization. Exploiting our optimized CRISPR-Cas9 protocol (Gundry *et al.*, Cell Reports 2016), we electroporated OCI-AML3 cells with an sgRNA spanning the 4bp insertion of mutA (NPM1c sgRNA). As a control, we targeted coding regions of *PTPRC* and *CD19*. Editing efficiency was assessed by high throughput amplicon sequencing. The dynamics of the nuclear re-localization of NPM1c upon drug treatment was studied using a CRISPR-Cas9 engineered NPM1c-GFP OCI-AML3 line generated in house.
Results: While the NPM1mutA allele showed 70-90% indel frequencies, the NPM1wt allele was intact. The novel edited alleles could direct nuclear localization of a reporter GFP-NPM1 fusion construct, and re-localization cytoplasmic NPM1 in edited cells was confirmed by immunofluorescence. Return of NPM1 protein to the nucleus was followed by significant impairment of cell growth (~4 fold decrease in cell counts), colony forming ability (16-20 fold reduction) and engraftment in xenograft models. Flow cytometry analysis showed terminal differentiation and cell cycle arrest in G1 phase (controls 45□3%, NPM1c sgRNA 68□1.5%) 9 days after NPM1c sgRNA transfection. Furthermore, transcriptome analysis on NPM1mutA-targeted and control OCI-AML3 cells revealed a fast and deep downregulation of the HOXA and HOXB cluster genes as well as MEIS1 in treated cells (4 to 5 fold average reduction). In order to verify that nuclear re-localization of NPM1c accounted for the dramatic changes, we treated OCI-AML3 cells with the nuclear export inhibitor selinexor (KPT-330). The impact of KPT-330 treatment mirrored the genome editing experiments, resulting in clear growth arrest, differentiation, and NPM1c localization with similar dynamics. Importantly, selinexor produced an almost complete loss of HOXA, HOXB and MEIS1 expression after 6 days of treatment.
Summary/Conclusions: Allele-specific editing is a powerful tool to probe mechanistic aspects of oncogene dependencies. By achieving nuclear re-localization of mutant NPM1, we demonstrated that cytoplasmic localization of NPM1c is necessary for OCI-AML3 cells to maintain their leukemic phenotype. Drugs promoting mutant NPM1 nuclear localization are attractive candidates for clinical success in *NPM1* mutated AML.

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THE LONG NON-CODING RNA HOXB-AS3 REGULATES RIBOSOMAL BIOGENESIS IN NPM1-MUTATED ACUTE MYELOID LEUKEMIA

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Background: Background: The prognostic significance of long non-coding RNA expression (lncRNAs) in older (≥60 years) patients (pts) with cytogenetically normal acute myeloid leukemia (CN-AML) was recently reported (Garzon *et al.*, 2014). The lncRNA *HOXB-AS3*, which is embedded in the *HOXB*-locus, was identified among the lncRNAs that associated with mutated *NPM1* (*NPM1*mut) in CN-AML.
Aims: Aims: Our aims were to evaluate the biologic significance of *HOXB-AS3* expression in *NPM1*mut AML.
Methods: Methods: *HOXB-AS3* expression profiling was performed by real-time PCR. Knock-down (KD) of *HOXB-AS3* was performed *in vitro* and *in vivo* [in a pt-derived xenograft (PDX) model] with locked nucleic acid-modified gapmers. Comparative proteomic analysis was conducted with a modified version of the RNA antisense purification (RAP) protocol (McHugh *et al.*, 2015). Direct visualization of the *HOXB-AS3* was performed using custom-designed Basecope probes (Advanced Cell Diagnostics, Newark, CA) according to the manufacturer's instructions.
Results: Results: Of 6 AML cell lines that were tested, only OCI-AML3 cells, which harbor *NPM1*mut, showed detectable levels of *HOXB-AS3* expression. Five- and 3-prime Rapid Amplification of cDNA Ends (RACE) assays in OCI-AML3 cells identified 3 previously annotated (NR_033201/NR_033203/ENST000491264) and 1 novel variant of *HOXB-AS3*. *NPM1*mut pt samples exhibited higher expression of *HOXB-AS3* compared to those with wild-type (WT) *NPM1* (*P*=.001*)* and healthy donors (*P*=.001). *In vitro* KD of *HOXB-AS3* led to decreased proliferation of OCI-AML3 cells, as measured by BrdU-based cell cycle analysis (S-phase average% in control *vs* KD: 24% *vs* 16%, *P*=.02). *HOXB-AS3* KD also led to a reduction in the number of formed colonies by OCI-AML3 cells in colony-forming assays (*P*=.002). *HOXB-AS3* KD in *NPM1*mut pt blasts (n=3) led to a decrease in the number of formed colonies (*P*<.001). To evaluate the effect of *HOXB-AS3* KD *in vivo* we generated a murine PDX model by engrafting NSG mice with blasts of a *NPM1*mut AML pt. Treatment of the engrafted mice with nanoparticle-formulated anti-*HOXB-AS3* gapmers led to significant prolongation of survival compared to treatment with non-targeting control gapmers in 2 independent experiments (*P*=.01 and *P*=.005). Mass spectrometry and comparative proteomic analysis of *HOXB-AS3*- and *U1*-specific RNA-protein complexes identified EBP1 and NPM1 as candidate *HOXB-AS3*-binding proteins. RNA-immunoprecipitation experiments validated the interaction of *HOXB-AS3* with EBP1 (20-fold increase of *HOXB-AS3* abundancy in EBP1-precipitate compared to normal IgG control, *P*=.001). Direct visualization of *HOXB-AS3* showed co-localization of the lncRNA and WT NPM1 in the nucleolus. EBP1 has been previously shown to interact with NPM1 and to regulate ribosomal biogenesis and growth of AML cells (Nguyen *et al.*, 2016). We hypothesized that *HOXB-AS3* could affect the EBP1-NPM1 interaction and impact on the ribosomal biogenesis process. In consistency with this hypothesis, *HOXB-AS3* KD led to a decrease in the transcription of rRNA species in OCI-AML3 cells (*P*=.001) and *in vivo*-treated blasts of 2 *NPM1*mut pts (*P*<.001). *HOXB-AS3* KD also led to a reduction of protein synthesis in the AML cells, as measured by incorporation of fluorochrome-tagged tracers in newly translated polypeptides.

Summary/Conclusions: Conclusions: *HOXB-AS3* is strongly associated with *NPM1* mutations in AML. *HOXB-AS3* interacts with EBP1 and NPM1 and regulates ribosomal biogenesis in the leukemic blasts. From a therapeutic standpoint, *HOXB-AS3* constitutes a promising target, as *in vivo* anti-*HOXB-AS3* treatment prolonged survival in a murine PDX model.

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A DUAL BH3-MIMETIC APPROACH TARGETING BOTH BCL-2 AND MCL1 IS HIGHLY EFFICACIOUS AND WELL-TOLERATED IN ACUTE MYELOID LEUKEMIA

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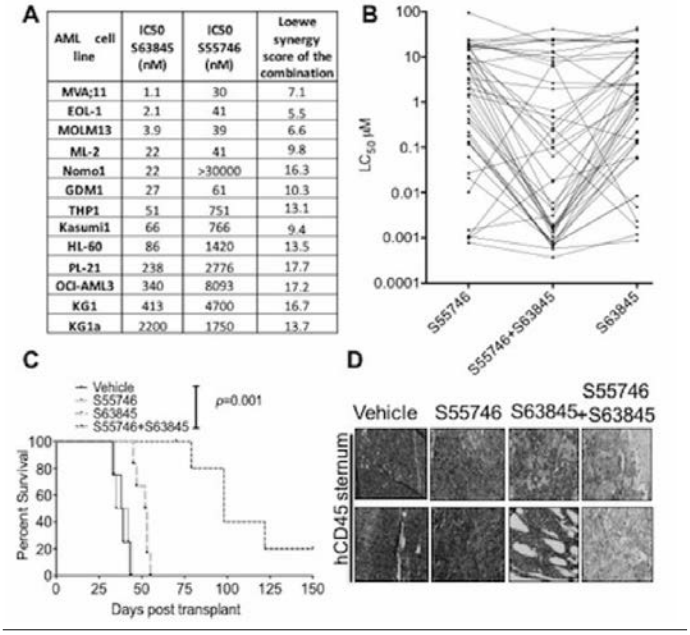
Background: Identification of a chemotherapy-free option for acute myeloid leukemia (AML) represents a highly desired and important research objective. Perturbation of cell survival is an essential hallmark of cancer now amenable to precision targeting by small molecule BH3-mimetics able to inhibit pro-survival BCL-2 (*e.g.* Souers *et al* Nat Med 2013 and Roberts *et al.,* NEJM 2016), BCL-X_L (Lessene *et al.,* Nat Chem Biol, 2013) and MCL1 (Kotschy *et al.*, Nature 2016). We hypothesize that simultaneous pharmacological targeting of BCL-2 and MCL1 will enhance apoptotic death of AML blasts, without increased toxicity to non-malignant cells.
Aims: To assess the feasibility and efficacy of targeting multiple BCL-2 pro-survival proteins using small molecule BH3-mimetics in pre-clinical models of AML
Methods: AML cell lines were obtained from ATCC or DSMZ. S55746 (BCL-2 inhibitor) and S63845 (MCL1 inhibitor with 6-fold higher affinity to human than mouse Mcl1) were obtained from Servier and A1155463 (BCL-X_L inhibitor) from Guillaume Lessene (WEHI). Primary AML cells were obtained from patients providing informed consent. For *in vivo* experiments, NSG; NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) or NOD/Rag$^{-/-}$/*Il2rg*^{tm1Wjl} (NRGS) mice were used.
Results: S55746 and S63845 showed strong synergy (Loewe score >5) in 13 AML cell lines tested, suggesting this dual BH3-mimetic targeting approach was highly efficacious (Figure 1A). S55746 and S63845 lowered the LC_{50} in primary AML samples by 10-1000-fold in the majority of cases tested, confirming remarkable anti-leukemic activity across a spectrum of AML cases with diverse cytogenetic and molecular pathologies (Figure 1B).



AML cell line	IC50 S63845 (nM)	IC50 S55746 (nM)	Loewe synergy score of the combination
MVA;11	1.1	30	7.1
EOL-1	2.1	41	5.5
MOLM13	3.9	39	6.6
ML-2	22	41	9.8
Nomo1	22	>30000	16.3
GDM1	27	61	10.3
THP1	51	751	13.1
Kasumi1	66	766	9.4
HL-60	86	1420	13.5
PL-21	238	2776	17.7
OCI-AML3	340	8093	17.2
KG1	413	4700	16.7
KG1a	2200	1750	13.7

Figure 1. (A) Loewe Score (0 → Additive, >1 → Weak Synergy, >2 → Synergy, >5 → Strong Synergy) in AML cell lines (Lehar, Nat. Biotech 2009). (B) LC50 of primary AML after 48hr treatment (C) NSG mice engrafted with MV4;11 AML and treated with i) vehicle, ii) S55746 100 mg/kg/d (x 4 wks), iii) S63845 25mg/kg IV (2x/wk x 4 wks) or iv) combined S55746 and S63845. (D) hCD45+ staining of NRGS sternums showing 2 representative examples of PDX AML one week after treatment with i) vehicle x5d, ii) S55746 100 mg/kg/d x 5d, iii) S63845 25mg/kg IV x 2d and iv) S55746+S63845.

A smaller fraction of AML samples were also sensitised to combined A1155463 and S63845 therapy. Bioluminescent imaging showed rapid and sustained clearance of xenografted MV4;11 AML (*FLT3*-ITD mutant and *MLL* re-arranged) cells, translating into significant prolongation of survival (Figure 1C) from combined S55746+S63845, but not from treatment with either BH3-mimetic alone. Similar *in vivo* efficacy was observed with xenografted OCI-AML3 cells harboring mutant *NPM1* and *DNMT3A*. Patient-derived xenograft models revealed rapid reduction of established AML in the bone marrow one week of treatment with S55746 and S63845 (Figure 1D). Safety and tolerability of this approach was confirmed using normal CD34+ stem and progenitor cells in short-term cell culture (48h) and long-term (2-3 weeks) clonogenic assays and from histological and biochemical examination of mice receiving treated for up to 8 weeks at doses shown to be highly efficacious against AML.
Summary/Conclusions: Dual BH3-mimetic targeting of BCL-2 and MCL1 induces rapid and synergistic cytoreduction of human AML cell line and primary AML samples *in vitro* and *in vivo* and across a diverse range of AML genotypes. We therefore report for the first time, that dual pharmacological inhibition of both BCL-2 and MCL1 represents a novel approach to treating AML without need for additional chemotherapy and with an acceptable therapeutic safety margin. Our results support the translational investigation of dual BH3-mimetic targeting of BCL-2 and MCL1 in the clinic for the treatment of patients with AML.

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THE PMLC62A/C65A KNOCK-IN MOUSE MODEL PROVIDES EVIDENCE FOR THE ROLE OF NUCLEAR BODY DISRUPTION IN THE PATHOGENESIS OF ACUTE PROMYELOCYTIC LEUKEMIA

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Background: Acute promyelocytic leukemia (APL) is driven by the oncogene *PML-RARA* which is generated by fusion of the promyelocytic leukemia (*PML*) and retinoic acid receptor alpha (*RARA*) genes, and which strongly interferes with downstream signalling and the architecture of multiprotein structures known as PML nuclear bodies (NBs). NB disruption is a diagnostic hallmark of APL; however, the importance of this phenomenon has only been studied *in vitro*.
Aims: The aim of this study was to decipher the impact of Pml NB disruption in APL pathogenesis.
Methods: We engineered a knock-in mouse model with NB disruption achieved through mutation of key zinc-binding cysteine residues (C62A/C65A) in the Pml RING domain.
Results: While no leukemias or tumors developed in Pml$^{C62A/C65A}$ mice, the forced dimerization of RARα - mediated artificially by linking RARα to the dimerisation domain of the NFκB p50 subunit - in cooperation with NB disruption was associated with doubling in the rate of leukemia (p<0.0001), with a reduced latency period (p=0.008). Moreover, response to targeted therapy with ATRA significantly improved the survival of mice transplanted with Pml^{WT}-p50-RARα or PML-RARα leukemic blasts, but not with Pml$^{C62A/C65A}$-p50-RARα, revealing the essential role of NBs for an effective response to differentiating drug. While formation of the *PML-RARA* fusion is considered an initiating event in APL pathogenesis, it is insufficient for the full leukemic phenotype. Moreover, whole exome sequencing analyses have consistently identified presence of cooperating mutations. Since Pml and Pml NBs have established roles in DNA repair and in the maintenance of genomic stability, we speculated that loss of NB integrity could affect these functions. Here, whole exome sequencing revealed a trend of higher genomic instability in Pml$^{C62A/C65A}$-p50-RARα leukemia as compared to Pml^{WT}-p50-RARα, with detection of mutations found in human APL, including *Kras*, *Ptpn11* and *Usp9y*. Using DNA repair reporter assays, we demonstrated that DNA repair via both non-homologous end joining (NHEJ; p=0.01) and homologous recombination (HR; p=0.006) pathways was less efficient in Pml$^{C62A/C65A}$ primary cells than in Pml^{WT} cells. Importantly, using a PML-RARα-inducible cell line, comparable defects in the NHEJ and HR pathways, which were PML-RARα dependent, were identified. These data were also supported by an increase in sister-chromatid exchange (p<0.0001) and chromosome abnormality (p=0.0002) rates in the context of Pml$^{C62A/C65A}$ *versus* Pml^{WT}. Interestingly, the kinetics of repair of ionising radiation (IR)-induced DNA double-strand breaks, assessed by analysis of γH2AX foci formation and clearance, was not affected. None of the DNA repair players analysed (*e.g.* Blm, Rad51 and 53BP1) failed to form foci in response to IR. However, their basal levels of foci were significantly greater in the presence of Pml$^{C62A/C65A}$ (p<0.04; quantified using Amnis ImageStreamX Mk II imaging flow cytometer). Additionally, we found that Rad51 foci showed a defect in localisation post-IR when Pml$^{C62A/C65A}$ was expressed, with impairment of Rad51 co-localisation and interaction with γH2AX.
Summary/Conclusions: Our study highlights the importance of re-formation of NBs for an efficient response to targeted therapy, the significant contribution

of Pml NB to the effectiveness of DNA damage repair processes, and the manner in which their disruption mediated by the PML-RARα oncoprotein can assist APL pathogenesis.

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DECIPHERING THE ONCOGENIC NETWORK OF PRC2 LOSS GUIDED LEUKEMOGENISIS

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Background: Loss of function mutations in EZH2 (including the chromosomal abnormalities -7/-7q) and other PRC2 subunits have been identified in adults with MDS, MPN and AML. Moreover children with JMML and up to 30% of children with Down syndrome related AML present with mutations in PRC2 subunits. Since myeloid neoplasms are elicited by accumulation of cooperating mutations and the study of isolated mutations is unlikely to unveil the molecular processes guiding transformation, we set out to decipher the oncogenic network guided by loss of PRC2-activity.
Aims: Through identification of collaborating mutations driving AML with loss of PRC2 function followed by molecular profiling we aimed to identify novel therapeutic targets in AML.
Methods: To model the complex interplay of mutational networks we performed CRISPR-Cas9 screenings with oncogene/tumor suppressor pools *in vitro* and *in vivo*. Cellular resources generated were subjected to mutational and molecular profiling.
Results: To this end, a 96-well based CRISPR-Cas9 immortalization assay allowing fast and quantifiable genetic cooperation screenings was established. Four out of six CRISPR-Cas9 pools tested –comprised of five genes each and representing 148 mutation combinations- reproducibly transformed LSK cells with distinct clonal output. Transplantation of *in vitro* immortalized clones yielded robust engraftment with multi-lineage contribution in mice but no overt leukemia was detected, indicating that induced mutations select for a preleukemic state *in vitro*. We thus tested every oncogene/tumor suppressor pool from the *in vitro* setting in a murine bone marrow transplantation model with freshly transduced LSK cells which resulted in robust induction of leukemia. Analysing the mutational spectrum of derived clones we were able to raise a list of potential partners cooperating with Ezh2 loss, which highlighted *Nf1(Ras-signaling)*, loss of *Dnmt3a,* and loss of *Runx1* as cooperating partners, whereas loss of cohesin complex subunits (*Smc3, Stag2*) seems to be dispensable during the induction of Ezh2-loss guided leukemogenesis. To define oncogenic dependencies in myeloid malignancies with PRC2-loss we analysed gene expression spectra of the generated samples. While *in vitro* transformed clones presented with distinct expression signatures clearly separating from controls a partially overlapping expression signature could be established. Through identification of these collaborating mutations and the resulting gene expression signature, which will be validated in a CRISPR-Cas9 knock-out screening we aim to identify novel therapeutic targets in AML.
Summary/Conclusions: Our study highlights the power of the CRISPR-Cas9 system to probe oncogenic interaction. Mutational CRISPR screenings *in vivo*, and a newly established *in vitro* CRSIPR-Cas9 immortalization assay for high throughput screening of sgRNA pools, delivered potential cooperating partners of Ezh2 loss in AML, and provides rich cellular resources to identify molecular mechanisms of oncogenic synergies and dependencies.

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Abstract withdrawn.

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ACUTE MYELOID LEUKEMIA EVOLUTION CAN BE RECONSTRUCTED BY ANALYSIS OF NON-LEUKEMIC CELLULAR SUBCOMPARTMENTS AND MULTI-LINEAGE ENGRAFTED MICE

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Background: Hematopoietic Stem Cells (HSC) isolated from patients with Acute Myeloid Leukemia (AML) have been shown to carry leukemia-specific mutations leading to the concept of pre-leukemic HSC. In order to understand the evolution from multi-potent pre-leukemic HSC to fully transformed AML, an accurate molecular comparison of patient matched HSC and leukemic cells is essential. Recently we have shown that functionally normal HSC can be separated from a subgroup of AML patients using the surface marker combination $CD34^+CD38^-$ and high ALDH enzyme activity ($CD34^+CD38^-ALDH^+$).
Aims: In this study we aim to understand the leukemic evolution from pre-leukemic HSC to fully transformed AML.
Methods: Whole exome sequencing (WES) of 12 diagnostic AML samples with the matched germ-line controls (T cells or buccal swab) was performed. Leukemia-specific mutations were identified according to specific criteria (*Allele Frequency* (AF) >0.20, *Sorting Intolerant From Tolerant* (SIFT)-Score <0.05, coverage >10 reads, support >2 reads, and GMAF <0.05) and validated. Identified AML-specific mutations were tracked in different cellular compartments (T- and B-cells) as well as in single HSC colonies derived from diagnostic AML samples. To test the functional properties of pre-leukemic HSC *in vivo*, we transplanted bulk AML in NOD/SCID-IL2R^{γnull} (NSG) mice and analyzed human subpopulations (myeloid and lymphoid) of multi-lineage engrafted animals for the presence of leukemia-specific mutations.
Results: WES identified 64 AML-specific mutations. Most cases (8 out of 12) showed 4-6 AML specific mutations per sample (1-18 mutations/ AML) including mutations in genes that are recurrently mutated in AML (DMNT3A, IDH1, IDH2, NRAS and KIT). Tracking of AML-specific mutations in non-leukemic T- and B-cells showed that some AML mutations like DNMT3A, IDH1, IDH2, EZH2 and ZNF536 were already detectable in T- and B-cells indicating their pre-leukemic status. Furthermore, analysis of multi-lineage engrafted xenografts detected leukemia-specific mutations in human myeloid and lymphoid sub-compartments suggesting that these animals were engrafted from functionally normal pre-leukemic HSC. To reconstruct the sequence of pre-leukemic mutations single-cell HSC were seeded and the resulting colonies analyzed for the presence of the respective leukemia specific mutations. Based on the different mutational data, combined with the cellular context in which these were detectable the leukemic evolution of most patients could be reconstructed. In one patient we detected a DNMT3A mutation in myeloid and lymphoid cells, whereas NPM1 and FLT3-ITD mutations were only detectable in leukemic cells proving the pre-leukemic status of DNMT3A in this case. In another patient we found DNMT3A and IDH2 in T- and B-cells whereas Trisomy 8 and a STAG2 deletion were only detectable in leukemic cells. By analyzing colonies from single cell HSC we were able to detect complex pre-leukemic hierarchies with one example in which a ZNF536 mutation could be identified as initiating event that hasn't been described in leukemia yet.
Summary/Conclusions: WES can identify leukemia specific mutations including mutations in genes that haven't been described in AML yet. Tracking of these mutations in various non-leukemic cellular compartments including HSC and multi-lineage engrafted mice allows reconstruction of the individual leukemic evolution. A better understanding of these processes may pave the way for new treatment strategies with the aim to target the relevant leukemic mutations.

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THE ESSENTIAL ROLE OF THE ENHANCERS OF POLYCOMB EPC1 AND EPC2 IN MLL-AF9 ACUTE MYELOID LEUKAEMIA IS A 'COMPLEX' STORY

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Background: The Enhancers of Polycomb (EPC) proteins EPC1 and EPC2 are required for the survival of MLL-rearranged acute myeloid leukemia (AML). Most importantly, loss of EPC1 or EPC2 in MLL leukemia stem cells, but not normal hematpoietic stem cells and progenitor cells, leads to the induction of cellular apoptosis. To date little is known about the functional contribution of EPC1 and EPC2 in AML. EPC1 is an essential component of the highly conserved NuA4 histone acetyltransferase complex. Additionally, EPC1 has been found in complexes with the Enhancer of zeste homolog 2 (EZH2), a catalytic core subunit of the histone methyltransferase Polycomb repressive complex 2 (PRC2). NuA4 and PRC2 are two major chromatin modifying complexes encompassing opposing epigenetic activities and both are known to be deregulated in AML.
Aims: A systems biology approach to understand the essential contribution of the homologous chromatin regulatory proteins EPC1 and EPC2 in AML in search for novel therapeutic targets.
Methods: Mass spectrometry (MS) analysis was performed on immunoprecipitated protein using EPC1 antibody from human THP1 *MLL-AF9* AML cell extracts. Chromatin immunoprecipitation (ChIP) was performed using HighCell ChIP Kit and iPure kit V2 (Diagenode) followed by NextSeq500 Illumina sequencing in THP1 cells. ChIP enriched regions were identified using SICER peak calling and ChIPpeakAnno. Lentiviral supernatants were prepared and THP1 cells were infected with viral particles containing pLKO.1 puro lentiviral vectors expressing shRNAs. RNA was extracted 72 hr following lentiviral transductions and whole transcriptome sequencing was performed. DESeq2 was used for differential expression analysis.
Results: MS analysis identified the core NuA4 complex components (TIP60, ING3, RUVBL1, RUVBL2, EP400 and DMAP1) and also revealed additional chromatin modifying proteins (HAT1 and HDAC2) copurify with EPC1. ChIP sequencing analysis on THP1 cells for EPC1 and EPC2 revealed both proteins bind in close proximity to genes enriched for the PRC2-associated repressive histone H3K27 trimethylation signature. Next we examined the genome-wide

histone methylation and acetylation profiles following lentiviral shRNA knockdown (KD) of *EPC1* or *EPC2* in THP1 cells. Interestingly, we find significant changes in histone H3K27 trimethylation levels as well as changes in the levels of histone H3 and H4 acetylation following *KD* of either *EPC1* or *EPC2* expression. Notably, the identified regions demonstrating changes in histone H3K27me3 levels are enriched for PRC2 target genes. RNA sequencing followed by gene-set enrichment analysis indicated significant transcriptional changes in PRC2 regulated genes following lentiviral shRNA knockdown of *EPC1* or *EPC2*. Meta-analysis of this PRC signature identified a sub-group of genes that are directly regulated by the EPC complex which include the monocytic differentiation inducer *MAFB*, the H2A ubiquitin ligase *TRIM37* and the pro-apoptotic tumor suppressor *CMTM3*.

Summary/Conclusions: Our data suggests that EPC1 and EPC2 are required for the recruitment of certain chromatin proteins to form EPC-associated complexes which are essential for the maintenance of an AML epigenetic signature and an aberrant transcriptional profile that supports leukemia stem cell survival. We have identified and characterized the EPC complex components in human AML. Additionally, we have refined a subgroup of PRC target genes that are regulated by the EPC complex which represent potential novel therapeutic targets in human AML. Overall we present a comprehensive analysis of the aberrant epigenomic landscape of THP1 *MLL-AF9* AML cells in relation to EPC1 and EPC2 and provide new insight into their deregulated role in AML.

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STROMA-DERIVED FACTORS STIMULATE JAK/STAT SIGNALING IN AML CELLS RESULTING IN RESISTANCE TO BCL2 INHIBITOR VENETOCLAX

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Background: The bone marrow (BM) microenvironment is known to protect AML cells from drug therapy. We showed earlier that conditioned medium (CM) from the BM stromal cell line HS-5 increased cell viability and led to resistance to specific drug classes.
Aims: Here, we investigate the mechanisms mediating the BM stromal cell induced resistance to venetoclax and its reversal by ruxolitinib.
Methods: Phospho-flow analysis was done by stimulating AML patient cells with GM-CSF, G-CSF, IL-6, IL-8 or MIP-3α (10 ng/mL) for 20 min, after which the cells were stained with Alexa 647-anti-phospho-Stat5 (pY694), PE188 CF594-anti-phospho-Stat3 (pY705), BV421-anti-phospho-Akt (pS473) and PE-anti-phospho-Erk1/2 (pT202/pY204). For co-culture and transwell assays AML cells were added directly to MSCs from AML patients or separated by a 0.4 µm pore membrane. Vehicle (DMSO), ruxolitinib (300 nM), venetoclax (100 nM) or their combination were incubated for 48h and AML cells labeled with PE-Annexin V, 7AAD, PE-Cy7-CD34, BV605-CD45. *In vivo* drug efficacy was tested on NSG mice inoculated i.v. with MOLM-13^{luc}AML cells. Mice were divided into control, venetoclax (25 mg/kg, i.p.), ruxolitinib (50 mg/kg BID, p.o) and combination groups (all n=6) and treated for 3 weeks, 5 days a week with 2 days off.
Results: To identify the factors contributing to BM mediated drug resistance of AML cells, we analyzed the effect of IL-6, IL-8, MIP-3α, GM-CSF and G-CSF, cytokines enriched in the HS-5 CM, on proliferation of MNCs collected from AML patients. GM-CSF and to some extent G-CSF alone conferred resistance to venetoclax similar to CM that we showed earlier to reduce sensitivity to BCL2 inhibitors. To identify the impact of stroma-derived factors on cellular signaling we stimulated AML patient cells with CM and analyzed the phosphorylation of STAT3, STAT5, ERK and AKT. Compared to control conditions, CM rapidly induced phosphorylation of STAT5 in primary AML cells. When the effect of individual cytokines was tested, we noted that GM-CSF and G-CSF alone could mimic the effect of CM on cellular signaling. Gene expression data showed the receptor for GM-CSF (CSFR2A) is more highly expressed in AML patient cells compared to healthy controls. Taken together, these results show that cytokines such as GM-CSF from BM stromal cells increase JAK/STAT signaling, which may lead to enhanced survival of AML cells. To determine whether the protective effect of stroma on BCL2 inhibition was dependent on cell-to-cell interactions we cultured AML patient cells either in direct contact with MSCs or separated from stroma with a 0.4 µm pore membrane. 48h treatment with 100 nM venetoclax did not result in significant reduction of CD34+ AML cells regardless of whether AML cells were directly cultured with stroma or separated by a membrane, further indicating that stroma-derived soluble factors are sufficient to reduce sensitivity to venetoclax. Since the most abundant cytokines secreted by HS-5 cells, GM-CSF and G-CSF led to increased phosphorylation of STAT5, a downstream effector of JAKs, we tested a combination of venetoclax and JAK1/2 inhibitor ruxolitinib. We found that ruxolitinib potentiated sensitivity to venetoclax when tested with AML patient cells in HS-5 CM and in co-culture and transwell assays. Significantly, the combination was more effective at reducing tumor burden in a xenograft mouse model of AML than either drug alone.
Summary/Conclusions: In conclusion, our data demonstrate that BM secreted soluble factors drive cytoprotection against BCL2 antagonist venetoclax that can be overcome by combined blockade of JAK/STAT and BCL2 pathways with ruxolitinib and ventoclax in *ex vivo* co-culture models and *in vivo* in an AML mouse model.

P182

IDENTIFICATION OF NOVEL GENE FUSIONS IN ACUTE MYELOID LEUKEMIA WITH COMPLEX KARYOTYPE BY TRANSCRIPTOME ANALYSIS USING RNA SEQUENCING

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Background: Acute myeloid leukemia with complex karyotype (CK-AML), defined as having ≥3 acquired cytogenetic aberrations in the absence of WHO-designated recurring translocations or inversions, represents about 15% of

adult AML cases. Despite having poor outcomes, CK-AML is the least understood at the molecular level, except for the finding that about two-thirds of cases carry *TP53* alterations. In particular, because cytogenetic alterations appear to be distinct among different patients, it is unclear whether they are cause of leukemogenesis, or merely reflect a state of genomic instability.
Aims: We have hypothesized that cytogenetic aberrations in CK-AML create gene fusions that, although not recurrent across patients, nonetheless deregulate cancer genes that contribute to leukemogenesis in individual patients.
Methods: We performed a transcriptome analysis using Illumina paired-end (101bpx2) RNA sequencing of 65 CK-AML cases to identify gene fusions using multiple independent algorithms (as paired reads that flank, or single-reads that span fusion junctions). Identified gene fusions were in part independently validated by array-based genomic profiling and/or long range PCR followed by use of long-read Oxford Nanopore sequencing technology.
Results: We identified 54 gene fusion events in 30 of the 65 cases (46%) with up to four fusions per case. All fusions are supported by 10-50+ junction-spanning reads, and most are independently supported and/or validated by evidence of genomic DNA breakpoints from array-based genomic profiling and/or long range PCR, respectively. About 35% of the fusions were in-frame, encoding chimeric proteins. The remainder encoded either C-terminally truncated 5' fusion partners, or else N-terminally truncated (or rarely full-length) 3' fusion partners in instances where the 5' partner contributed only the 5'UTR. In many instances, the fusions are predicted to lead to the overexpression or chimeric activation of known or putative novel cancer genes. Of the 54 fusions, only three (*RUNX1-MECOM*, *MN1-ETV6*, and *ETV6-MN1*) were previously reported in AML. The most frequently affected genes were *RUNX1* (n=5), *KMT2A*, and *MECOM* (n=3 each). Based on the affected genes the fusions can be categorized into six functional fusion clusters. Many of the fusions contained at least one known AML gene (n=16; *e.g. RUNX1*, *MECOM*, *DEK*, *ETV6*, *KMT2A*) together with a novel fusion partner, clearly suggesting pathogenic relevance. Other fusions were predicted to disrupt known tumor suppressors (n=4; *e.g. TP53*, *NF1*), or to activate known oncogenes (n=3; *e.g. MYB*). Others encoded chimeric proteins of unclear pathogenic relevance, but that could nonetheless encode novel epitopes created by the fusion junction (n=26).
Summary/Conclusions: Detailed molecular characterization of CK-AML revealed a high incidence of novel gene fusions in about 50% of cases. The affected genes suggest a more general role in leukemogenesis than reflecting a state of genomic instability. Furthermore, identifying gene fusions in each individual patient might lead to more effective, personalized treatments that target the gene fusion itself, enable immunologic therapies against the fusion junction epitopes, and provide private patient-specific biomarkers to track leukemic burden for the monitoring of disease remission and relapse.

P183

H3K27ME3 LEVEL ON THE HIST1 CLUSTER: A POWERFUL EPIGENETIC BIOMARKER THAT STRATIFIES TWO GROUPS OF NPM1-MUTATED AML DIFFERING IN THEIR OUTCOME AND EXPRESSION PROFILE

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Background: *NPM1* mutation (*NPM1*mut) is the most frequent genetic alteration found in cytogenetically normal acute myeloid leukemia (CN-AML). Patients harboring *NPM1*mut without *FLT3* internal tandem duplication (*FLT3*-ITD) are considered to have favorable outcome. Yet, some of them relapse and become resistant to chemotherapy. Little is known about biological processes underlying treatment failure. Our group previously described a new epigenetic biomarker corresponding to an abnormal gain of the repressive H3K27me3 histone mark within the HIST1 locus on the 6.p22 referred as H3K27me3 HIST1^{high}. This epigenetic biomarker had an impact on clinical outcome as CN-AML patients with H3K27me3 HIST1^{high} had a higher overall survival (OS) and leukemia-free survival (LFS) than H3K27me3 HIST1^{low} patients (Tiberi *et al.*, 2015).
Aims: We studied the impact of H3K27me3 HIST1 in an *NPM1*mut CN-AML cohort. Firstly, we asked whether H3K27me3 HIST1 could help to stratify *NPM1*mut CN-AML patients independently of known genetic alterations. Secondly, we studied gene expression profile (GEP) related to H3K27me3 HIST1 to explore biological pathways associated with treatment failure.
Methods: Blood or bone marrow samples with at least 70% blast involvement collected from 2005 to 2014 were recovered from the Paoli-Calmettes Cancer Institute Biobank and analyzed as training set. A validation set of samples collected during the conduct of two GOELAMS clinical trials (LAM2006IR and LAM2007SA) was used for validation. We performed H3K27me3 HIST profiling by chromatin immunoprecipitation followed by quantitative polymerase chain reaction (ChIP-QPCR) on five histone genes of the HIST1 cluster. We also sequenced frequently mutated genes in AML (*FLT3*, *CEBPα*, *DNMT3A*, *IDH1*, *IDH2*, *MLL*, *EZH2*, *TET2*, *P53*, *WT1* and *ASXL1*). GEP was done on Affymetrix whole-transcript DNA microarray and we used gene set enrichment analysis (GSEA, Subramanian *et al.*, 2005) along with the Gene Ontology database as statistical methodology.
Results: We pooled the 103 samples of patients with *NPM1*mut CN-AML of the training and validation sets together. Median age was 60 [37-76] and median leucocyte count was 76 G/L [10-352]. ChIP-QPCR Profiling identified 74 H3K27me3 HIST^{high} and 29 H3K27me3 HIST1^{low} patients. *FLT3*-ITD was found in 33 (43%) of H3K27me3 HIST1^{high} and 18 (38%) of H3K27me3 HIST1^{low} patients. We confirmed that H3K27me3 HIST1^{high} was associated with higher 5-year OS and LFS rates: 37% and 44% *versus* 17% and 19% (p=.005 and .01) for the H3K27me3 HIST1^{high} and the H3K27me3 HIST1^{low} patients, independently of other genetic alterations. Combining our biomarker with *FLT3* mutational status, we identified two subgroups of patients with very different outcome: 49% and 56% *versus* 18% and 18% (p=.004 and .01) for the H3K27me3 *FLT3*wt HIST1^{high} and the *FLT3*wt H3K27me3 HIST1^{low} patients, respectively (Figure 1). We performed GEP for 27 *NPM1*mut patients (12 H3K27me3 HIST1^{low} and 15 H3K27me3 HIST1^{high}). GSEA analysis revealed a strong enrichment in immune functions and leucocyte activation in the H3K27me3 HIST1^{high} group, evoking differentiated AML. While H3K27me3 HIST1^{low} samples had GSEA associated with chromatin remodeling factors and DNA replication. Considering only *FLT3*wt patients, the H3K27me3 HIST1^{low} subgroup had a gene expression signature characterized by a high expression level of genes from the HIST1 cluster which expression is known to be upregulated during S-phase of cell cycle.

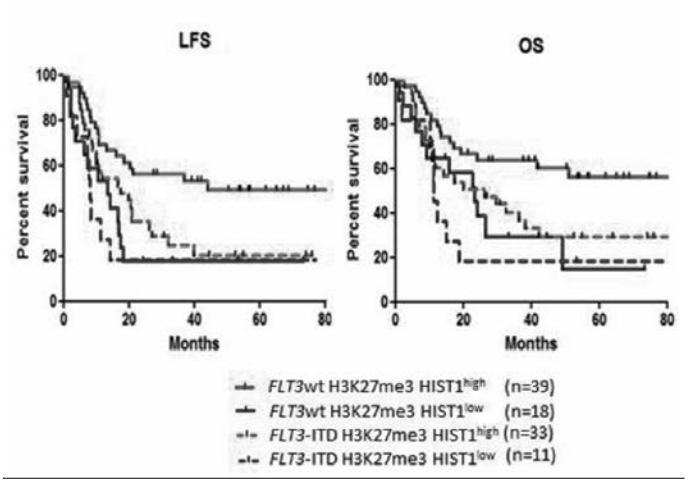

Figure 1.

Summary/Conclusions: the biomarker H3K27me3 HIST1^{high} is correlated with a better LFS and OS in *NPM1*mut CN-AML patient, independently of other known genetic alterations in particular *FLT3* ITD. The worse outcome of *FLT3*-wt H3K27me3 HIST^{low}patients is concomitant with high expression of replication-dependent HIST1 genes that could explain treatment failure.

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FUNCTIONAL ASSESSMENT OF NOVEL DIAGNOSTIC FLT3 MUTATIONS AND INHIBITION BY KINASE INHIBITORS

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Background: Somatic mutations in *FLT3* are among the most common events in AML, with *FLT3*/ITD mutations in the juxtamembrane domain (JMD) as well as D835 missense mutations in the kinase domain (KD) the predominant events. Sequencing of *FLT3* in a cohort of 788 children with *de novo* AML treated on contemporary Children's Oncology Group protocols demonstrated that in addition to the previously described *FLT3* mutations (ITD and D835), numerous other variants, including several novel variants, were present in 8% of patients at diagnosis, leading to a cumulative *FLT3* mutation prevalence of 27% in children and young adults. These variants mostly occurred in the JMD and KD and were predicted to activate FLT3, therefore increasing the number of patients who might be amenable to FLT3 inhibitor therapy.
Aims: We evaluated the oncogenic capability of each of these mutations by assessing their ability to result in aberrant FLT3 and STAT5 phosphorylation, as well as response to the tyrosine kinase inhibitors crenolanib and quizartinib.
Methods: Point mutations were introduced into HEK293 cells using retroviral transduction. Following transduction, phosphorylation status of FLT3 (pFLT3) and downstream STAT5 (pSTAT5) were evaluated by immunoblotting. Phosphorylation status was quantified by chemiluminescence analysis and the quan-

tity of protein expression was normalized to actin. That ratio of phosphorylated protein to total protein for FLT3 and STAT5 was determined and normalized to that observed in the D835Y mutation as a positive control. A value of >10% pFLT3 was considered positive. All mutations that resulted in FLT3 phosphorylation were subsequently evaluated for inhibition by crenolanib and quizartinib following 60-minute exposure to the compounds.
Results: A total of 24 non-ITD and non-ALM *FLT3* mutations were evaluated for autonomous FLT3 and STAT5 phosphorylation. Eleven mutations resulted in pFLT3 and pSTAT5, including 4 mutations with >50% pFLT3. All mutations that demonstrated aberrant pFLT3 also had aberrant pSTAT5, however a direct correlation of pFLT3 and pSTAT5 was not always observed. Overall, 87% (n=86 patients) of all non-ITD mutations evaluated resulted in autonomous FLT3 activation. Excluding D835 mutations, 64% (n=39) of patients harbored an activating mutation. Many of the mutations that were not found to be activating had the lowest prevalence, often present in only one patient. Evaluation of inhibition of phosphorylation demonstrated that in every case of aberrant activation, crenolanib resulted in potent inhibition of phosphorylation of FLT3 and STAT5 with an IC50 range of 1.3-13.9 nM and 0.6-6.5 nM respectively. Many of the mutations tested were exquisitely sensitive to crenolanib, with 9 of 10 mutations tested demonstrating an IC50 of pFLT3 inhibition ≤5.6 nM. Inhibition of downstream kinases are necessary for optimal efficacy of any FLT3 inhibitor and phosphorylation of STAT5 was potently inhibited by crenolanib in all cases. Quizartinib inhibited pFT3 and pSTAT5 with an IC50 range of 1.8-151.7 nM and 1-33.9 nM respectively, demonstrating less effective inhibition specifically at mutations including D835Y, D839E, N676K, M664I.
Summary/Conclusions: We have previously presented that *FLT3* mutations, including novel mutations in addition to the FLT3/ITD and D835, are prevalent in children and young adults with AML. Here we demonstrate that many of the non-ITD/835 mutations also result in aberrant FLT3 phosphorylation and are amenable to inhibition by FLT3 inhibitors. Crenolanib resulted in potent inhibition of FLT3 and downstream STAT5 in all mutations tested. This data supports expanding the cohort of pediatric patients with activating FLT3 mutations who may benefit from FLT3 inhibitor therapy beyond those with *FLT3*/ITD.

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Abstract withdrawn.

P186

THE BCL-2 INHIBITOR VENETOCLAX INHIBITS NRF2 ANTIOXIDANT PATHWAY ACTIVATION INDUCED BY HYPOMETHYLATING AGENTS IN ACUTE MYELOID LEUKEMIA

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Background: The selective Bcl-2 inhibitor Venetoclax (ABT-199) has shown potent antileukemic activity against Acute Myeloid Leukemia (AML) in preclinical and early clinical studies and impressive results have been achieved using the combination of hypomethylating agents (HMA) with venetoclax suggesting synergy between these agents.
Induction of Reactive Oxygen Species (ROS) is important for the cytotoxicity of various AML therapies including HMA. Induction of ROS by various cytotoxic therapies concurrently activates the Nrf2 antioxidant response pathway which in turn results in induction of antioxidant enzymes that neutralize ROS. Upon ROS induction, the transcription factor Nrf2 is released from its adaptor protein Keap 1 in the cytoplasm whereby Nrf 2 enters the nucleus and binds to antioxidant response element sequences in the promoters of various genes. Nrf 2 pathway activation has been shown to mediate chemoresistance in various cancers including AML. Low ROS levels have been shown to be a hallmark of leukemia stem cells and are critical to their self renewal capacity. In this study, we examined whether Nrf2 inhibition is an additional mechanism responsible for the marked antileukemic activity in AML seen with the combination of HMAs and venetoclax.
Aims: To determine the effect of veneoclax on ROS levels after HMA exposure in AML cells and to examine the effect of Bcl-2 inhibition on Nrf-2 antioxidant pathway activation in response to HMA
Methods: The effect of combination of venetoclax and HMA on ROS levels and apoptosis was measured by flow cytometry. Effcet of venetoclax and HMA on Nrf2 nuclear translocation was analyzed by immunostaining after cellular fractionation. Effcet of venetoclax treatment on the association of Bcl2 with Nrf2 Keap 1 complex was assessed by western blot analysis, immunoprecipitation and *in vitro* assay for ubiquitination.
Results: Our results demonstrated that combination of HMA with venetoclax augmented cellular and mitochondrial ROS induction and apoptosis compared to treatment HMA alone. Treatment of AML cell lines as well as primary AML cells with the hypomethylating agent decitabine resulted in increased nuclear translocation of Nrf2 (Figure 1) and induction of downstream antioxidant enzymes including HO-1 and NQO1. Immunofluoresence studies confirmed the inhibition of nuclear translocation of Nrf2 by venetoclax. Immunoprecipitation studies indicated that Bcl-2, Keap 1 and Nrf2 associate in a protein complex in the cytoplasm and that treatment with venetoclax leads to dissociation of Bcl-2 from the Nrf2/Keap 1 complex and targets Nrf2 to ubiqitination and proteosomal degradation.

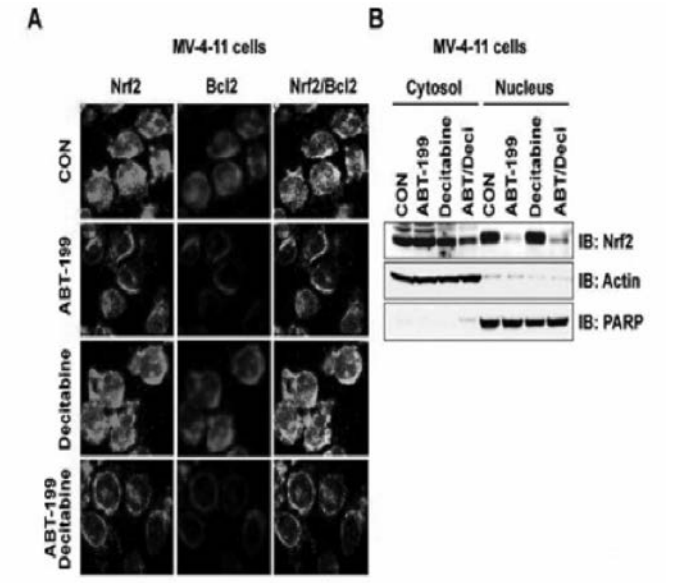

Figure 1.

Summary/Conclusions: In conclusion, inhibition of Nrf2 pathway may explain the marked potentiation of HMA activity by venetoclax that is observed in clinical trials. We show that ROS induction at least partially mediates the cytotoxicity of HMA and ROS induction after HMA treatment is augmented by venetoclax. We demonstrate for the first time that venetoclax is a potent inhibitor of Nrf2 activation via disruption of the association between Nrf2, Keap-1 and Bcl-2.

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UNRAVELING EPIGENOMIC REGULATION IN THE EVOLUTION OF RELAPSING PEDIATRIC AML

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Background: In comparison with pediatric acute lymphoblastic leukemia, pediatric acute myeloid leukemia (AML) is characterized by a high relapse rate (~30%), and lower overall survival rates of 60-70%. It is therefore crucial to increase our insights in pathophysiological mechanisms underlying AML relapse, including chemotherapy resistance, clonal evolution, and clonal selection. There is increasing evidence that epigenetic deregulation is involved in the initiation and progression of cancers, including adult AML. Epigenetic regulation involves the activity of non-coding regulatory DNA elements such as enhancers, which interact with promoters to fine-tune gene expression. Importantly, epigenetic signatures at enhancers are highly cell state specific. Since little is known concerning the epigenetic landscape of pediatric AML, it is crucial to gain more insights into the epigenome of relapsed and non-relapsed AML in children.
Aims: To identify differential epigenomic regulatory pathways involved in AML relapse by exploring the epigenome of relapsed (RP) and non-relapsed pediatric AML patients (NRPs).
Methods: The epigenome of 20 AML patients, harboring known molecular aberrations (including MLL-rearrangement, CBF-related and Flt3-ITD), was analyzed to identify active regulatory pathways. Acetylation of lysine 27 on the tail of histone H3 (H3K27ac) marks active regulatory DNA elements and was therefore used to identify active promoters and enhancers using Chromatin-Immunoprecipitation-sequencing (ChIP-seq) experiments. Additionally, single-cell RNA-seq data were generated for selected AML patients to analyze clonal heterogeneity.
Results: All genomic regions that were significantly enriched by H3K27ac were analyzed, resulting in ~30.000 active promoters and enhancers per sample. Genome-wide Pearson correlation of all enriched regions showed subclustering of patients based on molecular aberration. Interestingly, epigenomic analysis showed that the initial diagnosis (Dx) and the patient's relapse (Rel) sample were highly correlated. Also, single-cell RNA-seq analysis identified two highly identical homogeneous populations at Dx and Rel. Following the fact that no major differences were observed between AML cells at diagnosis and relapse, NRPs were analyzed. Here striking differences in H3K27ac enrichment were observed in MLL-rearranged patients between NRPs and RPs. Enhancers and promoters were differentially enriched at diagnosis, of which Sphk1, a kinase involved in proliferation and survival, was significantly more enriched in RPs, while the promoter of transcription factor ELF1 and nearby located enhancers were active in NRPs only.

Summary/Conclusions: Analysis of promoters and especially enhancers is a highly useful approach to identify cell state specific regulation. Here, we analyzed pediatric AML patients at diagnosis and at relapse to gain more insight into specific cell states which are involved in relapse. Our data revealed high similarity between diagnosis and relapse samples, while, strikingly, in the WHO intermediate-risk group containing MLL-rearranged patients, differential epigenomic regulation was observed between NRPs and RPs. Taken together, our preliminary data suggests that already at diagnosis, AML cells display an epigenomic fingerprint associated with the development of AML relapse during the course of disease. We are currently validating these data.

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MECHANISTICALLY INFORMED COMBINATIONS OF SY-1425, A POTENT AND SELECTIVE RARA AGONIST, WITH HYPOMETHYLATING OR ANTI-CD38 TARGETED AGENTS IN AML AND MDS

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Background: The complex pathogenesis of cancer often necessitates combination therapies to optimize patient benefit. Thus, we investigated preclinical combinations of SY-1425 (tamibarotene) and other agents to build on the monotherapy strategy with SY-1425 in biomarker selected AML and MDS patients (Phase 2 study, NCT02807558). Based on the RARα mediated myeloid gene activation of SY-1425, epigenetic priming with hypomethylating agents (HMAs) and CD38 induction were explored.
Aims: We sought to investigate mechanistically informed combinations of SY-1425 with standard of care agents and with potential novel agents in AML. We hypothesized that the HMA azacitidine could prime AML cells for SY-1425 mediated reprogramming by relieving aberrant methylation of RARα target genes and that strong upregulation of the maturation marker CD38 in AML cells by SY-1425 could induce sensitivity to the anti-CD38 therapeutic antibody daratumumab (DARA).
Methods: HMA synergy was tested *in vitro* in AML cell lines over a range of concentrations for SY-1425 and azacitidine. *In vivo* studies used a disseminated patient derived xenograft (PDX) model of AML expressing high levels of RARA. SY-1425 induction of CD38 was assessed by H3K27ac ChIP-seq, RARα ChIP-seq, RNA-seq and flow cytometry. Antibody dependent cell-mediated cytotoxicity (ADCC) was tested in an *ex vivo* co-culture model of human NK cells and AML cell lines.
Results: RARα acts as a repressive transcription factor until bound by SY-1425 leading to potent, targeted activation of myeloid genes. HMAs can further prime this activation by depleting repressive methylation of these target genes. The combination of SY-1425 and azacitidine showed synergy in RARA-high AML cell lines, but not in RARA-low AML cell lines, with combination indices less than 0.5. Co-administration in a RARA-high AML PDX demonstrated superior reduction of tumor burden (<1% detectable tumor cells) *vs* either treatment alone (7% with SY-1425 and 8% with azacitidine). Various combination regimens evaluated in the PDX model over two cycles (56 days) found that 1 week of azacitidine followed by 3 weeks of SY-1425 maximized for anti-tumor activity (<5% AML cells in periphery, bone marrow and spleen) and tolerability (<8% weight loss). RARα binds directly to the *CD38* locus and induces H3K27 acetylation in response to SY-1425 causing CD38 to be one of the most upregulated mRNA transcripts in RARA-high models. SY-1425 treatment of four RARA-high AML cell lines and three RARA-high primary AML patient samples induced cell surface CD38 to high levels comparable to those of DARA sensitive multiple myeloma cells. In contrast, no CD38 induction was observed in RARA-low cell lines. RARA-high AML cell lines treated with SY-1425 and DARA were six fold more sensitive to NK cell mediated ADCC compared to single agent controls and exhibited a 5-10 fold increase in NK cell-dependent activation measured by IFNγ secretion.
Summary/Conclusions: The RARA biomarker dependent synergy with azacitidine and SY-1425 is hypothesized to work through hypomethylation based priming of myeloid differentiation by SY-1425 agonism of formerly repressed RARα target genes. Since CD38 is one of the most strongly induced RARα target genes in response to SY-1425, AML blasts can be sensitized to DARA in a biomarker dependent manner. The preclinical synergistic effects and anticipated non-overlapping clinical toxicity profiles of the respective agents provide a strong rationale for clinical evaluation of each SY-1425 combination in biomarker selected AML and MDS patients.

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FLT3 INHIBITION OVERCOMES RESISTANCE TO THE BCL-2 SELECTIVE ANTAGONIST, VENETOCLAX, IN FLT3-ITD MUTANT AML MODELS

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Background: FLT3 internal tandem duplication (ITD) mutations account for ~20-25% of adult AML cases and are associated with worse prognosis. Although FLT3 inhibitors show clinical activity, relapse occurs quickly. Venetoclax is a potent, selective inhibitor of the anti-apoptotic protein BCL-2 that demonstrated monotherapy activity in relapsed/refractory AML (ORR 19%); however, no activity was seen in FLT3 mutant cases (Konopleva, Can Disc 2016). FLT3-ITD regulates expression of the anti-apoptotic proteins BCL-X_L and MCL-1, but not BCL-2, and FLT3 inhibition synergizes with the dual BCL-2/BCL-X_L inhibitor ABT-737 *in vitro* in FLT3-ITD+ cells (Kohl, Leukemia 2007).
Aims: Expression of BCL-X_L and MCL-1 are known resistance factors to venetoclax, therefore targeting pathways that regulate BCL-X_L or MCL-1 in combination with venetoclax may enhance cell death and improve efficacy. Based on this hypothesis, we interrogated if selective inhibition of BCL-2 by venetoclax in combination with quizartinib, a potent FLT3 inhibitor, resulted in synergistic anti-tumor effects in FLT3-ITD+ AML models.
Methods: FLT3-ITD+ (Molm13 and MV4;11) and wild type (wt; HL60 and OCI-AML3) cell lines were evaluated *in vitro*. Proliferation was measured by cell titer glo and apoptosis by Annexin V staining. *In vivo* efficacy was determined in a MV4;11 xenograft model.
Results: Sensitivity to venetoclax was initially assessed *in vitro*. Dose dependent growth inhibition and induction of apoptosis was observed in the MV4;11, Molm13 and HL60 cell lines following 48hr venetoclax treatment, with the MV4;11 cell line most sensitive. Modulation of BCL-2, BCL-X_L and MCL-1 expression by FLT3 inhibition was determined following 8-24hr treatment with quizartinib. Quizartinib reduced BCL-X_L and MCL-1 protein, but not BCL-2, in the FLT3-ITD+ cells. Quizartinib had no affect on expression of these three proteins in FLT3 wt cells. To interrogate the combination of quizartinib and venetoclax *in vitro*, cell lines were treated for 48hrs with venetoclax, quizartinib or the combination. Combination treatment led to significant reduction in proliferation and increased apoptosis in the FLT3-ITD+ cells compared to either single agent. FLT3 wt cells were not sensitive to quizartinib as a single agent or in combination with venetoclax. The combination translated *in vivo* as synergy between quizartinib and venetoclax was observed in the MV4;11 xenograft model. Together, this data underscores the dependency of these cells on the FLT3-ITD mutation for growth and its use as a predictive biomarker of venetoclax resistance. To determine the dependence of FLT3-ITD+ cells on other anti-apoptotic proteins, cell lines were treated with selective antagonists to BCL-X_L (A1331852) or MCL-1 (A1210477) in combination with venetoclax. Treatment with either antagonist in combination with venetoclax reduced the growth of the cell lines compared to venetoclax alone. However, co-inhibition of BCL-X_L or MCL-1 and FLT3-ITD did not reduce cellular growth compared to quizartinib alone, indicating that maximum anti-tumor responses may be achieved when all three anti-apoptotic proteins are targeted.
Summary/Conclusions: Quizartinib treatment in FLT3-ITD+ AML models decreased expression of the anti-apoptotic proteins BCL-X_L and MCL-1 and synergized with venetoclax *in vitro* and *in vivo* at clinically relevant doses for each compound. These data suggest that co-targeting FLT3-ITD with selective inhibitors and BCL-2 with venetoclax induces apoptosis to a greater extent than FLT3 inhibition alone. Importantly, our preclinical data supports further clinical investigation of this combination to treat FLT3-ITD+ AML.

P190

SPECIFIC TARGETING OF ACUTE MYELOID LEUKEMIA STEM CELLS BY INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN 7

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Background: Only 30-40% of acute myeloid leukemia (AML) patients survive five years after diagnosis. This extreme poor prognosis is mainly caused by treatment failure due to chemotherapy resistance. Leukemic stem cells (LSCs) are thought to be major determinants of AML recurrence due to their potential for self-renewal and chemotherapy resistance. LSCs co-reside with normal $CD34^+CD38^-$ hematopoietic stem cells (HSCs) in the AML bone marrow. Increasing the dose of chemotherapy might eliminate these chemotherapy resistant cells, however will inevitable result in the non-specific elimination of HSCs, delaying or even preventing the recovery of normal hematopoiesis after therapy. To significantly improve the outcome of AML patients, the discovery of alternative therapies that specifically eliminate LSCs while sparing HSC are urgently needed. To develop these specific anti-LSC therapies, identification of genes differentially expressed between LSCs and HSCs and between LSCs and the AML bulk is crucial.
Aims: To identify specific therapeutic strategies that have the potential to eliminate AML relapse-initiating cells.
Methods: We generated gene expression profiles of HSCs, LSCs and leukemic progenitors all derived from the same AML bone marrow and identified Insulin growth factor binding protein 7 (IGFBP7) as one of the top differentially expressed genes. As low IGFBP7 expression is a feature of LSCs, we hypothesized that

decreased expression of IGFBP7 might be associated with decreased chemotherapy sensitivity. To this end, we generated cell lines with IGFBP7 knockdown and subjected the cells to chemotherapy. Furthermore, to test whether increasing the IGFBP7 levels might be a strategy to deplete leukemic (stem) cells, we overexpressed IGFBP7 in or added recombinant human IGFBP7 (rhIGFBP7) to primary AML cells and measured clonogenic capacity, differentiation and cell survival *in vitro*. To study the effect of IGFBP7 on AML cell survival and engraftment potential *in vivo*, primary AML cells were transplanted into immune deficient mice and the mice were subsequently treated with rhIGFBP7. To study the effect of rhIGFBP7 on LSC survival, human AML cells derived from the first transplanted mice were re-transplanted into secondary recipients and engraftment and survival of the mice were monitored.

Results: Knockdown of IGFBP7 results in reduced sensitivity to chemotherapy and comparing matched diagnosis and relapsed AML samples showed that IGFBP7 expression is frequently downregulated at relapse, suggesting a survival advantage of IGFBP7^{low}AML cells during chemotherapy treatment. Importantly, enhancing cytoplasmic or extracellular IGFBP7, by overexpression or addition of rhIGFBP7, resulted in induction of differentiation and apoptosis, increased sensitivity to chemotherapy and inhibited AML blast and leukemic stem/progenitor cell survival *in vitro* and *in vivo*. IGFBP7 had no influence on the survival of normal hematopoietic (stem) cells. Moreover, treatment with rhIGFBP7 can add to chemotherapy treatment by elimination of chemotherapy resistant refractory AML (stem) cells.

Summary/Conclusions: Altogether, these data suggest that addition of IGFBP7 to the currently used chemotherapy regimens might be a promising strategy to specifically eradicate LSCs and decrease AML relapse rates.

Acute myeloid leukemia - Clinical 1

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ONGOING PHASE 2 CLINICAL TRIAL OF SL-401 IN PATIENTS WITH BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM: STAGE 1 AND STAGE 2 RESULTS

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Background: SL-401 is a targeted therapy directed to interleukin-3 receptor α (CD123), a target overexpressed on a variety of cancers including blastic plasmacytoid dendritic cell neoplasm (BPDCN), a highly aggressive malignancy with poor outcomes and unmet medical need.
Aims: This Phase 2 trial is a single-arm, open-label, study designed to generate efficacy and safety data to support potential registration in BPDCN
Methods: In this ongoing Phase 2 single-arm trial, patients with BPDCN (n=32) or relapsed/refractory (R/R) AML (n=48) received SL-401 as a daily IV infusion at 7, 9, 12, or 16 ug/kg/day for days 1-5 of a 21-day cycle in stage 1. In stages 2 and 3, patients received SL-401 at the dose determined in stage 1.
Results: 32 adult BPDCN patients received SL-401 in stage 1 (n=9) and stage 2 (n=23), including 19 first-line and 13 R/R patients. Stage 3 patients will be reported separately. Median age was 72 years (range: 30-85 years). In stage 1, 12 ug/kg was the highest tested dose for BPDCN; MTD was not reached in BPDCN. Median follow-up was 4.3 months (range: 0.5-22.9 months). ORR of 84% (27/32) was observed in all patients: 95% (18/19) in first-line and 69% (9/13) in R/R. 88% (14/16) of first-line patients treated at 12 ug/kg had a complete remission: CR (n=10), CR with incomplete hematologic recovery (CRi) (n=1) or clinical CR (CRc; residual skin disease) (n=3) based on investigator assessment. 56% (9/16) of these patients were progression free for 4 to 22.9 months (ongoing), including 3 patients on SL-401 in remission (4 to 18 months, ongoing) and 7 patients who were bridged to stem cell transplant (SCT; 3 auto-SCT and 4 allo-SCT). A R/R patient was also bridged to allo-SCT. Overall, most common ≥Grade 3 treatment-related AEs were transaminase elevation (22%) and thrombocytopenia (16%). Safety precautions, including daily monitoring of albumin and body weight during study drug infusions, have been implemented to minimize risk of severe capillary leak syndrome (CLS). Three patients had Grade 5 CLS: BPDCN (7 ug/kg); R/R AML (16 ug/kg); BPDCN (12 ug/kg) out of 118 patients who received SL-401 across all trials and regimens; 3/89 (3.4%) patients of which were enrolled in this clinical trial.
Summary/Conclusions: SL-401 continues to demonstrate single agent activity, including multiple CRs, in patients with BPDCN, with 25% (8/32) of patients bridged to SCT after a major response from SL-401. SL-401 side effect profile consists largely of transaminitis and thrombocytopenia. CLS can be fatal. Side effects have generally tended to decrease in frequency and severity with increasing cycles. Updated data, including detailed safety analysis across all ongoing SL-401 studies will be presented at the meeting.

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PROGNOSTIC IMPACT OF SOMATIC MUTATION CLEARANCE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Persistence of somatic mutations at the time of complete remission (CR) was associated with poor outcome in patients (pts) with AML.
Aims: To analyze differential pattern of mutation clearance based on the genes and affected pathway and to assess prognostic impact of mutation clearance in AML patients.
Methods: We studied 95 pts with AML who were treated with frontline induction and subsequently achieved CR. We sequenced pre-treatment and CR bone marrow samples by targeted capture sequencing of 295 genes (median 280x coverage). We defined 3 levels of mutation clearance (MC) based on variant allele frequency (VAF): 1) MC2.5, persistent mutation with VAF<2.5%, 2) MC1.0, persistent mutation with VAF<1%, and 3) complete mutation clearance (CMC).
Results: In the pre-treatment samples, we detected 597 mutations in 78 genes in 87 (92%) patients. In the matching CR samples, 62 (10%) and 82 (14%) mutations persisted at VAF≥2.5% and ≥1%, respectively, which corresponded to 43 (49%), 34 (39%), and 30 (34%) patients achieving MC2.5, MC1.0 and CMC, respectively. Table 1 shows the differential patterns of MC based on the mutations and pathways. Mutations associated with clonal hematopoiesis of

indeterminate potential (CHIP), DNA methylation, and splicing pathways had low rate of MC, whereas mutations in transcription factors or receptor tyrosine kinase (RTK) had high rate of MC. Pts who achieved MC1.0 (median 31.2 *vs* 12.5 months, P=0.04) or CMC (median 31.2 *vs* 12.5 months, P=0.049) had significantly better relapse-free survival (RFS).

Table 1.

Gene	MC2.5 (%)	MC1.0 (%)	CMC (%)	Pathway	MC2.5 (%)	MC1.0 (%)	CMC (%)
DNMT3A	21%	17%	14%	**CHIP associated**	33%	24%	22%
NPM1	100%	96%	96%	**DNA methylation**	39%	29%	26%
TET2	35%	35%	35%	**RTK pathway**	88%	87%	83%
FLT3	100%	100%	100%	**Transcription Factors**	94%	83%	77%
CEBPA	100%	89%	89%	**Chromatn-Cohesin**	67%	53%	53%
IDH2	38%	44%	38%	**Splicing**	33%	17%	17%
GATA2	100%	100%	91%				
NRAS	92%	92%	92%				
RUNX1	67%	44%	56%				
WT1	75%	75%	63%				
PTPN11	89%	89%	89%				
TP53	25%	0%	0%				
ASXL1	0%	0%	0%				
NF1	63%	50%	38%				
STAG2	57%	43%	43%				
BCOR	100%	50%	50%				
SRSF2	17%	17%	17%				
IDH1	100%	40%	40%				
SMC3	100%	100%	100%				
KRAS	100%	100%	75%				
SF3B1	100%	33%	33%				

Summary/Conclusions: Somatic mutations associated with CHIP, DNA methylation, and splicing pathways persisted frequently in CR samples suggesting preleukemic origin. Pts with deeper MC had significantly better RFS. Somatic mutation clearance may help risk prediction of AML.

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DO EDUCATION AND INCOME AFFECT TREATMENT AND OUTCOME IN ACUTE MYELOID LEUKEMIA IN A TAX-SUPPORTED HEALTH CARE SYSTEM? A DANISH NATIONAL POPULATION-BASED COHORT STUDY

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Background: No larger study has investigated the association between individual-level education or income level and clinical prognostic markers, treatment, and outcome in acute myeloid leukemia (AML). Understanding how socioeconomic status (SE) affects survival in AML patients may improve prognosis through targeted support among patients with different SE risk profiles.
Aims: We investigate effects of education as a knowledge-related SE factor and income as a measure of material resources in a tax-supported health care system linking individual-level SE information from Statistics Denmark to clinical data from the Danish National Leukemia Registry.
Methods: We conducted a nationwide population-based cohort study and included AML patients ≥25 years diagnosed in Denmark 2000-2014 (end of follow-up, Feb 2016). KM curves and Cox regression (Hazard ratios; HRs) was used to compare survival by education (low, medium, and high) and income level (tertiles). We repeated the survival analysis within educational groups by year of diagnosis (2000-2004, 2005-2009, 2010-2014), stratified by time period, and calculated crude survival (%) at 1, 3, and 5 years. We used logistic regression (odds ratios; ORs) to compare treatment intensity, chance of clinical trial inclusion, and complete remission (CR) between groups. Results were given crude and with different levels of adjustments for age and sex, SES factors, and clinical prognostic markers, overall and stratified by age (<60/≥60 years).
Results: Of 2992 patients, 1588 (53.1%) received remission induction chemotherapy. Forty-five percent (n=1336) completed a low-level education, 38% (n=1138) a medium education, and 17.3% (n=518) a higher education. Patients with higher education tended to be younger and to be male. In intensive therapy patients <60 years, survival was superior in high-education patients evident a year from diagnosis (1-year survival: high 65.2%, HR 1.0, medium 59.2%, adjusted HR 1.55 (CI=1-21-1.98), low 57.7%, 1.47 (CI=1.11-1.93)). Allogeneic transplantation rates in CR1 were significantly higher in high-education compared with low-education patients (16.3% *versus* 8.7%). Only survival in high-education patients improved over time; HR 0.78 (CI=0.61-0.99), medium 0.99 (CI=0.84-1.16), and low 1.03 (CI=0.84-1.27) increasing the survival gap between educational groups (Low: year 2000-2004 HR 1.28 (CI=0.88-1.85), 2004-2009 HR 1.55 (CI=1.01-2.44) 2010-2014 HR 2.09 (CI=1.27-3.44), high 1.0); Figure 1). In older patients, low education was associated with lower chance of intensive chemotherapy (30% *versus* 48%; adjusted OR 0.65 (CI=0.44-0.98)) compared to high-education, however neither CR rates in intensive therapy patients nor survival overall or in intensive therapy patients was affected. Low-income patients where less likely to be enrolled in clinical trials (low-income 22.8%, adjusted OR 0.55 (CI=0.39-0.79) medium 28.2%, adjusted OR 0.71 (CI=0.53-0.94)) compared to high-income (37.2%, HR 1.0), however, income was not associated with therapy intensity, chance of CR, or survival (intensive therapy-only; high income adjusted HR 1.0, medium 0.96 (CI=0.82-1.12), low 1.06 (CI=0.88-1.27)).

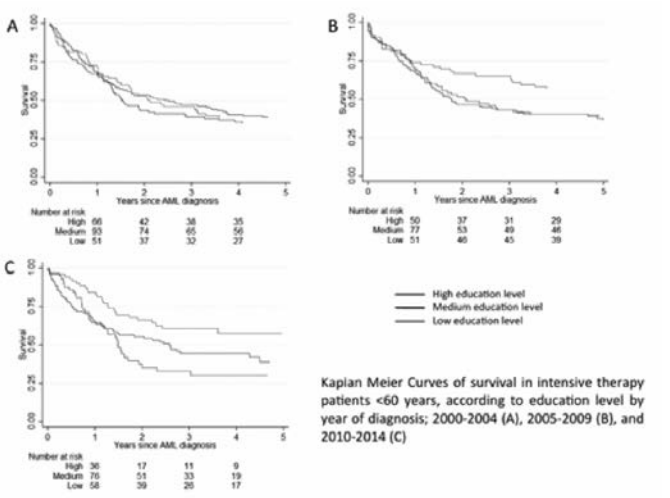

Figure 1.

Summary/Conclusions: In Denmark where health-care is free and uniform, high SE status does not affect treatment intensity in younger patients or response to therapy. However, educational level, but not income, influences alloHSCT rates and has a major impact on survival in younger AML patients. Since 2000, survival improvements have exclusively benefitted well-educated patients and additional attention during treatment and follow-up towards low-educated patients may increase transplantation rates and improve survival.

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IDENTIFICATION OF PATTERNS IN CO-OCCURRING MUTATIONS IN AML PATIENTS WITH GERMLINE AND SOMATIC RUNX1 MUTATIONS

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Background: RUNX1 plays a vital role in leukemogenesis through its interaction with core binding factor-β complex and other genes involved in hematopoiesis (1,2). Familial platelet disorder with predisposition to acute myeloid leukemia (FPD/AML) is linked to germline *RUNX1* mutations (3). This autosomal dominant disorder is characterized by thrombocytopenia and potential for transformation to AML. AML patients with somatic *RUNX1* mutations have a poor prognosis (6,7) independent of other risk factors. The role of co-occurring mutations in leukemogenesis in FPD/AML patients with germline *RUNX1* mutations and AML patients with de novo somatic *RUNXl* mutations is not fully understood.
Aims: In order to further characterize co-occurring mutations in patients with both germline and somatic *RUNX1* mutations, we analyzed a large cohort of AML tumor samples along with several paired normal tissue samples.
Methods: We sequenced a cohort of 482 diagnostic bone marrow or peripheral blood samples from AML patients by deep whole-exome sequencing. Samples were collected through the "Beat AML" project, an ongoing program at Oregon Health & Science University in collaboration with the Leukemia & Lymphoma Society. *RUNX1* mutations were classified using VarScan which defined somatic and germline mutations as follows: somatic if p <0.1 and germline if not called as somatic and normal variant allele frequency >0.1.
Results: Twenty AML samples had 21 germline *RUNX1* mutations with a total of 6 different germline variants; 31 other patient samples had 38 somatic *RUNX1* mutations with 31 unique somatic variants. One sample had 2 *RUNX1* germline mutations; 6 samples had >1 somatic RUNX1 mutations. The most common germline variant, missense mutation p.L56S, was found in 16 (76%) of the 21 HCG *RUNX1* mutations identified. Significantly, the germline variants occurred mutually exclusive of the somatic variants. Out of 20 patients with germline *RUNX1* mutations, 16 had co-occurring known pathogenic mutations in AML-related genes. Most significantly, 62% (10/16) and 51% (14/27) of patients with germline or somatic *RUNX1* mutations, respectively, had 7 co-occurring AML-related pathogenic mutations that were exclusive to their cohort (Table 1). Both germline and somatic *RUNX1* mutational cohorts had 12 overlapping co-occurring mutations. The most common mutations, for both groups, were in *FLT3* (14/43), *ASXL1* (8/43), and *IDH2* (7/43) (Table 1). Patient demographics and treatment-related outcomes were similar for both cohorts.

Table 1.

Frequency of germline or somatic *RUNX1* mutation variants and co-occurring mutations identified in 482 AML patients.

Germline versus Somatic *RUNX1* mutation variants				
Mutation Type	Germline Mutation Variants	Number of mutations seen in germline cohort	Somatic Mutation Variant	Number of mutations seen in somatic cohort
Missense	p.L56S	16	p.R162K	2
	Other[a]	3	Other[b]	12
In Frame Deletion	p.S167_R169del	1	None	0
Frame Shift Insertion	p.S322FfsX278	1	p.R250LfsX13	2
			Other[c]	6
Nonsense	None	0	p.E223X	2
			p.R166X	2
			p.R201X	3
			p.R320X	2
			Other[d]	3
Frame Shift Deletion	None	0	Other[e]	4

Co-occurring mutations identified in patients with either somatic (n = 27) or germline (n=16) *RUNX1* mutations						
Seen in germline cohort ONLY	No of mutations seen in germline cohort	Seen in somatic cohort ONLY	No of mutations seen in somatic cohort	Seen in both germline and somatic cohorts	No of mutations seen in germline cohort	No of mutations seen in somatic cohort
NPM1	3	CBL	2	FLT3	7	7
WT1	2	JAK2	2	DNMT3A	3	2
CHEK2	1	MLL	2	ASXL1	2	6
CCND3	1	APC	1	CEBPA	2	3
SUZ12	1	FBXW7	1	ZRSR2	2	1
GATA1	1	IDH1	4	BCOR	2	3
KIT	1	EZH2	2	SRSF2	2	4
				TET2	2	4
				IDH2	1	6
				U2AF1	1	3
				PHF6	1	1
				NRAS	3	3

a: Three different missense variants were identified in three patients with germline *RUNX1* mutations; b: Twelve different missense variants were identified in twelve patients with somatic *RUNX1* mutations; c: Six different in-frame deletion variants were identified in six patients with somatic *RUNX1* mutations; d: Three different nonsense variants were identified in three patients with somatic *RUNX1* mutations; e: Four different frameshift deletion variants were identified in four patients with somatic *RUNX1* mutations.

Summary/Conclusions: The incidence of *RUNX1* mutations seen in our 482-patient Beat AML cohort (4.3% germline, 6.4% somatic) is consistent with results from other studies (8). Our study suggests that germline and somatic *RUNX1* mutations in AML patients are mutually exclusive, as are several co-occurring pathogenic mutations that contribute to leukemogenesis. Our study adds to the already described mutually exclusive mutations in germline *RUNX1* by identifying *WT1*, *CHEK2*, *CCND3*, and others. Similarly, in samples with somatic *RUNX1* mutations, we found mutually exclusive mutations in *CBL*, *JAK2*, *MLL*, *EZH2* and others, in addition to the previously described *IDH1* (8).Further characterization of these results and analyses of additional samples using our whole-exome sequencing and our bioinformatics platform will help us better elucidate the molecular events underlying AML progression and help us establish novel prognostic/therapeutic markers aimed at early intervention in patients, or their family members, who carry *RUNX1* mutations.

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Abstract withdrawn.

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MULTIPLE LEUKEMIC STEM CELL MARKER EXPRESSION IS ASSOCIATED WITH POOR PROGNOSIS IN DE NOVO ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) is believed to originate from a small population of leukemic stem cells (LSCs). Current chemotherapy regimens target the majority of more mature leukemic blasts, but cannot efficiently eliminate LSCs, resulting in early treatment failure and relapse. Thus, the expression of LSC-specific markers could be used as a predictive factor of clinical outcomes in AML patients. Recently, the clinical impact of individual LSC markers has been documented in several reports, but the combined effect of different LSC markers remains unexamined.
Aims: This study aimed to estimate the prognostic impact of the expression of multiple LSC markers on the outcome of AML patients.
Methods: Ninety consecutive patients diagnosed with *de novo* AML at our institution and eligible for intensive chemotherapy were enrolled from September 2010 to March 2016. We excluded 10 patients with acute promyelocytic leukemia. This study was approved by the institutional review board of the Ethics Committee and complied with the Declaration of Helsinki. We analyzed the expression of three LSC markers, CD25, CD96, and CD123, in *de novo* AML patients. The expression of these markers on gated leukemic blasts was evaluated using 6-color flow cytometry. When over 20% of leukemic blasts were positive for any marker, the sample was defined as positive for that marker. We stratified *de novo* AML patients into two groups: $LSC^{High}$was defined as positivity for two or three LSC markers, and $LSC^{Low}$was defined as negativity for all markers or positivity for a single LSC marker. The primary endpoint was overall survival (OS). The secondary endpoint was progression-free survival (PFS). OS and PFS were estimated using the Kaplan-Meier method, and assessed using the log-rank test. Multivariate analysis using Cox proportional hazard ratio was performed for OS and PFS.
Results: The median follow-up for patients still alive at the end of the study was 38.9 months (range: 1.5-64.8 months). The median patient age was 60 years (range: 17-78 years). There was no statistical significance between LSC^{High} patients (n=30) and LSC^{Low} patients (n=50) in sex, age, laboratory data, NPM1 mutation, or European Leukemia Net karyotype risk group. FLT3 mutation was associated with the LSC^{High} group (p=0.003). Three-year OS and PFS were significantly better in the LSC^{Low}group than in the LSC^{High}-group (Figure 1) (OS: 65.0% *vs* 18.2%, p <0.001; PFS: 49.3% *vs* 19.4%, p <0.001). In multivariate analysis controlled for age and karyotype (Table1), being in the LSC^{High}group was an independent prognostic factor for OS (hazard ratio: 3.17; 95% CI: 1.64-6.15; p <0.001) and PFS (hazard ratio: 2.25; 95% CI: 1.24-4.08; p=0.007). Being in the LSC^{High}group had incremental value for OS compared with the karyotype risk (Harrell's C index: 0.80 *vs* 0.70; p = 0.028). Moreover, this classification based on LSC marker expression allowed subgroups with unfavorable prognosis to be identified among patients in the intermediate karyotype risk group (3y-OS 54.6% *vs* 14.5%, p=0.013), as well as those in the favorable karyotype risk group (3y-OS 94.1% *vs* 50.0%, p=0.021).

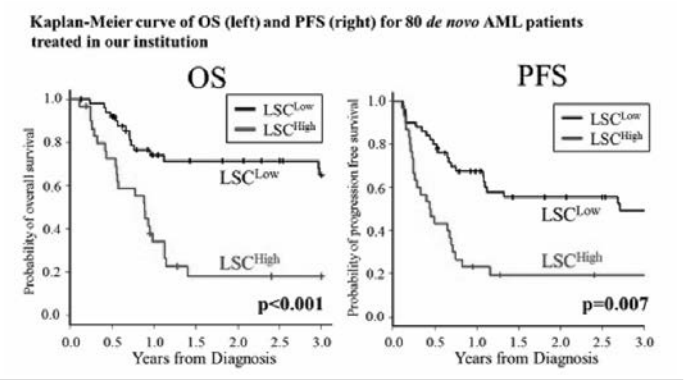

Figure 1.

Table 1. Univariate and multivariate analysis for OS.

	Univariate (OS)		Multivariate (OS)	
Variable	HR (95% CI)	P-value	HR (95% CI)	P-value
Sex	1.04 (0.55-1.97)	0.90		
Age(> 65 years)	1.79 (0.93-3.46)	0.083		
WBC (> 50×10³/μL)	1.67 (0.87-3.18)	0.12		
ELN genetic risk group	3.48 (2.02-6.00)	**<0.001**	3.55 (1.96-6.44)	**< 0.001**
FLT3-ITD^{mut}	2.10 (1.05-4.18)	**0.035**	0.92 (0.42-1.99)	0.83
LSC^{High} group	3.80 (1.96-7.36)	**< 0.001**	3.51 (1.68-7.39)	**<0.001**

Summary/Conclusions: We demonstrated that multiple LSC marker expression predicts poor clinical outcomes in newly diagnosed *de novo* AML patients, and may facilitate better stratification even among patients with intermediate-risk and favorable-risk karyotypes.

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NEXT GENERATION SEQUENCING TARGETED PANEL FOR MINIMAL RESIDUAL DISEASE MONITORING IN ACUTE MYELOID LEUKEMIA

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Background: Many personalized therapies for acute myeloid leukemia (AML) have been developed targeting specific biomarkers. Unfortunately, the efficacies of these therapies are inconsistent while the need to determine successful therapies prior to patient relapse is critical. Minimal residual disease (MRD) monitoring can help determine effective treatments and predict potential relapse. While there are now several MRD tests available on the market, most target single or small numbers of biomarkers, which can limit detection of residual AML heterogeneity. Thus, full characterization of a sample may require testing with multiple MRD assays, which can be impractical in a clinical setting. We have developed a target capture-based assay (MyMRD™), which allows characterization of the entire therapeutic AML biomarker repertoire and can inform

the molecular remission status of a patient's malignancy. This targeted panel can identify the mutations in driver clones that cause relapse in ~90% of all AML patients, as well as common drivers in myeloid prolific neoplasms (MPN) and myelodysplasic syndromes (MDS).

Aims: To establish a sensitive and reliable targeted NGS assay to comprehensively detect and monitor the majority of known driver mutations in AML and other myeloid malignancies.

Methods: Whole genome libraries, made from DNA extracted from cell lines and clinical samples, were hybridized with MyMRD probes targeting mutation hotspots in 23 genes associated with AML. In addition to single nucleotide variants (SNVs) and indels in 21 of these genes, 5 structural variant (SV) breakpoints within 3 genes were also targeted. Enriched libraries were sequenced with the MiSeq® platform and analyzed using proprietary Invivoscribe (IVS) MyInformatics™ software. To validate mutations detected by the MyMRD assay, samples were additionally tested with IVS developed capillary electrophoresis (CE) assays and NGS-based assays targeting common mutations in *FLT3* and *NPM1*.

Results: The linearity and limit of detection (LOD) of the MyMRD assay were assessed using data generated from contrived cell line DNA containing known AML driver mutations with a range of variant allele frequencies (VAFs). The assay shows strong linearity (R^2=0.96 – 0.99) in the entire range of tested VAFs (0.1– 20%). Overall, we established a LOD of 0.5% for >95% of the targeted sites in the assay with lower LODs for specific mutations of interest (*e.g.* 0.1% for a 30bp *FLT3* ITD and 0.2% for *FLT3* p.D835Y). In addition, using clinical samples the MyMRD assay shows excellent concordance with the standard *FLT3* CE assays for variants with VAFs above the CE detection threshold (5%). Samples below the CE detection threshold were additionally evaluated with IVS *FLT3* ITD MRD and *NPM1* MRD amplicon assays which showed 100% concordance with the MyMRD panel assay for variants with VAFs above the MyMRD LOD.

Summary/Conclusions: The IVS developed MyMRD targeted panel is a sensitive and reliable assay to monitor residual AML driver mutations. The assay is shown to have excellent linearity and a LOD of 0.5% (tenfold lower than the standard CE assay LOD) at >95% of the targeted sites. Additionally, specific mutations of interest, such as those used for residual disease monitoring (*e.g. FLT3* ITD), demonstrate LODs as low as 0.1%. The MyMRD assay provides an accurate method for detecting mutations in multiple targets in patients and can be used to effectively stratify patients for therapy and clinical trials.

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IS IT POSSIBLE TO RELIABLY DETECT CLINICALLY-RELEVANT BIALLELIC CEBPA GENE MUTATIONS USING NGS PANELS?

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Background: CEBPA gene encodes a leucine zipper transcription factor that is important for normal myeloid cell differentiation. Biallelic CEBPA (biCEBPA) mutations are associated with favourable prognosis in patients with acute myeloid leukaemia (AML); therefore, accurate molecular testing of this gene is crucial in the clinical setting. Molecular pathology labs routinely analyse CEBPA through fluorescence-based multiplex-PCR fragment analysis or, more frequently, Sanger sequencing. Lately, it is increasingly common to use next-generation sequencing (NGS) technology in the pathology labs, and CEBPA gene is indeed included in the majority of NGS panels commercially available for testing of patients with neoplasias of the myeloid lineage.

Aims: We set ourselves to compare the performance of two different NGS targeted panels and of direct Sanger sequencing for detection of CEBPA molecular aberrations, with a particular focus on biCEBPA mutations.

Methods: DNA specimens from 173 myeloid cases were subjected to Sanger (n=92) or to NGS (n=81) sequencing, including the TruSight Myeloid Sequencing Panel (Illumina) (n=59), and the Ion AmpliSeq AML Community Panel (Thermo Fisher Scientific) (n=22). Cases showing two variants were further analysed through cloning of the whole length of CEBPA and subsequent Sanger sequencing of at least 10 colonies from each case.

Results: We called 10 CEBPA variants affecting 7 samples through NGS. Both NGS panels are designed to cover CEBPA through overlapping amplicons (6 or 9). However, we found that an average of 3.5 amplicons were covered <500x, and more worryingly, we realised that at least one of those amplicons was shallowly (<100x) covered in 97% of the cases. Indeed both panels showed significantly lower average coverage levels of this gene compared to the panel as a whole (Figure 1). This might not be surprising, since CEBPA is encoded within a CpG-rich region, and therefore its amplification needs tailored PCR conditions, hard to address in the multiplexed PCR step included in their library prep protocols. Therefore, both NGS approaches are prone to miss variants. In contrast, Sanger sequencing protocol (which includes optimized PCR conditions for correct amplification of the CEBPA gene) managed to cover the whole length of the gene. We were able to detect 26 variants affecting 20 AML cases through Sanger sequencing. Cases showing two variants were manually curated (through Chromas or IGV tools) to confirm if they affected different alleles. However, in 6 cases both mutations laid on different amplicons, which made not possible to univocally conclude if they were biallelic. These inconclusive cases were subjected to DMSO-Pfu-PCR in order to amplify the whole length of CEBPA coding region, followed by cloning. Colony sequencing showed independent clones harbouring different variants (*i.e. bona fide* biCEBPA mutations) in the majority of the cases, but crucially, not in all of them. This result highlights the need of implementing techniques able to accurately assess CEBPA biallelism, others than plain calling of more than one variant.

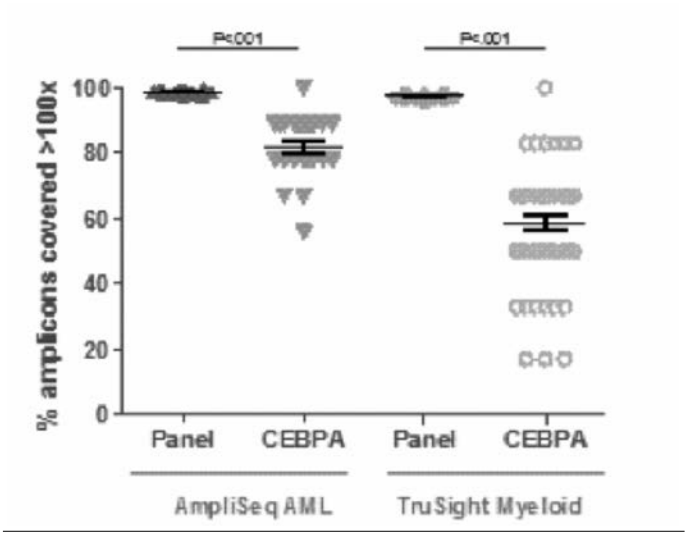

Figure 1.

Summary/Conclusions: Since AML patients with biCEBPA mutations have relatively favourable overall survival, it is important in the clinical setting to accurately assess CEBPA molecular status. In our study, we have tested the ability of three different assays to detect CEBPA mutations in 173 samples. Sanger sequencing was the only method actually covering the entire coding region of CEBPA. Both NGS amplicon-based panels failed to fully cover the coding region of the gene, and therefore have likely missed mutations. Crucially, even when any of the three methods detected more than one variant, cloning studies confirmed biCEBPA mutations only in a fraction of the cases. In summary, none of the amplicon-based tested methods can reliably determine if multiple mutations affect two different alleles; therefore biCEBPA mutations would still need additional confirmation. We are currently exploring the ability of capture-based NGS approaches coupled to appropriately tailored bioinformatic analysis of sequencing data to detect biCEBPA mutations.

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EXPERIENCE WITH MINIMAL RESIDUAL DISEASE MONITORING IN AML WITH RUNX1-RUNX1T1: A STUDY ON 186 PATIENTS

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Background: The cure rate in AML is dependent on patient's age and performance status, cytogenetics, early blast clearance and sustainable first complete remission. Investigation of minimal residual disease (MRD) is possible by multiparameter-flow cytometry (MFC) or molecular techniques. Recent findings have further depicted a broad spectrum of molecular markers in AML in 99% of pts (TCGA, NEJM, 2013). This broadens the set of targets for MRD and will hopefully help to better individualize treatment strategies. In this analysis we focused on MRD monitoring in *RUNX1-RUNX1T1* positive AML in an unselected cohort.

Aims: To understand the clinical use of PCR based MRD monitoring in AML with *RUNX1-RUNX1T1* fusion.

Methods: Between 2005 und 2017 we investigated a total of 186 intensively treated AML patients with *RUNX1-RUNX1T1* fusion, 130 of them diagnosed at our laboratory and 56 with follow up samples available. 1448 individual samples were analyzed during the course of disease. We applied quantitative real-time PCR to detect *RUNX1-RUNX1T1*/*ABL* ratios. Complete molecular remission (CMR) was defined as one valid qPCR ratio of 0, while low MRD was assigned to patients with a >0 but <0.01 ratio and high MRD was assigned to all patients with a ratio above 0.01. As a comparator log fold change to baseline was independently assessed. Median age was 51 years

(18-83 years). All patients were treated with standard induction and consolidation protocols.

Results: Median time between two investigations was 2.8 months (range for all 0.1-115 months). A complete molecular remission was reached in 90/130 pts (69%) after a median of 5 months. 19/130 (14.6%) pts reached low level MRD and 20/130 (15.4%) high level MRD. Median event free survival (EFS) of patients with CMR was not reached (EFS at 2 years 82%). 16 (18%) of those patients relapsed in the course of follow up with a median time to relapse of 12.7 months (range 4.1 to 38.3 months). Median EFS for MRD low and MRD high patients was 18.4 months and 10.8 months respectively (all 3 groups, p<0.0001). For patients with CMR, rising MRD levels accurately predicted relapse with a median latency of 5.5 months from loss of CMR to relapse. We next used the widely accepted log fold change from baseline to define high and low risk patients in our cohort. 123/130 (95%) patients reached a >3 log fold reduction in *RUNX1-RUNX1T1/ABL* ratio within the first 200 days following first diagnosis. Median EFS for those patients was not reached (EFS at 2 years 66%). The 7/130 (5%) patients with a <3 log fold reduction had a median EFS of 14.7 months (2 groups, p=0.017). A total of 59/185 patients received allogeneic SCT. Among the 130 patients diagnosed at our laboratory 34 (26%) received allogeneic SCT, 12 (9%) were transplanted in first CR and 17 (13%) were transplanted for relapse. Following allogeneic SCT 11/17 patients (65%) reached a second CR with CMR.

Summary/Conclusions: Our data shows that MRD testing is routinely performed in *RUNX1-RUNX1T1* AML outside of clinical studies. Defining MRD levels by *RUNX1-RUNX1T1*/*ABL* ratios resulted in a better classifier for high and low risk patients than log fold change. However, despite CMR 16/90 (18%) patients relapsed with a maximum time from first achievement of CMR of 38.3 months. We conclude that 1) MRD monitoring could serve to guide BMT decisions in *RUNX1-RUNX1T1* positive AML, 2) allogeneic BMT can rescue the majority of relapsed patients and 3) molecular monitoring can reliably identify patients with high risk for relapse.

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NUMBER OF TP53 ABNORMALITIES AND THEIR CLINICAL RELEVANCE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA AND MYELODYSPLASTIC SYNDROMES

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Background: Mutations in *TP53* can be detected in up to 16-19% patients with acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS). *TP53* mutations confer adverse prognosis irrespective of currently available therapies. The clinical impact of the type and number of *TP53* abnormalities is unclear.
Aims: To evaluate the prognostic impact of the number of *TP53* abnormalities in AML and MDS.
Methods: We evaluated 1401 patients with previously untreated AML or MDS treated at The University of Texas MD Anderson Cancer Center from 2012 to 2016. Sequencing data was obtained by use of a 28 or 53-gene targeted PCR-based next generation sequencing platform. Response was defined following 2003 IWG criteria for patients with AML and 2006 revised IWG criteria for patients with MDS. Generalized linear models were used to study the association of overall response (OR), complete response (CR) and risk factors. Kaplan-Meier produce limit method was used to estimate the median overall survival (OS).
Results: A total of 593 (42%) patients had MDS and 808 (56%) had AML. In a total of 984 (70%) patients, data on therapy with sufficient follow up and response evaluation was available, with 494 (35%) patients receiving therapy with hypomethylating agents (HMAs) and 373 (27%) with chemotherapy regimens. A total of 384 mutations in *TP53*, involving 208 unique mutations, were detected among 300 (21%) patients with *R273H*, *R248W*, *Y220C* and *R175H* being the most prevalent. Overall frequency of *TP53* mutations was higher among patients with MDS (25%, n=146) compared to AML (19%, n=154) (p=0.012) with 251 (84%) of detected mutations happening in patients with complex karyotype (p<0.001). Among patients with *TP53*-mutant disease, 221 (74%) had 1 detectable mutation, 76 (25%) had 2 and 3 (1%) had 3. Additionally, 188 (13%) patients had *TP53* deletions evidenced by presence of monosomy 17 or del(17p). In 167 (89%) of these patients, chr17 abnormalities were detected in the context of a complex karyotype and in 127 (42%) a co-occurring *TP53* mutation was detected. Correlation between *TP53* mutations and deletions (r=0.443, p<0.001) was observed with 172 (12%) patients having 1 *TP53* abnormality, 169 (12%) having 2 and 20 (1%) having 3 abnormalities. Patients with multiple detectable *TP53* mutations were less likely to have co-occurring chr17 abnormalities (79% *vs* 22%, OR 0.28, CI 0.15-0.50, p=0.03). Median follow up was 8.6 months (range 0-167 months). Presence of a *TP53* mutation adversely impacted OS (MDS: 12.4 *vs* 111.7 months, HR=5.98, CI 4.28-8.35, p<0.001; AML: 5.3 *vs* 16.9 months, HR=2.81, CI 2.26-3.50, p<0.001). Increasing number of *TP53* abnormalities negatively impacted OS of patients with AML (Figure 1A) but not that of patients with MDS (Figure 1B). No difference in survival was observed between patients with two *TP53* mutations and those with *TP53* mutation+deletion (p=0.730). Presence and number of *TP53* mutations did not predict for response (OR: 60 *vs* 63%, p=0.498; CR: 34 *vs* 36%, p=0.695) to HMAs, but was associated with significantly lower likelihood of response to intensive chemotherapy (OR: 41 *vs* 86%, p<0.001; CR: 33 *vs* 75%, p<0.001).

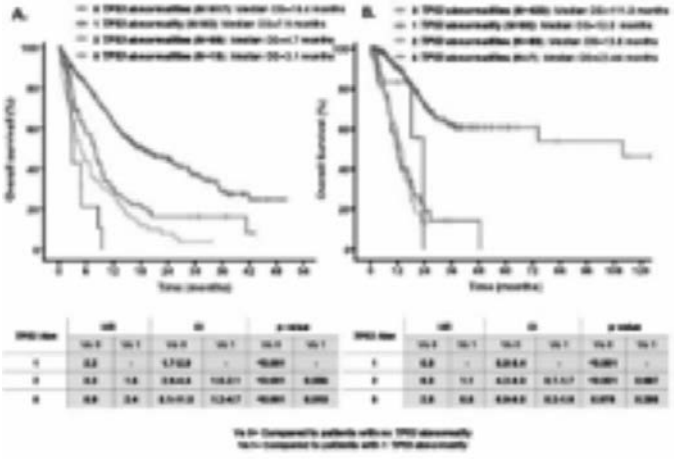

Figure 1.

Summary/Conclusions: Presence of multiple *TP53* abnormalities can be observed in up to 13% patients with AML and MDS. Second *TP53* abnormalities more commonly involve *TP53* deletions with additional *TP53* mutations being less common and generally mutually exclusive with *TP53* deletions. The number of *TP53* abnormalities impacts the survival of patients with AML but not that of patients with MDS. Presence and number of *TP53* mutations do not seem to impact response to HMAs but are associated with lower responses to chemotherapy.

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VADASTUXIMAB TALIRINE PLUS HYPOMETHYLATING AGENTS: A WELL-TOLERATED REGIMEN WITH HIGH REMISSION RATE IN FRONTLINE OLDER PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Treatment of AML among the elderly is challenging. HMAs are commonly used, but yield suboptimal response rates and modest survival. Deep remissions are difficult to achieve; in a study of MRD response by flow cytometry in patients treated with single-agent HMA therapy at MD Anderson Cancer Center, only 13/58 (22%) responding patients achieved minimal residual disease (MRD) negativity (F Ravandi, MD, unpublished data, Jan2017). Vadastuximab talirine (SGN-CD33A; 33A) is a CD33-directed antibody conjugated to 2 molecules of a pyrrolobenzodiazepine (PBD) dimer. Upon binding, 33A is internalized and transported to the lysosomes where PBD dimer is released via proteolytic cleavage of the linker, crosslinking DNA, and leading to cell death.
Aims: A cohort in a phase 1 study (NCT01902329) was designed to evaluate the safety, tolerability, PK, and antileukemic activity of 33A in combination with an HMA.
Methods: Eligible patients (ECOG status 0-1) had previously untreated CD33-positive AML. One dose of 33A (10 mcg/kg) was administered outpatient IV every 4 weeks on the last day of HMA (azacitidine or decitabine [5-day regimen], standard dosing). CRi required either platelet count of ≥100,000/μL or neutrophils of ≥1,000/μL (Cheson 2003). MRD was measured by multiparameter flow cytometry.
Results: Fifty-three patients (median age 75 years [range, 60-87]) were treated with 33A+HMA. Patients had adverse (38%) or intermediate (62%) cytogenetics (per MRC); patients were either unfit for (40; 75%) or declined (13; 25%) intensive therapy. The median treatment duration is currently 19.3 weeks (range, 2-86) with 8 patients still on treatment; no DLTs were reported. Adverse events (AEs) ≥Grade 3 reported in ≥15% of patients were thrombocytopenia (55%), febrile neutropenia (49%), anemia (46%), neutropenia (42%), pneumonia (19%), and leukopenia (17%); no ≥Grade 4 bleeding events were observed. Treatment-emergent (TE) liver lab elevations (≥Grade 3) were rare: ALT (8%), AST (2%), and total bilirubin (2%). Other non-heme TEAEs reported in >25% of patients regardless of relationship to study treatment were fatigue (60%), nausea (49%), constipation (43%), peripheral edema (42%), decreased appetite (40%), dyspnea (34%), pyrexia (32%), diarrhea, vomiting (28% each), and dizziness (26%). Thirty- and 60-day mortality rates were 2% and 8%, respectively, with no treatment-related deaths reported. A total of 39% (103/263) of doses were delayed due to AEs mostly from myelosuppression (neutropenia 18%, thrombocytopenia 7%, and febrile neutropenia 3%). High remission rates (37/49 [76%] CR+CRi) were maintained across adverse disease subsets including adverse cytogenetics (16/18, 89%), *TP53*-mutated (6/7, 86%), secondary AML (18/22, 82%), and age ≥75 years (18/26, 69%). Of all responding patients, 19/37 (51%) achieved MRD negativity. Two patients went on to subsequent allo-HSCT, and no SOS/VOD was observed. The median relapse-free survival was 9.1 months (range, 0.1-19.4+) and OS continues to evolve with 15 patients (28%) alive (11.3 month median follow-up) (Figure 1).
Summary/Conclusions: 33A+HMA is well tolerated with a safety profile consistent with on-target myelosuppression. The CR+CRi rate of 76% and low early mortality in older AML patients with poor risk factors is particularly encouraging, and activity appears markedly improved compared to the historical experience of HMA monotherapy. The MRD clearance rate among responding patients who received 33A+HMA is higher than the rate observed with single

agent HMAs. Survival data are evolving and compare favorably to historical controls. CASCADE, a phase 3 trial investigating 33A+HMA v. HMA alone in older AML patients, is enrolling (NCT02785900).

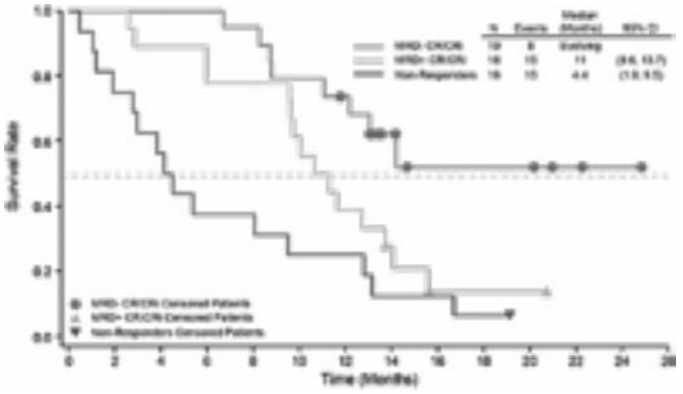

Figure 1.

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ACUTE MYELOID LEUKEMIA WITH INTERMEDIATE-RISK CYTOGENETICS AND A FAVORABLE GENOTYPE: PROGNOSTIC FACTORS AND RESULTS IN PATIENTS TREATED ACCORDING THE SPANISH CETLAM PROTOCOLS

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Background: Acute myeloid leukemia (AML) with intermediate-risk (IR) cytogenetics includes a substantial proportion of patients with favorable molecular profile (FMP); in which AML cells harbor the NPM1 mutation or CEBPA biallelic mutation without internal tandem duplication of the FLT3 gene (FLT3-ITD). The role of allogeneic hematopoietic transplantation (allo-HCT) in first complete remission (CR) in these patients remains controversial.

Aims: To analyze the results and prognostic factors of IR-FMP AML patients in a large series of patients treated by the Spanish CETLAM group.

Methods: Patients with primary AML diagnosed at 19 institutions from the Spanish CETLAM group and treated between 2003 and 2017. Induction chemotherapy included idarubicin and cytarabine (standard or intermediate-dose) in all cases, consolidation with intermediate or high-dose cytarabine (HDAC) and, depending on the protocol, additional HDAC, autologous or allogeneic hematopoietic transplantation.

Results: Two-hundred twenty-one patients were analyzed. Median age of the series was 54 years (range 18 to 72). 152 patients had an age up to 60 years and 69 (31%) were older. Median WBC count was 19x10e9/l (range 0.55-282). One-hundred eighty-two patients had a normal karyotype and it was abnormal in 34 (5 patients no metaphases). One hundred ninety-one patients had NPM1 mutated and FLT3-ITD wild type (NPM1+/FLT3-ITD-) and 30 CEBPα biallelic mut /FLT3-ITD wild type (CEBPα+/FLT3-ITD-). There were no significant differences in the main clinical or biological parameters in these two groups. The CR rate in the overall group was very high (92%) without significant differences between the two molecular groups. Chemo-resistance was observed in only 2 patients of the NPM1+/FLT3-ITD- group (1%). Death during induction was observed in 16 patients (7%), all of them with NPM1+/FLT3-ITD-. Induction results according to age were similar in both groups. Event-free survival and overall survival are reported at 8 years and were 52±8% and 70±4%, respectively. In univariate comparisons, better EFS and OS was observed in CEBPα+/FLT3-ITD- patients compared to those with NPM1+/FLT3-ITD- (p=0.03 and p= 0.02, respectively) . When analyzing post-remission treatment, patients treated with HDAC only had an excellent prognosis, even better than those receiving an autologous or allogeneic transplantation. One patient died in CR in the HDAC group, another in the autologous transplant group and 7 in the allo-HCT group (p<0001). In multivariate analysis of pretransplant characteristics, age up to 60 years and CEBPα+/FLT3-ITD- associated to improved EFS (RR=0.42) and OS (RR=0.29). Interestingly, in a subgroup of 123 patients with data on MRD after consolidation chemotherapy (flow citometry, cut-off: 0.12%), positivity was associated with worse EFS (0.02). Despite age was a prognostic factor, patients older than 60 years with IR-FMP AML had remarkable EFS of 36±3% and OS 54±10% at 8 years (Figure 1).

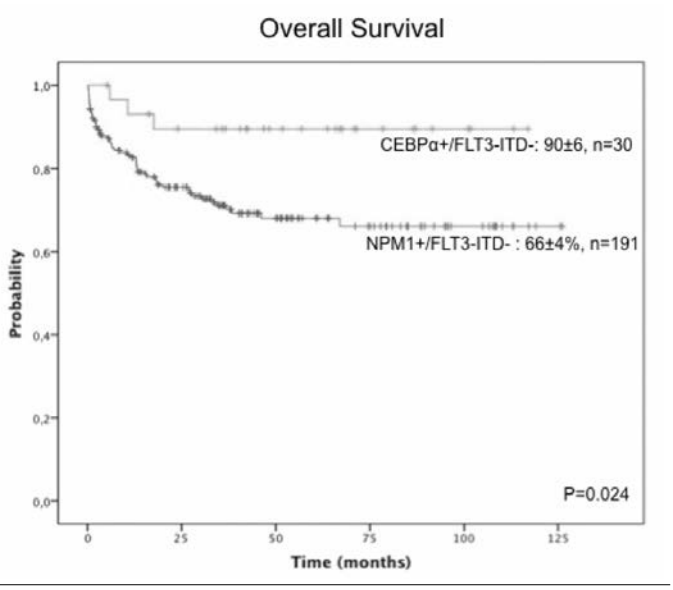

Figure 1.

Summary/Conclusions: Patients with primary AML, IR cytogenetics and FMP have a good outcome. Best results are achieved in patients with CEBPα+/FLT3-ITD-, particularly if age is up to 60 years. In this subset, OS at 8 years is 96±7%, comparable to current results achieved in acute promyelocytic leukemia. Patients above 60 years treated intensively may achieve a long term survival of more than 50%. Chemotherapy without subsequent transplantation is a valid option. MRD monitoring after treatment has to be taken into account since in the subset of patients analyzed this vas an independent prognostic factor for EFS.

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GMI-1271, A POTENT E-SELECTIN ANTAGONIST, COMBINED WITH INDUCTION CHEMOTHERAPY IN ELDERLY PATIENTS WITH UNTREATED AML: A NOVEL, WELL-TOLERATED REGIMEN WITH A HIGH REMISSION RATE

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Background: The outcomes for elderly patients (pts) with acute myeloid leukemia (AML) remain poor due to limited tolerance of intensive cytotoxic chemotherapy and low response rate, therefore newer and less toxic therapies are urgently needed. The binding of E-selectin (E-sel), an adhesion molecule expressed in the vasculature of the bone marrow, to the leukemic cell surface activates survival pathways and promotes chemotherapy resistance. GMI-1271, a novel E-sel antagonist, disrupts these survival pathways and enhances chemotherapy response (Becker ASH 2013; Winkler ASH 2014). Protection from common toxicities (neutropenia and mucositis) has also been observed in preclinical models, affording survival benefit (Winkler ASH 2013). Additionally, preclinical toxicology studies have indicated a benign safety profile. We report interim Phase 2 data for GMI-1271 plus anthracycline-based induction chemotherapy in elderly untreated pts with AML.

Aims: A Phase 2 open label trial of patients ≥60 yrs with untreated AML assessed safety, tolerability, pharmacokinetics (PK), pharmacodynamics (PD), and antileukemic activity of GMI-1271.

Methods: Eligible pts had ECOG 0-2, WBC <40K/uL, no active CNS disease, and adequate renal and hepatic function. Prior treatment of MDS was allowed. GMI-1271 (10 mg/kg) was given 24 hrs prior, then every 12 hrs during and for 48 hrs post induction with infusional cytarabine and idarubicin (7+3). Two cycles of induction were allowed and responders could receive consolidation with GMI-1271 plus intermediate dose cytarabine. Dose-limiting toxicity (DLT), defined as myelosuppression in the absence of disease or related Grade 3 (Gr) non-hematologic toxicity beyond day 42, was assessed in the first 3 pts. Baseline E-selectin ligand expression on leukemic blasts in the bone marrow (CD45/SSC by flow) is reported.

Results: 24 pts have been enrolled to date and 17 are evaluable for response. The median age was 68 years (range, 60-79) with 58% male pts, and 25% with high-risk cytogenetics (by SWOG). 50% (12/24) were pts with secondary AML (sAML), half of whom had prior hypomethylating therapy (50%; 6/12), . This study had a rolling safety run-in and the first 3 pts had no DLT, allowing enrollment to proceed. Common Gr 3/4 AEs included febrile neutropenia (47%), pneumonia (20%), pulmonary edema (13%) and non-fatal respiratory failure (13%). 2 pts died of sepsis within 60 days. The remission rate (CR/CRi) was 12/17 (71%). CR/CRi rate was 75% for pts with *de novo* disease and 67% for pts with sAML. The PK profile in this elderly population was consistent with that of younger adults (median age <60 years) with relapsed or refractory AML in Phase 1 (DeAngelo, EHA 2016); no accumulation or evidence of drug-drug interactions were apparent. The median E-sel ligand expression at baseline was 29% (range, 2-67%) of blasts in the bone marrow.

Summary/Conclusions: The addition of a novel E-selectin antagonist, GMI-1271, to anthracycline-based induction chemotherapy in untreated elderly pts with AML, including patients with secondary AML, demonstrates a high remission rate with acceptable side effect profile resulting in low induction mortality. This study compares favorably to previous studies (Lancet, ASCO 2016). E-selectin ligand was expressed on leukemic blasts in the majority of pts, therefore supporting its relevance as a target. A randomized trial is being planned.

P204

A PHASE 2 STUDY OF GLASDEGIB (PF-04449913) IN COMBINATION WITH CYTARABINE AND DAUNORUBICIN IN UNTREATED PATIENTS WITH ACUTE MYELOID LEUKEMIA OR HIGH-RISK MYELODYSPLASTIC SYNDROME

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Background: Glasdegib, a selective, once-daily (QD), oral Smoothened (SMO) inhibitor, demonstrated significant improvement in overall survival (OS) when used in combination with low-dose cytarabine (LDAC) *vs* LDAC alone in a randomized (2:1) open-label trial in 132 patients (pts) not suitable for induction chemotherapy (ICT). Preclinical studies showed that glasdegib limits leukemia stem cell proliferation and provided evidence of glasdegib synergy with chemotherapy.

Aims: Primary objective of this open-label, single-arm Ph 2 study (NCT01546038) was to determine complete remission (CR) rate with glasdegib in combination with cytarabine and daunorubicin in untreated AML or high-risk MDS pts suitable for ICT. OS was the key secondary endpoint.

Methods: Pts suitable for ICT (ECOG PS 0-1, creatinine ≤1.3 mg/dL, no severe cardiac disease) gave informed consent and received glasdegib 100 mg QD from day -3 in combination with cytarabine 100 mg/m^2 CI for 7 days and daunorubicin 60 mg/m^2 IV for 3 days, followed by 2-4 consolidation cycles (cytarabine 1 g/m^2 Q12 hrs on days 1, 3, 5). Maintenance (up to 6 months) included glasdegib 100 mg QD. Pts were assessed for efficacy, safety and tolerability.

Results: All Pts: As of 1 Dec 2016, 71 pts (66 AML, 5 MDS) were enrolled and 69 pts received glasdegib and ICT (2 pts not treated due to ineligibility). Among AML pts (47 de-novo; 19 secondary), 20% had favorable, 32% intermediate (int)-I, 21% int-II and 26% adverse cytogenetic abnormalities (1 pt not assessed). Among MDS pts (5 de-novo), 20% had good, 40% int and 40% poor risk cytogenetic abnormalities. Median age was 64 (27-75) years. Median treatment duration was 48 (10-502) days. The most common NCI-CTC v4.0 grade 3-4, non-hematologic all-cause adverse events (AEs) included: hypokalemia (13%), hyponatremia (11.6%) and hypertension (10.1%). Grade 5 AEs within 28 days from last dose (5 pts, 7.2%) included pneumonia, sepsis, septic shock (1 pt each) and disease progression (2 pts). The observed steady-state plasma exposures for glasdegib were as expected at the 100 mg dose level. Based on investigator's assessment, CR was 41% (80% CI 33.2-47.9) and CR/CRi was 49%. CR for good/int. risk pts (n=49) was 49% and for poor risk pts (n=19) 21%. Forty-one (59%) pts died (33 [48%] due to disease progression) with median follow up of 30.1 months; 24 (35%) remain in follow up. The median OS (mOS) was 14.9 months (80% CI 13.4-19.3); 16.3 months for AML pts and 13.0 months for MDS pts. Twenty-three (33%) pts received a transplant and mOS censored for transplant was 17.7 months. AML Pts >60 yrs: The mOS for 44 AML pts age >60 yrs is presented in the Table 1, in the context of historical control.

Table 1. mOS in Pts >60 Yrs Stratified by European Leukemia Net (ELN) Risk Criteria

Risk Group	ICT (Historical Röllig et al, 2011) months	ICT + Glasdegib (n=44*) months	Increase in mOS (%) (29 events)
Favorable	14.6	Not reached (n=9)	Not estimable
Int-1	9.5	15.7 (n=12)	65.3
Int-2	9.2	13.4 (n=12)	45.7
Adverse	4.8	8.5 (n=10)	77.1

*1 pt was not classifiable by ELN risk.

Summary/Conclusions: Although the CR rates do not appear to be higher than those reported historically for AML pts receiving ICT, the mOS for AML pts >60 yrs stratified by subgroup compares favorably by adding glasdegib. It is possible that this is a result of the effect of glasdegib on the leukemia stem cells. The combination of glasdegib with ICT was well tolerated, with a safety profile consistent with that in AML pts receiving standard ICT. Further studies are warranted.

P205

CM942 IS A NEW SMALL MOLECULE THAT TARGETS SET-PP2A INTERACTION AND INHIBITS GROWTH OF ACUTE MYELOID LEUKEMIA CELLS

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Background: Acute myeloid leukemia (AML) is a heterogeneous malignant disorder of hematopoietic progenitor cells in which several genetic and epigenetic aberrations have been described. Nevertheless, outcome for most patients is poor, and it is necessary to develop more effective treatment strategies. Our group showed that the inactivation of the tumor suppressor PP2A is a recurrent event in AML, and that overexpression of SET, an endogenous inhibitor of PP2A, is a poor prognostic factor in this disease. Furthermore, the anticancer activity of FTY720, a PP2A-activating drug (PAD), depends on its interaction with SET. FTY720 is a relatively nontoxic drug currently used in patients with relapsing multiple sclerosis; however, this drug cannot be used in cancer patients due to its toxicity at the needed anti-neoplastic dose. Therefore, investigation of alternative agents for reactivation of PP2A is warranted.

Aims: To test the efficacy of CM942, a FTY720 analogue, on AML cell lines and primary patient samples, and investigate its mechanism of action.

Methods: AML cell lines and 29 de novo AML samples were analyzed by treatment with FTY720 and CM942, MTS (viability), apoptosis, cell cycle and PP2A activity assays, and western blot.

Results: CM942 exhibited notable cytotoxicity on all human AML cell lines with SET overexpression (n=10). By using phosphatase assays we confirmed that CM942 treatment activated PP2A on cell lines, similarly to FTY720. Immunoprecipitation of PP2Ac in untreated cells confirmed that SET interacts with PP2Ac, and that treatment with CM942 effectively disrupted this association. Furthermore, CM942 had a caspase-dependent pro-apoptotic effect, and decreased phosphorylation of the PP2A target ERK1/2. Microarray data from vehicle-treated and CM942-treated HL-60 cells showed a high correlation between the gene expression profiles of the samples. This analysis identified up-regulated and down-regulated genetic pathways by treatment with CM942, providing mechanistic insights into the anti-tumor mechanism of this small molecule. Our analyses in primary AML samples showed that 7 out of 29 (24%) samples treated with CM942 had a significant reduction in proliferation. By western blot analyses we found that those patients responding to CM942 treatment had SET overexpression. Of note, treatment of peripheral blood mononuclear cells from healthy donors with CM942 had no effects on cell viability. Therefore, although FTY720 and CM942 have similar effects inhibiting cellular proliferation, CM942 was less toxic when assayed on normal peripheral blood cells.

Summary/Conclusions: CM942 inhibits growth of AML cells in both cell lines and primary patient samples, exerting its antileukemic effects through reactivation of PP2A activity. Although treatment with FTY720 was somewhat more effective than CM942 in primary samples of AML, fewer cytotoxic effects were observed after CM942 treatment in peripheral blood from healthy donors. Further experiments would be necessary to confirm the *in vivo* antitumor activity of CM942 in AML models. New compounds have been developed for the treatment of AML, although few have been translated into clinical practice; nevertheless, it is unlikely that any of these compounds, when used as single agents, will cure the disease, which suggests the need for combinatorial therapy. Our results indicate that PADs may be a valid therapeutic option for AML, especially for treating leukemias characterized by SET-dependent inactivation of PP2A.

P206

CLONAL HETEROGENEITY IN LEUKEMIC STEM CELLS FROM PATIENTS WITH ACUTE MYELOID LEUKEMIA

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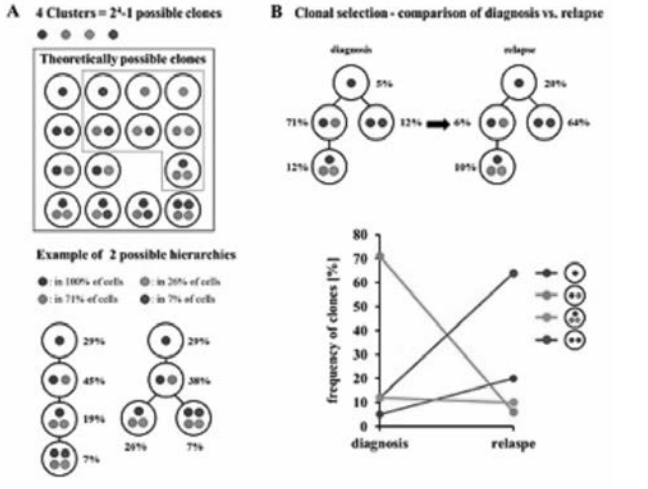
Background: Clonal heterogeneity occurs in many cancers, including Acute Myeloid Leukemia (AML). In cases of relapse, chemotherapy has triggered clonal selection with minor or evolved sub-clones driving relapse. A better understanding of the underlying clonal architecture, the extent of genetic heterogeneity and its response to therapy is necessary to better understand mechanisms of therapy escape and relapse.
Aims: In this study we aim to define the clonal architecture of AML during the course of therapy and in leukemia propagating cells.
Methods: We sequenced 12 AML samples at the time of diagnosis and in one case also at the time of relapse with at least 80% blasts per sample. 6/12 patients displayed a normal karyotype while the other 6 patients showed various cytogenetic abnormalities (inversion 16 (2), trisomy 8 (1), add(19)(p13.3) (1), complex aberrant karyotype (2)). Whole-exome sequencing (WES) was performed with the appropriate germ line controls. WES data were clustered using empirical Bayesian clustering.
Results: WES identified more than 3000 variants in total. By setting distinct filtration criteria (20% allele frequency (AF), ≥10 reads coverage, ≥2 reads support of the detected variant, SIFT-score <0,05 and GMAF <5%) 64 leukemia specific mutations were detected (1-18 mutations/AML). As expected, these included recurrently mutated genes like DNMT3A (in 4 patients), IDH1 and 2 (each in one patient), KIT and NRAS (both in 2 patients). Categorization of identified mutations showed that these mutations affected genes involved in various cellular processes including transcriptional regulation (15), cell differentiation (6), cell cycling (5), apoptosis/survival signals (5), proliferation (3), cell growth (3) and splicing (3). Empirical Bayesian clustering of all detected variants according to their respective AF resulted in 2-5 different clusters per AML. Based on this cluster analysis we were able to predict the founding cluster/clone. Assuming that most of the mutations are heterozygous and considering the blast percentage at diagnosis, mutations of the biggest clusters are present in every cancer cell and the mutations of the smaller clusters in proportionately smaller fractions. Based on the clustering information we were able to model the potential clonal hierarchies. Using a combinatorial approach, clonal modelling can identify which theoretically existing clones (2^n-1; n= number of clusters) were present at the time of diagnosis and in which order they evolved (Figure 1). Through comparison of clusters from diagnosis and relapse clonal selection can also be detected and via modelling the most likely clonal architectures can be identified. By assigning our 64 identified leukemia-specific mutations to the defined clusters we can now track the different clusters/clones in phenotypically distinct subpopulations and during xenotransplantations by targeted sequencing. An update of this analysis will be presented at EHA.



Modelling of potential clonal architectures according to clustering data

A) Example of the clonal make-up in an AML sample with 4 detectable clusters. In theory 2^n-1 clones are possible. In the case of 4 clusters 15 combinations are possible. If the purple cluster contains the founding mutations - clones in the dotted area are therefore unlikely to occur, since this cluster is present in every cancer cell. On the bottom two possible clonal hierarchies are shown that are based on the presented cluster frequencies.

B) Through comparison of clusters from diagnosis and relapse we can detect clonal selection and via modelling we can identify the most likely clonal architectures of both time points. By comparing the modelled clonal hierarchies from diagnosis and relapse (one example is shown) we can evaluate which model fits best to explain the clonal make up of both time points allowing an estimation of the clonal complexities.

Figure 1.

Summary/Conclusions: WES can identify leukemia specific mutations that are involved in various cellular functions including mutations that have been shown to be recurrently mutated in AML like DNMT3A. Sequencing data can also be used in combination with mathematical modelling approaches to reconstruct the clonal architecture of AML at the time of diagnosis and relapse allowing estimations of the clonal complexity at these time points.

P207

TREATMENT OF PRACINOSTAT AND AZACITIDINE IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML): CORRELATION BETWEEN MUTATION CLEARANCE AND CLINICAL RESPONSE

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Background: In a phase 2 study of 50 elderly patients (≥65 years) with AML who were not eligible for intensive chemotherapy, treatment with the investigational HDAC inhibitor pracinostat+azacitidine (AZA) was well tolerated and led to 42% complete remission (CR) rate and a median overall survival (OS) of 19.1 months (Blood 2016; 128:100). Responses were durable (median CR+CRi 17.2 months), blast clearance was rapid (median 8 weeks), and maximum clinical benefit required prolonged therapy (>6 months) in some patients.
Aims: Our aim was to understand the impact of somatic mutations and their clearance on disease response and survival outcomes in AML patients treated with pracinostat+AZA.
Methods: 88 samples from 41 study patients were sequenced. Pre-treatment samples were available for analysis from all 41 patients, and a median of 3 longitudinal samples were analyzed from 19 patients between Cycle 2 and 9. Leukemia mutations were detected by SureSelect targeted capture exon sequencing (Agilent) of 295 genes that are recurrently mutated in hematologic malignancies (median coverage 507x [range: 111-777x]). Longitudinal mutation clearance was analyzed by tracking variant allele frequency (VAF). Informed consent was obtained from all patients.
Results: At baseline, 96 mutations in 28 genes were detected in 38 (93%) patients, with the most frequent being in *SRSF2* (27%), *DNMT3A* (20%), *IDH2* (17%), *RUNX1* (17%), and *TET2* (17%). The median number of mutations detected per patient was 2 (range: 0-6). Among the 33 patients with evaluable treatment response, CR was observed in 13 (39%) patients. The rate of CR was significantly higher in patients with mutations in *NPM1* or in one of the DNA methylation pathway genes, while patients with *TP53* mutation had a trend for poor CR (Table 1). The median follow up duration of the 41 patients was 23.8 months (95% CI: 20.4-27.1 months) with median OS of 18.1 months (95% CI: 10.1-26.1 months), patients with *CEBPA* mutation had a trend toward better OS, whereas patients with *NF1* mutation had significantly worse OS (Table). Considering mutations associated with AML ontogeny (Lindsley RC, Blood 2015;125:1367-76), median OS was 17.7 months in 20 patients with mutations typically associated with secondary AML and 18.1 months in 18 patients with mutations typically associated with de novo AML. Among the 19 patients whose longitudinal specimens were analyzed, 10 achieved CR. Of those 10 patients, 9 (90%) had persistently detectable mutations in their bone marrow at the time of CR, however, in 7 of them, continued exposure to pracinostat+AZA lowered the VAF or cleared residual mutations. Mutations in genes associated with DNA methylation, RNA splicing, clonal hematopoiesis of indeterminate potential (CHIP), and receptor tyrosine kinase (RTK) pathways had poor clearance of mutation, while transcription factors or cohesin had better clearance with pracinostat+AZA treatment. In 2 patients, relapsed samples were sequenced and showed re-expansion of the founder clone.

Table 1.

	CR rate (%)		
Gene; n (%)	**Mutated**	**Wild-type**	**P value**
NPM1; 6 (15)	83	30	0.025
DNA methylation pathway*; 15 (37)	60	22	0.027
CEBPA; 3 (7)	100	33	0.052
TET2 ; 5 (12)	80	32	0.066
RAD21; 3 (7)	100	33	0.052
TP53; 5 (12)	0	46	0.065
	OS (months)		
CEBPA; 3 (7)	Not reached	14.8	0.061
RUNX1; 6 (15)	7.9	18.1	0.078
NF1; 3 (7)	3.0	19	0.005

* *DNTM3A, IDH1, IDH2*, or *TET2*

Summary/Conclusions: Mutations in *NPM1*, and DNA methylation pathway were associated with a better response to pracinostat+AZA, while *TP53* mutation was associated with a trend toward poor response. Persistent mutation at the time of CR suggests residual preleukemic clonal hematopoiesis in this elderly population. Benefit of prolonged exposure to pracinostat+AZA was also confirmed at molecular level where continued decline of mutation VAF was seen after achieving CR.

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STABLE DISEASE WITH HEMATOLOGIC IMPROVEMENT IS CLINICALLY MEANINGFUL FOR OLDER PATIENTS WITH ACUTE MYELOID LEUKEMIA TREATED WITH AZACITIDINE

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Background: Effects on overall survival (OS) are of primary importance when evaluating AML treatments (Tx). Though complete remission (CR) rates are lower with azacitidine (AZA) than with intensive chemotherapy (IC), OS is similar with AZA and IC (Dombret *et al.*, *Blood*, 2015). The 2017 European LeukemiaNet (ELN) recommendations acknowledge that hypomethylating agents, including AZA, may alter the natural course of AML in some patients (pts) who do not achieve CR (Döhner *et al.*, *Blood*, 2017). According to IWG criteria for AML (Cheson *et al.*, *J Clin Oncol*, 2003), stable disease (SD) is considered non-response to Tx. Yet AML is a progressive disease; potentially, stable health status may reflect delayed disease progression and result in improved OS.
Aims: This *post hoc* analysis evaluated OS outcomes among older pts with AML treated with AZA or conventional care regimens (CCR) who maintained SD, with or without hematologic improvement (HI), in the phase 3 AZA-AML-001 study.
Methods: Pts aged ≥65 years with AML (>30% marrow blasts), ECOG PS score ≤2, NCCN-defined intermediate- or poor-risk cytogenetics, and WBC count ≤15x10^9/L received AZA (75mg/m^2 x7 days [d]/28d cycle) or a CCR (IC [standard 7+3 regimen], low-dose cytarabine [20mg BID x 10d/28d cycle], or best supportive care). OS was assessed using Kaplan-Meier methods for pts with SD at 2-, 4-, and 6-month landmarks. SD was protocol-defined as the absence of an IWG-defined AML response and no progressive disease (PD), whether or not HI was attained. Pts with SD could have had an IWG-defined response or PD at any time other than at the specified landmarks. OS was also evaluated in pts with HI as their best response; attainment of HI must have begun on or before, and been sustained past, each landmark, and lasted for ≥56 consecutive days.

Table 1.

Survival among patients with Stable Disease, with or without hematologic improvement, at 2-month study landmarks

Landmark	SD and HI status	N		Median OS, months (95%CI)		Difference in Median OS, AZA vs CCR	K-M Estimated 1-Year survival		Difference in 1-year survival, AZA vs CCR (95%CI)
		AZA	CCR	AZA	CCR		AZA	CCR	
2 months	SD Overall	103	67	13.6 (12.0, 15.4)	11.1 (7.9, 14.1)	+2.5 months	59.2%	44.4%	+14.8% (-0.5%, 30.1%)
	SD/HI+	19	7	17.0 (6.9, 22.3)	12.2 (4.9, NR)	+4.8 months	63.2%	53.6%	+9.6% (-35.3%, 54.5%)
	SD/HI-	84	60	13.3 (11.5, 15.3)	11.1 (7.5, 14.1)	+2.2 months	58.3%	43.3%	+15.0% (-1.4%, 31.4%)
	Difference, HI+ vs HI-			+3.7 months	+1.1 months		+4.9%	+10.3%	
4 months	SD Overall	106	59	13.5 (12.1, 16.3)	11.3 (9.1, 14.1)	+2.2 months	60.4%	45.4%	+15.0% (-0.8%, 30.8%)
	SD/HI+	30	10	20.5 (15.3, NR)	11.1 (4.9, 26.6)	+9.4 months	80.0%	46.7%	+33.3% (-2.2%, 68.8%)
	SD/HI-	76	49	12.6 (9.7, 14.1)	11.3 (7.5, 14.1)	+1.3 months	52.6%	44.9%	+7.7% (-10.2%, 25.6%)
	Difference, HI+ vs HI-			+7.9 months	-0.2 months		+27.4%	+1.8%	
6 months	SD Overall	70	42	14.3 (12.6, 19.0)	12.2 (10.3, 15.0)	+2.1 months	67.1%	51.7%	+15.4% (-3.3%, 34.3%)
	SD/HI+	25	7	20.8 (15.3, NR)	15.1 (9.1, NR)	+5.7 months	84.0%	51.4%	+32.6% (-9.9%, 75.0%)
	SD/HI-	45	35	12.9 (10.4, 14.3)	12.2 (10.0, 14.5)	+0.7 months	57.8%	51.4%	+6.3% (-15.6%, 28.3%)
	Difference, HI+ vs HI-			+7.9 months	+2.9 months		+26.2%	0%	

95%CI, 95% confidence interval; AZA, azacitidine; CCR, conventional care regimens; K-M, Kaplan-Meier; NR, not reached

Results: Median OS for all SD pts was 2.1-2.5 months longer with AZA *vs* CCR, and estimated 1-year survival was ~15% higher at each landmark in the AZA arm (Table 1). Hazard ratios for OS among all SD pts treated with AZA *vs* CCR ranged from 0.81–0.88. Median OS among pts with SD and no HI ranged from 12.6–13.3 months in the AZA arm and from 11.1-12.2 months in the CCR arm. Within Tx arms, AZA-treated pts with HI had meaningfully improved OS at all landmarks, ranging from 3.7 to 7.9 months longer than OS for pts without HI (Table 1). In contrast, HI attained with CCR did not largely influence OS; differences between pts who attained HI *vs* no HI ranged from -0.2 to 2.9 months. Median durations of HI in the AZA *vs* CCR arms, respectively, were 183 *vs* 166

days at 2 months, 176 *vs* 148 days at 4 months, and 176 *vs* 138 days at 6 months. Estimated 1-year survival within the AZA arm was 4.9%–27.4% greater for pts with HI than for pts with no HI, but for CCR-treated pts with HI, 1-year survival was 0%–10.3% greater. Between Tx arms, 1-year survival with AZA in pts with HI was 9.6%–33.3% greater than for CCR-treated pts with HI.
Summary/Conclusions: Maintaining SD during AZA or CCR Tx is associated with relatively favorable OS outcomes, as median OS in pts with SD exceeded that for all pts in the AZA-AML-001 trial (10.4 months with AZA *vs* 6.5 months with CCR; Dombret *et al*., *Blood*, 2015). Pts with SD who also attained HI during early AZA Tx had meaningfully improved OS, whereas similar CCR-treated pts did not, suggesting that HI with AZA is qualitatively different from HI with CCR. The prognostic relevance of HI in AML requires further study.

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A RANDOMIZED PHASE II STUDY OF IDARUBICIN AND CYTARABINE WITH EITHER CLOFARABINE OR FLUDARABINE IN ADULTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA

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Background: Fludarabine and clofarabine are purine nucleoside analogues with clinical activity in acute myeloid leukemia (AML).
Aims: We designed a randomized phase II trial to evaluate the efficacy and safety of idarubicin and cytarabine with either clofarabine (CIA) or fludarabine (FIA) in adults with newly diagnosed AML. The primary objective was to compare the EFS rates of the two regimens.
Methods: Adults with newly diagnosed AML deemed suitable for intensive chemotherapy were randomized using a Bayesian adaptive design to receive CIA or FIA. All patients (pts) received idarubicin 10 mg/m^2 IV on D1-3 and cytarabine 1 g/m^2 IV daily on D1-5. Clofarabine and fludarabine were given at doses of 15 mg/m^2 and 30 mg/m^2, respectively, IV daily on D1-5. Pts with *FLT3*-ITD mutations could receive concomitant sorafenib. Responding pts could receive up to 6 cycles of consolidation at attenuated doses. Outcomes were compared to a historical cohort of pts <60 years of age who received idarubicin and cytarabine (IA) without a nucleoside analogue.
Results: Between 8/2011 and 6/2016, 182 pts were enrolled (CIA, n=106; FIA, n=76; Table 1).

Table 1.

Baseline characteristics

Characteristic*	CIA (n=106)	FIA (n=76)
Age (years)	53 [20-66]	49 [18-66]
WBC (10^9/L)	3.7 [0.6-103.0]	4.9 [0.5-59.4]
Hemoglobin (g/dL)	9.5 [7.3-13.1]	9.1 [7.5-13.1]
Platelets (10^9/L)	37 [1-1069]	41 [5-399]
BM blasts (%)	52 [1-96]	54 [11-96]
Diagnosis		
AML	103 (97)	73 (96)
High-risk MDS	3 (3)	3 (4)
Cytogenetics		
Diploid	48 (45)	34 (45)
-5, -7 and/or complex	29 (27)	19 (25)
Others	29 (27)	23 (30)
s-AML/t-AML	13 (12)	12 (16)
FLT3-ITD mutation	22/103 (21)	15/76 (20)
ELN risk		
Favorable/intermediate-1	43/101 (43)	29/69 (42)
Intermediate-2/adverse	58/101 (57)	40/69 (58)

*Continuous variables are listed as median [range] and categorical variables as n (%)

CIA, clofarabine, idarubicin and cytarabine; FIA, fludarabine, idarubicin and cytarabine; WBC, white blood cell; BM, bone marrow; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; s-AML/t-AML, secondary- or therapy-related AML; ELN, European LeukemiaNet

The imbalance of the arms was due to the better performance of CIA during the initial period of the trial. Treatment arms were well-balanced after randomization. 12 pts (55%) in the CIA arm and 8 (53%) in the FIA arm received sorafenib. The composite CR/CRp rate was similar between the two arms (80% for CIA *vs* 82% for FIA; P=0.84). CR was achieved in 72% and 74% in the CIA and FIA arms, respectively. MRD negativity rates at remission by multiparameter flow cytometry were higher in the CIA arm (80% *vs* 65%; P=0.07). 37 pts (35%) in the CIA arm and 28 (38%) in the FIA arm underwent allogeneic stem cell transplant in first remission. The median duration of follow-up was 27 months (range, 1-58). Median EFS for pts who received CIA and FIA were 13 months and 12 months, respectively; the 2-year EFS rate was 44% in both arms (P=0.91). Median OS were 24 months and not reached, and the 2-year OS rates were 51% and 57%, respectively (P=0.23). No differences in EFS or OS were observed according to baseline factors, including cytogenetics, mutations or ELN risk group. CIA was generally associated with more adverse events compared to FIA, including a higher rate of transaminase elevation (29% *vs* 4%), hyperbilirubinemia (26% *vs* 9%), and rash (29% *vs* 12%). Early mortality was similar in the 2 arms (60-day mortality: 4% for CIA *vs* 1% for FIA; P=0.32). We compared outcomes of pts treated with either CIA/FIA to a historical cohort treated with IA (n=92). Pts in the CIA/FIA group with *FLT3* mutations who received sorafenib (n=20) were excluded from this analysis. The two cohorts were similar with respect to pretreatment characteristics analyzed, including age, cytogenetics, and ELN risk. No differences were observed in CR/CRp rates, EFS or OS between the two groups. However, among pts <50 years of age, the median EFS for pts who received FIA (n=36), CIA (n=28) and IA (n=34) was not reached, 10 months and 9 months, and the 2-year EFS rates were 58%, 33% and 30%, respectively (P=0.05 for FIA *vs* IA; P=0.79 for CIA *vs* IA). For these pts <50 years of age, the median OS was not reached, 22 months and 15 months, and the 2-year OS rates were 72%, 46% and 36%, respectively (P=0.009 for FIA *vs* IA; P=0.23 for CIA *vs* IA).
Summary/Conclusions: CIA and FIA have similar efficacy in younger pts with newly diagnosed AML, although FIA is associated with a better toxicity profile. FIA may improve outcomes compared to IA in pts <50 years of age.

P210

OVERALL SURVIVAL AND TRANSPLANTATION IN PATIENTS WITH FLT3 MUTATIONS: SUBGROUP ANALYSIS OF A PHASE 3 STUDY OF CPX-351 VERSUS 7+3 IN OLDER ADULTS WITH NEWLY DIAGNOSED, HIGH-RISK ACUTE MYELOID LEUKEMIA

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Background: Approximately 20% to 30% of patients with acute myeloid leukemia (AML) have FLT3 mutations; these patients often experience rapid post-induction relapse, highlighting the need for therapies that provide an improved bridge to stem cell transplantation. CPX-351 is a liposomal formulation that delivers a synergistic 5:1 molar ratio of cytarabine and daunorubicin. CPX-351 demonstrated significantly prolonged overall survival (OS) *versus* cytarabine/daunorubicin (7+3) in a randomized, open-label, controlled phase 3 trial in patients aged 60 to 75 years with newly diagnosed, high-risk AML (Lancet, *et al.* ASCO 2016). A study of the *ex vivo* cytotoxicity of CPX-351 found that AML blasts with the FLT3-ITD phenotype were 5-fold more sensitive to CPX-351 than those with wild type FLT3 (Gordon, *et al.* Leuk Res. 2017;53:39-49).
Aims: The current analysis of the phase 3 trial therefore investigated outcomes in the subset of patients with FLT3 mutations.
Methods: Enrolled patients were randomized 1:1 to receive induction with 1 to 2 cycles of CPX-351 (100 units/m2 [cytarabine 100 mg/m2+daunorubicin 44 mg/m2] on Days 1, 3, and 5 [2nd induction: Days 1 and 3 only]) or 7+3 (cytarabine 100 mg/m2/day x 7 days [2nd induction: x 5 days]+daunorubicin 60 mg/m2 on Days 1, 2, and 3 [2nd induction: Days 1 and 2 only]). Patients who achieved complete remission (CR) or CR with incomplete platelet or neutrophil recovery (CRi) could receive up to 2 consolidation cycles.
Results: Of the 274 patients who were assessed for FLT3 mutations and received study treatment, 22/138 (16%) patients in the CPX-351 arm and 20/136 (15%) patients in the 7+3 arm had baseline FLT3 mutations. AML subtypes in FLT3+ patients were: therapy-related AML (19%); AML after myelodysplastic syndrome (MDS) with (38%) or without (10%) prior hypomethylating agents; AML after chronic myelomonocytic leukemia (12%); and de novo AML with MDS karyotype (21%). In FLT3+ patients, median OS was longer with CPX-351 (10.25 months) *versus* 7+3 (4.55 months; hazard ratio=0.57 [95% CI: 0.24, 1.33]; P=0.093; see Figure 1), and the rate of CR+CRi was higher (68% *vs* 25%). A greater number of FLT3+ patients treated with CPX-351 were able to undergo stem cell transplantation (n=10/22 [45%]; 4 patients were alive as of this analysis, after a median post-transplant follow up of 692 days [range: 96-769]) compared with 7+3 (n=2/20 [10%]; neither patient was still alive). The adverse event profile (reported during treatment or within 30 days of discontinuation) of CPX-351 in FLT3+ patients was comparable to that of 7+3 and consistent with the overall study population. Serious treatment-emergent adverse events (TEAEs) were experienced by 7 (32%) FLT3+ patients in the CPX-351 arm and 10 (50%) patients in the 7+3 arm; individual serious TEAEs in ≥2 patients included febrile neutropenia (n=2 in each arm), respiratory failure (n=1 in each arm), cardiac failure (n=2 with CPX-351), and cerebral hemorrhage (n=2 with 7+3).
Summary/Conclusions: CPX-351 demonstrated numerical improvement in median OS in older patients with newly diagnosed, FLT3+ high-risk AML and

allowed more patients to undergo stem cell transplantation. The safety of CPX-351 in this subpopulation was in line with that of previous studies and the overall phase 3 population. This analysis was limited by small number of patients.

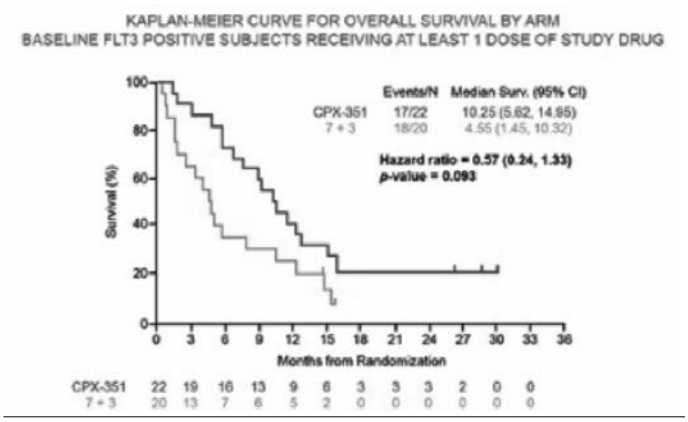

Figure 1.

P211

NIVOLUMAB MAINTENANCE THERAPY FOR PATIENTS WITH HIGH-RISK ACUTE MYELOID LEUKEMIA IN REMISSION

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Background: Dose intensification and newer drug combinations during induction have led to high rates of complete remission (CR) in pts with newly diagnosed AML. However, disease relapse remains a major source of failure. With the exception of allogeneic (allo) stem cell transplant (SCT), there are few options for post-consolidation maintenance of remission in high-risk pts. Prior attempts to develop maintenance therapy using cytotoxic drugs in AML have been unsuccessful. Immune mediated disease control by engaging tumor-specific cytotoxic T-cells may be important in suppressing leukemia relapse, as is seen with graft *vs* leukemia effect following allo SCT. Immune checkpoint inhibitors may be effective in restoring host immune surveillance in the setting of post-remission maintenance.
Aims: We designed a pilot phase II clinical trial studying the efficacy and safety of nivolumab (nivo) as maintenance therapy in AML pts with high-risk disease in remission, who were not being considered for SCT.
Methods: AML pts ≥18 years with a high-risk feature in 1st CR (CR1) or any patient in 2nd CR (CR2) who had received induction and at least 1 consolidation cycle were eligible for enrollment. Pts should be within 12 months of achieving CR, have PS ≤2, and adequate organ function. Pts were treated with nivo 3mg/kg IV every 2 weeks for 6 months. 1 cycle was 4 weeks. After 6 months, nivo could be given every 4 weeks until 12 months on study, and then every 3 months until relapse. All pts had baseline cytogenetic and molecular testing, and minimal residual disease (MRD) assessment by flow cytometry. Peripheral blood and bone marrow samples were collected at baseline and during treatment for immune correlative studies to explore immune cell repertoire and biomarkers for response.
Results: Eight pts have been treated, with a median age of 60 years (range, 49-71). 7 pts were in CR and 1 in CRi at the time of enrollment; 5 pts (63%) were in CR1, 2 pts (25%) were in CR2, and 1 pt (13%) in CR4 was inadvertently enrolled and treated on the trial. Baseline characteristics are outlined in Table 1. AML-related mutations detected at start of therapy include: IDH2 (n=2), NPM1 (2), TET2 (2), and 1 each of TP53, JAK2, ASXL1, and DNMT3a. High risk features at the time of enrollment were as follows: 2 (25%) persistent MRD, 2 (25%) adverse karyotype, 1 (13%) adverse mutational profile, and 3 pts (38%) in CR2 or beyond. Pts have received a median of 4 (1 – 13) cycles of therapy. With a median followup of 6+ months (1 – 14), the 6- and 12-month estimated RFS were 88% and 73%, respectively. The 6- and 12-month estimated OS were 100% (Figure 1). The one patient who died was discovered after enrollment to actually be in CR4. This patient relapsed approximately 8 months after achieving CR4. The regimen was well tolerated overall, with 4 pts having possible immune-related events. 1 patient had grade 3 thyroiditis leading to hypothyroidism, treated successfully with steroids and thyroid hormone supplementation, who continues on treatment. 1 patient had grade 4 transaminase elevation which responded to dose interruption and who continues on treatment. 2 pts had grade 3 possible pneumonitis treated successfully with steroids and dose interruption – both of whom continue on treatment (Table 1).
Summary/Conclusions: Nivo appears to be a feasible maintenance strategy in high-risk AML pts who are not candidates for SCT. The study continues to surpass the pre-specified expected rate of 6-month relapse-free survival of high-risk pts based on a historical cohort. Correlative studies profiling the immune repertoire of pts before and during treatment are being finalized and will be summarized.

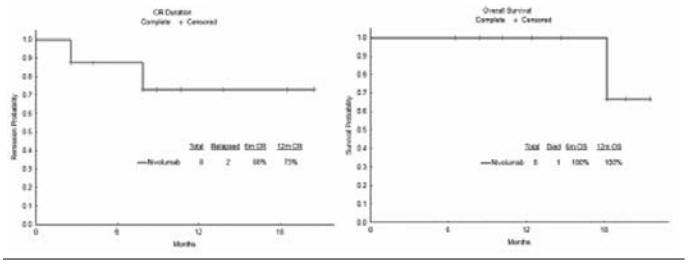

Figure 1.

Table 1.

Characteristic	Median (range)
Age	60 (49 - 71)
WBC [x10^9/L]	3.8 (1.3 - 8)
Platelets [x10^9/L]	125 (32 - 272)
LDH	465 (417 - 630)
Albumin	3.9 (3.4 - 4.6)
Bilirubin	0.5 (0.3 – 0.8)
Creatinine	0.7 (0.5 – 0.8)

P212

HIGHER EXPRESSION OF LONG NON-CODING RNA KIAA0125 IS ASSOCIATED WITH CHARACTERISTIC CLINICAL AND BIOLOGICAL FEATURES AND IS AN INDEPENDENT POOR PROGNOSTIC FACTOR IN ACUTE MYELOID LEUKEMIA

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Background: Long non-coding RNAs (lncRNAs) are non-protein coding RNAs longer than 200 nucleotides. Recently, a number of lncRNAs have been shown to play important roles in cancer biology. lncRNA *KIAA0125* is one of the 11 genes in an expression signature significantly associated with prognosis in cytogenetically normal acute myeloid leukemia (AML) patients as shown in our previous report. It is also among another set of 17 leukemia stem cell (LSC) genes, identified through xenotransplantation model in NSG mice, which predict inferior treatment response in AML.
Aims: *KIAA0125* gene is localized on chromosome 14q32.33; its functions remain unexplored. One study reported that it might be involved in neurogenesis including induction of astrocytosis, preventing formation of dopaminergic neurons. Another study showed that it could potentiate cell invasion and migration in gallbladder cancer. Its clinical significance in hematologic malignancies has not been explored yet. Since independent studies have reported *KIAA0125* as an important gene for unfavorable prognosis, in this study we aimed to investigate its clinical relevance in AML.
Methods: We performed global mRNA arrays for bone marrow samples from 347 newly diagnosed de novo AML patients in the National Taiwan University Hospital, who had adequate cryopreserved cells and detailed demographic, clinical, and genetic data for analysis. The *KIAA0125* expression level extracted from the array data was analyzed for its clinical relevance. We also validated our findings by analyzing the public databases of AML.
Results: The 347 patients were divided into two groups based on the median level of *KIAA0125* expression on the arrays. Higher *KIAA0125* expression was inversely associated with favorable karyotypes including t(8;21) and t(15;17). Patients with M1 by the French-American-British classification more frequently had higher *KIAA0125* expression (p <0.001), while those with M3 (acute promyelocytic leukemia) had significantly lower levels of *KIAA0125* expression (p <0.001). To investigate the association of gene mutations with *KIAA0125* expression in AML, we analyzed mutations of 17 AML-associated genes. We found that patients with higher *KIAA0125* expression had significantly higher incidence of *FLT3*-ITD (28.7% *vs* 19.7%, p=0.048), and mutations of *RUNX1* (18.4% *vs* 10.4%, p=0.034), and *DNMT3A* (24.1% *vs* 13.9%, p=0.015), compared to those with lower *KIAA0125* expression. Among the 227 patients who received standard chemotherapy, those with higher *KIAA0125* expression had a lower complete remission rate (61.2% *vs* 84.7%, p <0.001), and shorter overall survival (median OS, 23.7 months *vs* 116.8 months, p = 0.001) than those with lower *KIAA0125* expression after a median follow-up of 57.0 months. The prognostic significance could be validated in another two independent cohorts, TCGA and GSE12417. In multivariate analyses, higher expression of *KIAA0125* remained to be an unfavorable prognostic factor for OS independent of age, white blood cell counts, karyotype, *FLT3*-ITD, *CEBPA* double mutations,

RUNX1 mutation, *MLL*-PTD, *WT1* mutation, and *TP53* mutation (p=0.011).
Summary/Conclusions: Higher expression of *KIAA0125* in AML patients was correlated with mutations of *RUNX1, DNMT3A*, and *FLT3*-ITD but negatively associated with favorable karyotypes such as t(8;21) and t(15;17). Higher expression of *KIAA0125* appeared to be an independent unfavorable prognostic factor in our cohort, and its negative prognostic impact could be validated in another two large independent cohorts of AML. The close association of *KIAA0125* expression with LSC signatures might in part explain its unfavorable impact on the survival of AML patients.

P213

LEUKEMIC STEM CELLS CAN BE DETECTED IN A CONSIDERABLE PERCENTAGE OF PATIENTS WITH ACUTE MYELOID LEUKEMIA AT DIAGNOSIS AND IS A SIGNIFICANT PROGNOSTIC FACTOR

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Background: There is a growing interest on the identification of leukemic stem cells (SC) as a potential prognostic factor in patients with acute myeloid leukemia (AML). Several studies identify these cells as CD34+CD38-Lin-, although there is a controversy about its phenotypic identification and prognostic value.
Aims: To identify SC in a cohort of patients with AML and evaluate their prognostic value in a series of newly diagnosed AML patients.
Methods: The presence of SC (CD34+CD38-Lin-) in bone marrow samples was prospectively evaluated in a consecutive series of 67 newly diagnosed AML patients by flow cytometry, between may'13-oct'16. All patients receive intensive chemotherapy according to PETHEMA protocol. We evaluated response, relapse rate and overall (OS) and event free survival (EFS).
Results: Out of the 67 patients [34 men/33 women, median age 54 (0-78)], 58 (86.6%) have SC at diagnosis. 37.9% of them (n=22) achieved complete remission (CR) with a negative minimal residual disease (MRD) *vs* 77.8% (7/9) among patients without SC (p=0.03). Among patients who obtained CR with a negative MRD (n=29), no one suffer a leukemic relapse in the non SC *vs* 5/22 (22.7%) in the SC group (p=0.2). Considering the intermediate risk group according to cytogenetic / molecular features, 100% of patients without SC at diagnosis achieve a negative MRD (5/5) *vs* 14/41 (34.1%) among those in the SC group (p=0.008). OS at 9 months was 89 *vs* 56% (p=0.043), and the EFS 78 *vs* 48% (p=0.054) in the non SC and SC group, respectively (Figure 1).

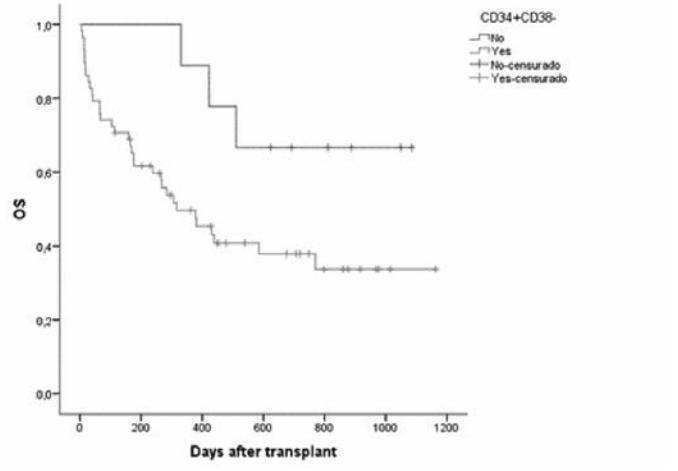

Figure 1.

Summary/Conclusions: SC can be detected in a considerable group of patients with AML at diagnosis. The presence of SC is a prognostic factor in terms of response, OS and EFS. Accordingly, SC detection could help to identify prognosis subgroups of patients with different prognostic among those in the intermediate risk group by genetics/molecular assays.

P214

POST-REMISSIONAL AND PRE-TRANSPLANT ROLE OF MINIMAL RESIDUAL DISEASE DETECTED BY WT1 IN ACUTE MYELOID LEUKEMIA: A RETROSPECTIVE COHORT STUDY

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Background: In acute myeloid leukemia (AML) the detection of residual leukemic cells at a submicroscopic level (minimal residual disease - MRD) is still under investigation. In about 30-40% of AML lacking a specific molecular target, quantitative real-time polymerase chain reaction (QRT-PCR) has been used to detect transcripts commonly overexpressed in AML. Among a large number of candidates, Wilms tumor gene 1 (*WT1*) has been proposed as a promising MRD marker.
After the standardization of QRT-PCR on behalf of the European LeukemiaNet (ELN), subsequent studies investigated the role of *WT1* expression in AML with controversial results.
Aims: To assess the role of *WT1* expression as a MRD marker after intensive induction chemotherapy and before allogeneic hematopoietic cell transplantation HCT (allo-HCT) in a large cohort of AML patients treated in a single institution.
Methods: The present retrospective cohort study included adult patients with untreated AML consecutively diagnosed between 2004 and 2014 in the Hematology Unit of the University-Hospital Città della Salute e della Scienza of Torino, Italy. The study was approved by the Ethical Committee and was registered at www.clinicaltrials.gov as NCT02714790. Among 255 enrolled patients, MRD was investigated in those in first complete remission (CR) with an available at diagnosis and at two further time-points: after induction (n=117) and prior allo-HCT (n=65). Patients with baseline *WT1* <250 copies were excluded. All patients underwent intensive induction chemotherapy with curative intent and subsequent consolidation chemotherapy according to the AML risk assessment (autologous peripheral stem cell transplantation for low risk and allo-HCT for intermediate and high risk patients). Effect of post induction *WT1* expression on disease-free survival (DFS) and overall survival (OS) and of pre allo-HCT *WT1* on the cumulative incidence of relapse (CIR) were investigated.
Results: Baseline BM *WT1* expression were not found significantly associated with demographic, clinical and disease biological features at diagnosis. Baseline BM *WT1* expression lacked even to show an association with response to induction chemotherapy (OR 1.16; 95% CI 0.90-1.50, p=0.244).
Median OS and DFS were significantly shorter in patients in first CR with >350 *WT1* copies after induction compared to those with ≤350 (OS 17 *vs* 95 months with HR 2.13; 95% CI 1.14-3.97, p=0.018 and 3-year DFS rates 15% *vs* 55% with a HR of 2.81; 95% CI 1.14-6.93, p=0.025).
Adding the BM *WT1* in the model along with other factors determines an increase of the C-statistic from 0.6996 to 0.7193 for OS (NRI=0.384) and from 0.7413 to 0.7920 (NRI=0.4037) for DFS. Before allo-HCT, patients with *WT1* >150 copies (n=18) had a significantly higher CIR compared to those with *WT1* ≤150 (n=47), HR 4.61; 95% CI 1.72-12.31, p=0.002.
Summary/Conclusions: The results of the present study showed that BM *WT1* is associated with survival in patients in CR in two decisive time-point for treatment planning: after induction treatment and before allo-HCT. The prognostic role of *WT1* resulted independent from other well-established risk factors. Therefore, *WT1* may represent an additional MRD tool for risk stratification in patients nowadays classified in CR, especially in the high risk MRD positive subgroup in which a risk-adapted approach may have a role. Published evidences available so far supported these suggestions, but mainly due to methodological issues, the role of *WT1* is still a matter of debate. Prospective randomized studies are required to confirm these results.

P215

DIFFERENTIATION SYNDROME ASSOCIATED WITH ENASIDENIB (AG-221), A SELECTIVE INHIBITOR OF MUTANT ISOCITRATE DEHYDROGENASE 2 (MIDH2)

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Background: Enasidenib (AG-221) is an oral, selective, small-molecule inhibitor of mIDH2 enzymes. Preclinical studies showed that exposing myeloblasts from patients (pts) with acute myeloid leukemia (AML) to enasidenib *ex vivo* resulted in differentiation of leukemic marrow blasts into mature, fully functional neutrophils (Yen *et al.*, *Cancer Discov*, 2017). Enasidenib can result in IDH-inhibitor-associated differentiation syndrome (IDH-DS) in treated pts, with manifestations akin to retinoic acid syndrome seen during therapy of acute promyelocytic leukemia.
Aims: To characterize the prevalence, characteristics, and course of IDH-DS in pts with relapsed or refractory (R/R) AML receiving enasidenib 100 mg daily in a phase 1 dose-escalation and expansion study (NCT01915498). This dose is currently under study in a multicenter, randomized, phase 3 trial comparing enasidenib with conventional care regimens in R/R AML pts (NCT02577406).

Methods: An independent Differentiation Syndrome Review Committee (DSRC) was formed to review potential cases of IDH-DS. The DSRC identified and agreed upon a series of signs and symptoms possibly characteristic of IDH-DS, including fever, lung infiltrates, pleural or pericardial effusions, rapid weight gain, edema, and azotemia. In all, 27 cases (8 of investigator-reported IDH-DS and 19 with characteristics suggestive of IDH-DS) were identified and retrospectively reviewed by the DSRC to determine their consistency with IDH-DS.
Results: The DSRC determined 13 cases (11.9% of 109 R/R AML pts in the enasidenib 100 mg/day dosing cohort) to be consistent with IDH-DS. Median time to onset was 30 days (range 7-116). Manifestations of IDH-DS in >2 pts were dyspnea (n=10), pyrexia (9), lung infiltrates (8), pleural effusion (5), and kidney injury (3). IDH-DS was effectively managed with systemic corticosteroids in 12/13 cases. Leukocytosis accompanied 4/13 cases, for which hydroxyurea was employed for cytoreduction. Enasidenib was interrupted for 9 pts (for a median of 7 days), but dose reductions or enasidenib discontinuation were not required for pts with IDH-DS. Six of the 13 pts had clinical responses (2 complete remissions [CR], 2 CRs with incomplete hematologic recovery, 1 partial remission, and 1 morphologic leukemia-free state), 6 pts had stable disease, and 1 pt had progressive disease.
Summary/Conclusions: Systemic corticosteroids, close hemodynamic management, and hydroxyurea (in the presence of leukocytosis) are effective IDH-DS management strategies; they should be administered promptly when IDH-DS is suspected, and continued until improvement. Enasidenib interruption can be considered if initial intervention is unsuccessful. IDH-DS represents a novel clinical finding in pts with m*IDH2* AML treated with enasidenib, and is likely due to its suggested mechanism of action, myeloblast differentiation.

Aggressive Non-Hodgkin lymphoma - 1st line

P216

Abstract withdrawn.

P217

OUTCOME OF PATIENTS WITH INTRAVASCULAR B-CELL LYMPHOMA, A RETROSPECTIVE STUDY CONDUCTED ON BEHALF OF THE LYMPHOMA STUDY ASSOCIATION GROUP

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Background: Intravascular large B-cell lymphoma (IVLBCL) is a rare type of extranodal large B-cell lymphoma characterized by the selective growth of lymphoma cells within the lumina of vessels, classically reported with poor responses to chemotherapy. Due to its low incidence and rarety of tumor cells, diagnosis of IVLBCL remains difficult and many issues remain unresolved, regarding both clinical features and therapeutic strategies.
Aims: Our work aims to describe clinical presentation and outcome of IVLBCL patients treated in French LYSA centers between 2000 and 2016.
Methods: All LYSA centers were asked to report and update clinical data about IVLBCL patients treated. No central pathology review was performed for the present study, but all cases were classified by LYSA pathologists. Local investigators reported disease characteristics and updated patients' outcome (clinical examination, standard biological parameters, bone marrow biopsy, CT scan at baseline, CT response evaluation and outcome).
Results: We identify 65 IVLBCL patients treated in 23 LYSA centers during the studied period. Median age was 67.8 years (range 22-91). In note, two third of patients presented with IPI score >3 (67%) and all patients had a stage IV disease. As expected in Western patients, cutaneous and CNS involvement were highly frequent, respectively 33% and 39%. But interestingly, hemophagocytic syndrome were observed in nearly half of the patients (41%), while it was mainly described in Asian series. Despite classically delayed diagnosis in IVLBCL, only 2 cases were confirmed post-mortem and almost all alive patients at diagnosis (n=58) were treated with rituximab-containing chemotherapy regimen (92%). Regarding first line treatment, 83% of patients were treated with anthracycline-based regimens, with CNS prophylaxis for half of them (47%), and seven patients underwent autologous stem cell transplantation upfront. The median progression free survival was 29.4 months and median overall survival was 63.8 months (Figure 1). Pathological features (including cell of origin characterization, C-MYC expression, adhesion protein expression level) investigation is ongoing and will be presented at the time of the meeting.

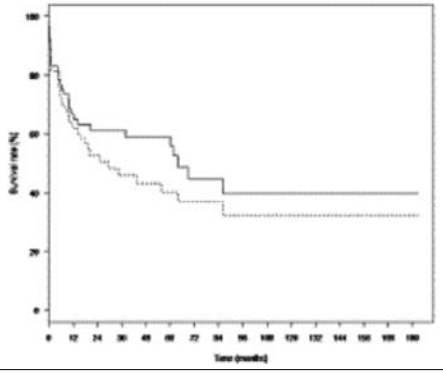

Figure 1.

Summary/Conclusions: The present study is the largest European IVLBCL series. It shows that despite the use of modern immune-chemotherapy, IVLBCL remains an aggressive lymphoma entity. In particular, these patients are highly exposed to early relapse and therefore should be considered for innovative frontline therapies.

P218

OUTCOME OF ELDERLY DLBCL PATIENTS (≥80 YEARS) TREATED WITH ANTHRACYCLINE BASED CHEMOTHERAPY; R-CHOP DOSE REDUCTION IS NOT NECESSARY FOR EVERYBODY

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Background: Management of elderly patients (above ≥80y) is difficult and only limited number of patients could be treated by curative approach with anthracycline based chemotherapy. Dose reduction of particular drugs is used very often and it varies based on pt's characteristics and center preferences. There is however lack of randomized or at least non-randomized historical comparisons.
Aims: The objective of this study is to analyze elderly DLBCL patients prospectively registered in NiHiL Lymphoma Project and treated anthracycline based regimen in real world outside of clinical trials.
Methods: Patients (pts.) with informed consent are prospectively followed in multicenter Lymphoma Project since 1999. Diagnostic, therapeutic and follow up data are prospectively collected. There were 399 DLBCL pts older than 80year diagnosed in period 1999-2014 identified. Among 372 pts. with pathology review and essential data there were 112 pts. (30.1%) treated with R-CHOPlike chemotherapy. Analysis of clinical prognostic factors, therapy and toxicity was performed. Pearson, Kaplan-Maier and log rank tests were used.
Results: Median age was 81 years (80-88), 51.8% of men. Proportion of pts ³85 was 14.3%, with PS ≥2 (ECOG) 34.0%, with higher LDH 64.3%, with high or intermediate high IPI 49.1%, with bulky disease (≥10 cm) 17.0%, with lower albumin 27.7%, with Charlson Comorbidity Score (CCS) ≥4 25%. According to treatment choice of physician (intent to treat), pts. could be divided into 3 groups R-CHOP (CH) (cyclophosphamide –CF 750 mg/m^2, adriamycin – A - 50 mg/m^2) or R-MiniCHOP (miniCH) (CF 400 mg/ m^2, A 25 mg/ m^2, Peyrade 2011) or modified R-CHOP (modiCH) (CF 750 mg/m^{2}and A 25 mg/m^{2}or any other dose between CHOP and miniCHOP). There were 21 pts (18.8%) treated with CH, 38 (33.9%) with miniCH and 53 (47.3%) with modiCH. There were no signficant differences between the subgroups, except higher proportion of bulk in modiCH *vs* miniCH and CH (35% *vs* 12.9% *vs* 7.7% resp.; p 0.04) and cardiac comorbidity (60.5% *vs* 33.3% *vs* 30.2% resp.; p 0.02). Six and more cycles were administered in 71.4%, 63.1% and 58.5% pts. in CH, miniCH and modiCH resp. Following proportion of pts. received >80% (>50%) of original CHOP dose. For cyclophosphamide it was 66.7% (81%), 0% (50%) and 62.2% (79.2%) resp. and for A it was 57.1% (76.1%), 2.6% (15.8%) and 13.2% (49%) resp. for CH, miniCH and modiCH resp. There were observed 11 treatment related deaths (6 cardiac toxicity and 4 infection), 5 in miniCH and 6 in modiCH groups. The overall response rate was 76.8% with 59.8% CR/CRu. Median PFS and OS were 2.8y and 3.5y resp. (Figure 1A) with median follow up of 3.3y. There were found high beta2microglobulin (HR 2.2, p 0.05), low albumin (HR 1.9, p 0.05) and PS (p 0.05) as the only factors correlated with OS as well as PFS (data not shown). Pts who achieved CR or PR have significantly better OS median (as well as PFS) compared to stable or progressive disease with 4.6 *vs* 3.5 *vs* 0.8 *vs* 0.5 y. There was numerically (not significantly) better OS median for R-CHOP (4.6y) *vs* R-miniCHOP (3.2y) and R-modiCHOP (2.9y) (Figure 1B).

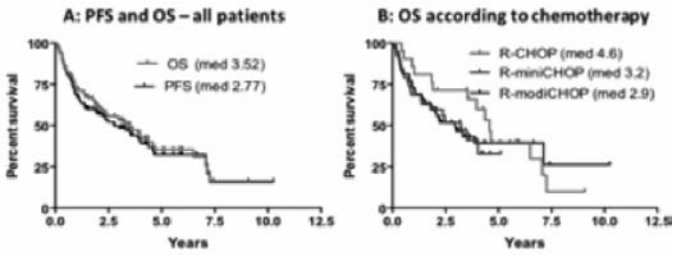

Figure 1.

Summary/Conclusions: Only one third of elderly DLBCL pts (≥80y) is treated with anthracycline based regimen. Performance status, albumin and beta2microblobulin levels were significantly associated with prognosis. In minority of these pts full dose of R-CHOP could be safely used and there is trend to better overall survival.
Supported by AZV 16-31092A.

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IMPROVED SURVIVAL IN PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA UP TO AGE 70 ONLY: A POPULATION-BASED STUDY ON INCIDENCE, PRIMARY TREATMENT AND SURVIVAL IN THE NETHERLANDS, 1989-2015

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Background: PCNSL is a rare, aggressive form of an extranodal non-Hodgkin lymphoma that exclusively affects the CNS. Recent findings from the few available prospective studies demonstrated improved outcome in PCNSL. However, the results from such studies are inherent to patient selection. Population-based studies that assess long-term patterns of incidence, treatment and survival in PCNSL are virtually lacking.
Aims: The aim of this comprehensive nationwide population-based study was to assess trends in incidence, primary treatment and survival among adult PCNSL patients (pts) diagnosed during a 27-year period in the Netherlands.
Methods: We selected all adult (≥18 years) pts diagnosed with PCNSL of the diffuse large B-cell type in the Netherlands between 1989-2015 from the nationwide Netherlands Cancer Registry with survival follow-up through February, 2016. Pts diagnosed without pathological or cytological confirmation (n=50) and pts diagnosed at autopsy were excluded (n=32). Age-standardized incidence rates (ASR) were calculated per 1,000,000 person-years and standardized according to the European standard population. Data on primary treatment (*i.e.* no therapy, chemotherapy (CT) alone, radiotherapy (RT) alone, and CT+RT) were available for individual pts. Pts were categorized into 4 periods (1989-1994, 1995-2000, 2001-2007 and 2008-2014) and 3 age groups (18-60, 61-70 and >70 years). We calculated relative survival (RS) and the relative excess risk of mortality as measures of disease-specific survival.
Results: We included a total of 1,673 newly diagnosed PCNSL pts in the study (median age, 65 years; age range, 19-89 years; 53% males). The ASR of PCNSL increased from 3.0 in the first period (1989-1995) to 4.4 in the last period (2009-2015), which was consistently higher among males than in females throughout the entire study (4.8 *v* 4.0 in the last period). The age-specific incidence rates were 2.3, 9.0 and 10.0 in the first period for the three age groups (18-60, 61-70 and >70 years), as compared with 2.7, 18.7 and 19.5 in the last period. The application of CT+RT increased exclusively among pts age 18-60. More specifically, the proportions for the three age groups were 26, 18 and 4% in the first period, as compared with 60, 10 and 4% in the last period. The use of RT alone among pts age >60 decreased with each period, following the wider use of CT alone over time, especially for pts age 61-70 years. The proportions of CT alone for the three age groups were 11, 8 and 2% in the first period, as compared with 31, 64 and 32% in the last period. Of note, 38 and 26% of pts age >70 received no therapy and RT alone in the last period, respectively. Five-year RS only improved for pts age 18-70 (Figure 1). Five-year RS (95% confidence intervals) was 22% (16%>30%), 13% (7%>22%), and 3% (1%>10%) in the first period for the three age groups, as compared with 56% (47%>64%), 35% (28%>43%) and 6% (2%>13%) in the last period. A multivariable survival model confirmed the adverse effect of older age on excess mortality and an improvement of survival over time. However, when information on treatment was added to that model, the effect of period lost statistical significance. This suggest that treatment contributed to the improved survival over time. Older age remained a predictor of poor prognosis.

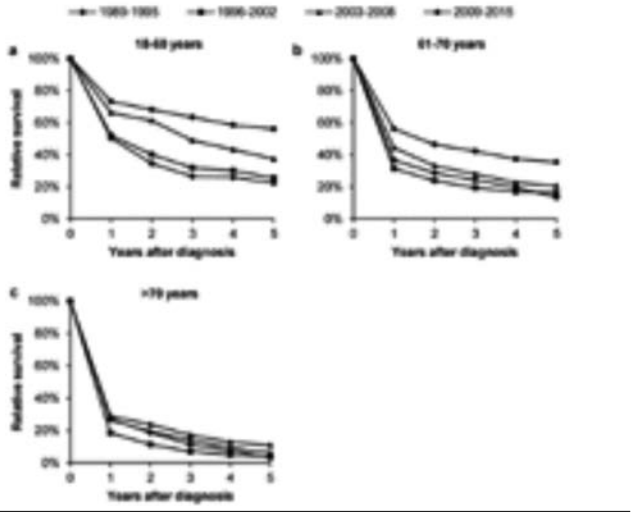

Figure 1.

Summary/Conclusions: The incidence of PCNSL steadily increases among

pts >60 years, which might in part be related to improved diagnostic practices among the elderly over time. RS increased over the past decades for pts age 70 or below. This is largely explained by the increased use of intensive therapy over time. Although the use of CT alone gradually increased among pts >70 years, their survival is still poor. Therefore, there is an urgent need to design specific trials for elderly PCNSL pts to improve their survival.

P220

CLINICAL CHARACTERISTICS AND LONG-TERM RESULTS OF TREATMENT OF DIFFUSE LARGE HEPATITIS C - ASSOCIATED NON-HODGKIN LYMPHOMA (DLBCL+C)

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Background: In the WHO classification (2008), hepatitis C virus distinguish as one of the etiological factors of multistage etiopathogenesis DLBCL.
Aims: The purpose of this study was evaluation of clinical features and results of treatment of diffuse krepnokletochnoy lymphoma associated with hepatitis C in comparison with a control group of patients with diffuse large lymphoma without viral hepatitis markers.
Methods: It was included 521 patients with DLBCL: 98 patients with DLBCL and markers of hepatitis C (DLBCL+C) and a control group of 422 patients with DLBCL without markers of hepatitis C (DLBCL-C).
Results: Patient's age ranged from 21 to 76 years (median was 47 years) in DLBCL+C; ranged from 23 to 81 years (median 61) in DLBCL -C (p=0.02). The male: female ratio was 1: 1.3 in patients with DLBCL +C; 1: 1.7 in the group DLBCL – C. Stage I and II were in 11% patients with DLBCL+C, and 48% patients with DLBCL - C; III and stage IV were detected in 89% patients with DLBCL+C and 52% of DLBCL-C (p=0.00002). Extranodal lesions detected in 72% in DLBCL+ C and in 26% in C DLBCL-C (p=0.006). In comparable groups localization of extranodal lesions was: spleen (52% to 23%), bone marrow involvement (43% and 27%), liver (18% and 1%). GCB / non-GCB histological variants ratio was 55% / 45% in DLBCL+C; 36% / 64% in DLBCL -C ratio GCB / (p=0.001). Hepatitis C virus RNA in blood was detected by PCR. Viral RNA was found in 78% (74 patients). High viral load was in 21% of patients. In 22% of cases markers of hepatitis C virus in blood were identified by ELISA. All patients received chemotherapy according to the scheme CHOP / R-CHOP. The frequency of complete remission was 60% in the group of patients with DLBCL+C and 63% of DLBCL-C. Median overall survival (OS) was 46 months in group DLBCL +C and 71 months in DLBCL -C (p=0.0003). Median progression-free survival (PFS) was 28 months in DLBCL+C 47 months in the control group (p=0.0002). According to the immunohistochemical variant of DLBCL: GCB DLBCL: median OS of 45 months in GCB DLBCL+C and 62 months in GCB DLBCL - C (p=0.002). Median PFS was 36 and 47 months in comparable group. Median OS was 18 months in non-GCB DLBCL+C and 70 months in non-GCB DLBCL-C (p=0.00001). Median PFS groups was 13 and 42 months, respectively. 58 patients received antiviral therapy after chemotherapy. Median OS was 63 months in GCB DLBCL+C with antiviral therapy and 28 months in GCB DLBCL+C without antiviral therapy (p=0.00002). Median PFS was 46 and 20 months, respectively. Median OS was 22 months in non-GCB DLBCL+C with antiviral therapy and 17 months in non-GCB DLBCL+C without antiviral therapy. Median PFS in the group was 11 and 15 months, respectively.
Summary/Conclusions: DLBCL+C characterized by aggressive course of the disease (younger age at onset of the disease, advanced stages, extranodal involvement), which is one more evidence possibility of separating DLBCL+C in a separate group. Although there is no difference in the effectiveness of the therapy. But disease-free survival in DLBCL patients+C was significantly worse.

P221

MAGNETIC RESONANCE IMAGING FOR EARLY DETECTION OF ANTHRACYCLINE CARDIOTOXICITY IN MALIGNANT LYMPHOMA

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Background: Doxorubicin is a cornerstone of curative lymphoma treatment. However, doxorubicin therapy is limited by cardiac side effects including high-mortality heart failure (HF). Signs of cardiotoxicity often appear too late to avoid irreversible myocardial damage.
Aims: The aim of our study is to investigate the value of rubidium 82 positron emission tomography (82Rb PET), iodine 123 metaiodobenzylguanidine (123I MIBG) and cardiac magnetic resonance (MR) imaging in early detection of doxorubicin-induced cardiomyopathy and prediction of HF in patients with malignant lymphoma. We aim to identify early signs of cardiotoxic injury that predict the formation of interstitial fibrosis and subsequent HF. Here we present our preliminary MR data. 82Rb PET and 123I MIBG data will be analysed later.
Methods: The study is a prospective, clinical, single-centre study. The study aims to include 70 consecutive chemotherapy-naive lymphoma patients scheduled for intended curative chemotherapy without planned mediastinal radiation therapy. All patients undergo routine clinical examinations, but with supplementary imaging, including 1) baseline 82Rb PET and MR (prior to treatment); 2) acute 82Rb PET and MR (within 1 week of the first treatment); 3) subacute 123I-MIBG (after 2-3 months of therapy) and 4) late MR (1 year after the start of treatment). 82Rb PET imaging is performed at rest and during pharmacological stress testing with adenosine. It is primarily used to evaluate the acute effects of doxorubicin on myocardial perfusion. 123I MIBG is used for detection of doxorubicin-induced subacute changes in the myocardial adrenergic neurons. Cardiac MR is performed with late gadolinium enhancement and provides information on acute and late changes in left and right ventricular function, atrial and ventricular volumes, myocardial mass and interstitial fibrosis. Statistical analyses were done in R (version 3.2.0) as paired difference tests using Wilcoxon signed rank test. P-values <0.05 were considered significant.
Results: As of March 1st 2017, 61 patients have been included. In 33 cases, the time of intended follow-up has been reached. Four patients died prior to follow-up, including one patient who died before the acute imaging procedures. Four patients were excluded due to compliance problems. One patient was excluded due to disease downstaging resulting in omittance of doxorubicin from the treatment plan. Of the 24 patients with complete data from both the baseline and late MR scans, 16 had lower LVEF values at follow-up: 0-5% (n=3), 6-10% (n=8), 10-15% (n=4) and >20% (n=1). Mean LVEF at follow-up was significantly lower (57.1%) compared to baseline LVEF (62.0%; p=0.01) and acute LVEF (64.3%; p=0.002). The LVEF decline from baseline to follow-up was paralleled by an increase in mean left ventricular end diastolic volume (LVEDV) of 10.0mL (p=0.03). Interestingly, an increase in LVEDV was already registered at the acute MR scan (7.3mL; p=0.03). The increase in LVEDV from the acute MR to follow-up was not significant. We also registered an acute increase of 7.4mL in mean stroke volume (SV) (p=0.02). However, from the acute MR to follow-up MR we found a significant decline in SV (p=0.02). There was no difference in SV from baseline to follow-up (p=0.7). The acute changes in LVEDV did not predict LVEF declines from baseline to follow-up (Figure 1).

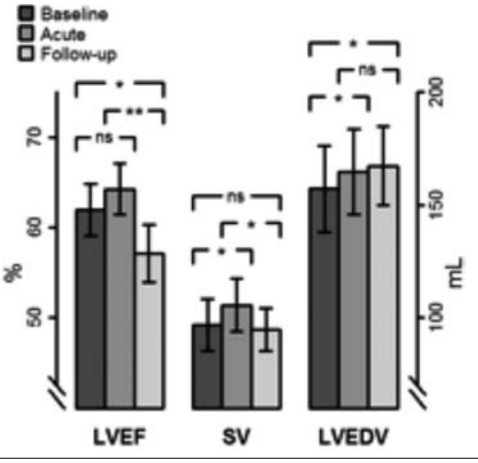

Figure 1.

Summary/Conclusions: Our preliminary show that cardiac MR can be used for detection of declining LV function 1 year after after doxorubicin exposure. It appears that cardiac MR may also provide information on acute functional changes in LVEDV and SV. We hope that our 82 Rb PET and 123I MIBG data will provide additional early signs of doxorubicin cardiotoxicity that can be used to predict subsequent development of HF.

P222

Abstract withdrawn.

P223

RELAPSE CHARACTERISTICS AND THE ROLE OF SURVEILLANCE COMPUTED TOMOGRAPHY IN AGGRESSIVE NON-HODGKIN LYMPHOMA

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Background: The use of surveillance computed tomography (CT) is usual practice for cases of complete remission (CR) in aggressive non-Hodgkin lymphoma (aNHL). However, there is a lack of evidence to support this strategy.
Aims: To determine whether surveillance CT could contribute to the improvement of survival in relapsed aNHL patients, we retrospectively analyzed our institutional lymphoma registry, which enrolled consecutive patients with lymphoma from June 1995 to October 2016. Of 1,385 aNHL patients in the registry, 664 patients achieved CR and received follow-up through clinical visits, with or without surveillance CT.
Methods: Patients who met the following inclusion criteria were selected: i) histologic diagnosis of aNHL (diffuse large B-cell lymphoma, Burkitt lymphoma, and B-cell lymphoblastic lymphoma, peripheral T-cell lymphoma not otherwise specified, angioimmunoblastic T-cell lymphoma, anaplastic large cell lymphoma, NK/T-cell lymphoma, and T-cell lymphoblastic lymphoma); ii) patients who achieved CR after frontline or salvage chemotherapy with curative intent; and iii) time from the date of diagnosis to the date of last follow-up longer than 12 months. All patients in CR after frontline therapy were followed-up with clinical visits (symptom assessment, physical examination, and blood tests) every 1 to 6 months. Surveillance CT covering the neck, chest, or abdomen were performed every 3 or 6 months or when clinically indicated in the first 2 years, and then every 6 or 12 months or when clinically indicated thereafter. The decisions regarding the surveillance strategy (clinical visit with appropriate blood chemistry with or without regular surveillance CT) were at the discretion of the treating physicians.
Results: Relapse was detected in 171 patients, of whom 152 had undergone surveillance CT during follow-up. Of these 152 patients, asymptomatic relapse was detected in 67 (44%) by surveillance CT and symptomatic relapse outside the surveillance interval was detected in the other 85 (56%). Detection of asymptomatic relapse by surveillance CT did not improve either the overall or post-relapse survival in the relapsed aNHL patients. In addition, the interval of surveillance CT (3 or 6 months) did not affect survival. No subgroups were identified that favored the use of surveillance CT to detect relapse. Additionally, we analyzed the impact of surveillance CT in patients with refractory or relapsed aNHL who achieved CR after salvage chemotherapy (CR2). Of 315 aNHL patients relapsed/refractory to frontline chemotherapy (144 refractory and 171 relapsed patients), 99 patients achieved CR after salvage chemotherapy (18 refractory and 81 relapsed patients) and these patients were followed with clinical visits, with or without surveillance CT. Relapse was detected in 42 patients (42/99; 42.4%). A total of 27 (64.3%) and 15 patients (35.7%) were identified as relapsed by methods other than CT scan and using surveillance CT, respectively. There was no significant difference in the median PRS between the two groups (12.5 months, 95% CI: 2.8 to 22.1 months *vs* 10.7 months, 95% CI: 0 to 41.5 months; p=0.182] (Table 1).
Summary/Conclusions: In conclusion, this study suggests that routine surveillance CT in aNHL patients for the detection of asymptomatic relapse might have a limited role in improving survival. Therefore, surveillance CT to identify relapse would only be recommended when relapse is clinically suspected.

Table 1.

Characteristics at relapse according to method of detection

Characteristics at relapse	All (*n* = 171)	Group 1* (*n* = 104)	Group 2* (*n* = 67)	p-value
Biopsy-proven relapse, *n* (%)	109 (63.7)	72 (69.2)	37 (55.2)	0.063[a]
Median time to relapse, months (range)	10.0 (1.2-97.4)	10.1 (1.2-97.4)	9.9 (2.2-49.5)	0.399[b]
Visit or surveillance interval, *n* (%)				**<0.001**[a]
<3 months	74 (43.3)	74 (71.2)	0 (0.0)	
Every 3 months	75 (43.9)	27 (26.0)	48 (71.6)	
Every 6 months	22 (12.9)	3 (2.9)	19 (28.4)	
Time to relapse, *n* (%)				
1st year of follow-up	91 (53.2)	54 (51.9)	37 (55.2)	0.673[a]
2nd year of follow-up	38 (22.2)	16 (15.4)	22 (32.8)	**0.007**[a]
3rd year of follow-up	17 (9.9)	14 (13.5)	3 (4.5)	0.055[a]
After 3rd year of follow-up	25 (14.6)	20 (19.2)	5 (7.5)	**0.033**[a]
Relapse site, *n* (%)				**<0.001**[a]
Involved fields at diagnosis	83 (48.5)	39 (37.5)	44 (65.7)	
Other new fields	88 (51.5)	65 (62.5)	23 (34.3)	
Histology, *n* (%)				0.107[a]
DLBCL	109 (63.7)	60 (57.7)	49 (73.1)	
PTCL	57 (33.3)	41 (39.4)	16 (23.9)	
Other	5 (3.0)	3 (2.9)	2 (3.0)	
Extranodal involvement, *n* (%)	104 (60.8)	76 (73.1)	28 (41.8)	**<0.001**[a]
Bone marrow involvement, *n* (%)	9 (9.6)	8 (13.6)	1 (2.9)	0.088[a]
LDH above normal limit, *n* (%)	76 (45.8)	46 (45.5)	30 (46.2)	0.939[a]
ECOG performance score, *n* (%)				0.115[a]
0–1	135 (78.9)	78 (75.0)	57 (85.1)	
2–4	36 (21.1)	26 (25.0)	10 (14.9)	
Disease stage, *n* (%)				0.980[a]
Limited (stages I–II)	107 (62.6)	65 (62.5)	42 (62.7)	
Advanced (stages III–IV)	64 (37.4)	39 (37.5)	25 (37.3)	

*Patients with relapse detected by methods other than surveillance CT comprise Group 1; patients with relapse detected by surveillance CT scans comprise Group 2.

Note: Bold indicates statistical significance.

[a]Chi-square test.

[b]Mann–Whitney U test.

Abbreviations: DLBCL, diffuse large B-cell lymphoma; PTCL, peripheral T-cell lymphoma; LDH, lactate dehydrogenase; ECOG, Eastern Cooperative Oncology Group.

P224

A MULTI-CENTER STUDY OF GLIDE CHEMOTHERAPY CONSOLIDATED WITH AUTOLOGOUS STEM CELL TRANSPLANTATION FOR NEWLY DIAGNOSED STAGE IV AND RELAPSED EXTRANODAL NATURAL KILLER/T-CELL LYMPHOMA PATIENTS

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Background: The prognosis of advanced-stage and relapsed extranodal NK/T cell lymphoma(ENKTL) is poor, with long term survival rate of 30%. Our previous study of GLIDE (gemcitabine, L-asparaginase, ifosfamide, dexamethasone and etoposide) chemotherapy reported complete response(CR) rate and 3-year overall survival(OS) of these patients were 57.1% and 56% respectively. We assumed autologous stem cell transplantation may further improve the prognosis of these patients.
Aims: We conducted this clinical trial to address the efficacy and safety of our treatment strategy, GLIDE induction followed by ASCT, in newly diagnosed stage IV and relapsed ENKTL.
Methods: We treated 60 patients with newly diagnosed stage IV(n=49) and relapsed(n=11) ENKTL from 2010 to 2016. The median age at recruitment was 38 years and the median follow-up period was 13.4 months. Patients were treated with GLIDE (gemcitabine 800 mg/m^{2}D1,5; *L*-asparaginase 6000 u/m^{2}D4,6,8,10,12 or peg-asparaginase 2500 u/m^{2}D4,11; *i*fosfamide 1000 mg/m^{2}D1-3; *d*examethasone 20mg D1-4; *e*toposide 100 mg/m^{2}D1-3) every 4 weeks, and responses were evaluated with PET/CT every 2 cycles. Patients achieving CR underwent ASCT or continued with GLIDE up to 6 cycles. Others finished 6 cycles of GLIDE. Overall response rate(ORR), CR, OS and progression free survival(PFS) were calculated using standard methods. Statistical analysis was done using Fishers exact test or Chi-square test / Kruskal-Wallis test. Kaplan-Meier method was used for time-to-event analysis including overall survival and progression free survival. The Log-rank test was used to evaluate the difference in time-to-event endpoints between patient groups.
Results: Fifty-seven patients had finished planed treatment with 1 withdraw of informed consent after cycle 1, and 2 death of sepsis during cycle 1 and cycle 2 respectively. Twenty-one patients underwent ASCT. The ORR was 81.4% and the CR was 69.5% with early CR (CR after 2 cycles) of 57.6%. Estimated 5-year OS and PFS rates of the whole cohort and patients underwent ASCT were 68.7%, 54.0%, 79.6% and 85.2% respectively. Univariate analysis revealed that ECOG ≤1, IPI≤2, early CR and ASCT were associated with less relapse and death. Multivariate analysis showed ECOG ≥2 was an independent risk factor for disease progression (HR=4.321, 95% CI 1.127~16.572, P=0.033) and death (HR=46.254, 2.150~993.190, P=0.014) and ASCT was associated with better PFS (HR=0.058, 95% CI 0.007~0.495, P=0.009) and OS (HR=0.019,95% CI 0.001~0.596, P=0.024). Figure 1 highlights the OS and PFS of whole cohort (A) and ASCT patients (B). Myelosuppression was the most common adverse reaction(AE). The incidences of level 4 neutropenia, thrombocytopenia and anemia were 46.6%, 28.6% and 5.3% respectively. The most common non-hematologic AE was fever with neutropenia (36.5% of total cycles), while others were mild and manageable.

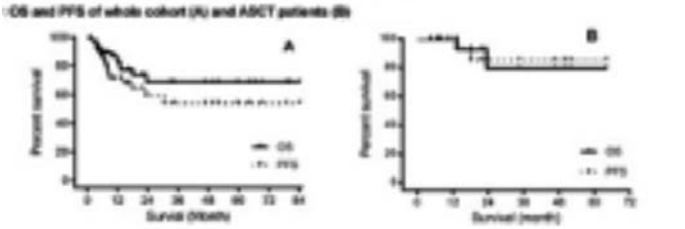

Figure 1.

Summary/Conclusions: GLIDE is an effective regiment for newly diagnosed stage IV and relapsed ENKTL. Up-front ASCT after achieving CR can reduce relapse and prolong survival. Treatment related adverse reactions and support care need concerns.

P225

LONG TERM FOLLOW-UP OF PATIENTS WITH PERIPHERAL T-CELL LYMPHOMAS TREATED WITH IFOSFAMIDE, ETOPOSIDE, EPIRUBICIN / INTERMEDIATE METHOTREXATE AND AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background: Despite improvement in the outcome for some subtypes of lymphoma, the prognosis of patients with peripheral T-cell lymphoma (PTCL) remains unsatisfactory. The recent data on dose-dense intensified cyclophosphamide, doxorubicine, vincristine and prednisone (CHOP) with addition of etoposide followed by autologous stem cell transplantation (ASCT) are encouraging. After achieving very promising results with in enteropathy associated T-cell lymphomas (EATL), we prospectively evaluated the alternative approach with more intensive treatment with ifosfamide, etoposide, epirubicin / methotrexate and ASCT (IVE/MTX–ASCT) in patients with other subtypes of PTCL.
Aims: To asses the long-term follow-up results of first line high-dose therapy IVE/MTX–ASCT in patients with PTCL.
Methods: The regimen was piloted for patients with a de-novo diagnosis of PTCL eligible for intensive treatment. The therapy delivers one cycle of CHOP, followed by 3 courses of IVE alternating with intermediate dose MTX. Stem cells are harvested after IVE and complete and partial remissions were consolidated with ASCT. The patients were evaluated with an intention-to-treat analysis for feasibility, response, progression free survival (PFS) and overall survival (OS) and late events.
Results: 30 patients were included: 17 peripheral T-cell lymphoma NOS, 6 anaplastic large cell lymphoma (ALCL) ALK positive, 4 extranodal NK/T-cell lymphoma nasal type, 2 ALCL ALK negative and 1 hepatosplenic T-cell lymphoma. The median age at diagnosis was 42 years (range 22 – 64), 37% patients were female, 28% presented with ECOG >1 and 57% with advanced stage disease. The age adjusted IPI was calculated for 22 patients with primary nodal disease and 41% of patients were in high intermediate and high risk. Three patients discontinued treatment prematurely due to disease progression and one due to poor general condition. Of the remaining 26 patients 19 went on to receive ASCT. ASCT was omitted due to: insufficient stem cell mobilisation in 4 patients, refractory disease in 2 and poor general condition in one. Toxicity was acceptable for such an intensive regimen. At final evaluation, complete remission (CR) was confirmed in 23/30 (77%) patients and partial remission (PR) in 2/30 (7%) patients. When the patients with ALCL ALK positive were excluded, the remission rates remained the same: CR 18/24 (75%) and PR 2/24 (8%); p>.999. During the study time 13/30 (43%) patients died, 11 due to lymphoma. For all patients 5-years PFS was 57% and OS 63%. These results were unchanged after the exclusion of ALCL ALK positive: 50% and 58%; p=.587 and p=.70; respectively. The 5-year PFS and OS of histological subgroups were as follows: ALCL, ALK positive: both 83%, PTCL NOS: 47% and 53%, ALCL, ALK negative: 0% and 50%, extranodal NK/T-cell lymphoma, nasal type: both 100% and for hepatosplenic T-cell lymphoma: both 0%. During the median follow-up time of 6.35 years, 13 patients had a relapse of underlying disease, six of them had received no treatment and died shortly after the diagnosed of relapse, further six received systemic chemotherapy and in one patient no information on relapse treatment was available. Among the six patients treated with systemic chemotherapy four received curative treatment including high-dose chemotherapy with allogenic stem transplant (alloSCT) in three patients and two received palliative treatment. Only two patients who received dexamethasone, high-dose cytarabine and cisplatin (DHAP) followed with alloSCT achieved a long lasting remission. Seven years post IVE/MTX-ASCT one patient developed secondary malignancy ALM/MDS and died shortly after the diagnosis.
Summary/Conclusions: IVE/MTX-ASCT significantly improves outcome in patients with PTCL, and has acceptable toxicities. Where feasible patients with PTCL could be considered for aggressive treatments, like IVE/MTX–ASCT as primary therapy, further studies are required.

Bone marrow failure syndromes incl. PNH - Biology

P226

IDENTIFICATION OF A NOVEL GERMLINE MECOM / EVI1 VARIANT THAT RUNS IN A PEDIGREE WITH RADIOULNAR SYNOSTOSIS AND AMEGAKARYOCYTIC THROMBOCYTOPENIA AND PREDISPOSES TO ADULT ONSET MYELOID MALIGNANCY

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Background: Radioulnar synostosis and amegakaryocytic thrombocytopenia (RUSAT), one of the rare bone marrow failure syndromes, is caused by a point mutation in *HOXA11*. In three simplex patients, *de novo* missense variants in *MECOM* have recently been reported as an alternative cause in individuals with RUSAT. *MECOM*, identified as a common ecotropic viral integration site 1 (*EVI1)* in murine myeloid leukemia, is known as a key transcriptional regulator in hematopoiesis and is frequently involved in sporadic myeloid leukemia.
Aims: To screen for the causative genetic alteration in a family with four affected individuals out of three generations with radioulnar synostosis, incompletely penetrant congenital thrombocytopenia, hearing impairment due to dysplastic middle ear bones, patellar hypoplasia, and hand and foot dysmorphisms. Notably, two of four affected individuals in our family developed adult onset myeloid malignancies (*i.e.* myelodysplastic syndrome (MDS) with excess blasts and MDS/myeloproliferative neoplasm-unclassifiable). No *HOXA11* mutation was identified in this family.
Methods: Whole exome sequencing was performed in three affected individuals using a *Nextera Rapid Capture kit* and a *NextSeq 500* instrument (Illumina, Munich, Germany). Identified sequence variants were filtered for those that are (i) called in all three subjects, (ii) predicted to be damaging (SIFT, Polyphen, and MetaLR), (iii) reported to have an allele frequency of ≤0.1% (1000G, ESP6500, ExAc), and (iv) not listed in our in-house database of recurrent variants.
Results: Following this approach, a novel MECOM missense variant (*i.e.* Cys766Gly, UniProtKB Q03112-1) was identified. The missense mutation affects a heavily conserved cysteine residue in C_2H_2-zinc finger motif 9 in the C-terminal zinc finger domain of MECOM. This residue is crucial for the tetrahedral coordination of a zinc ion stabilizing the zinc finger conformation and thus, is essential for DNA binding of the C-terminal zinc finger domain.
Summary/Conclusions: Our findings confirm the causality of *MECOM* missense mutations targeting the C-terminal zinc finger domain in subjects with RUSAT and indicate that *MECOM* needs to be considered in RUSAT pedigrees with no *HOXA11* mutation. We report here for the first time that *MECOM* germline mutations are associated with an increased risk for adult onset myeloid malignancies. This extends the RUSAT-associated phenotype and proposes that *MECOM* germline mutations can cause a genetic predisposition to adult onset myeloid malignancy.
[BZ and DS contributed equally to this work].

P227

LOSS OF THE HOMOLOGOUS RECOMBINATION GENE RAD51 LEADS TO FANCONI ANEMIA-LIKE SYMPTOMS IN ZEBRAFISH

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Background: Fanconi anemia (FA) is a hereditary DNA repair disorder characterized by various congenital abnormalities, progressive bone marrow failure and cancer predisposition. *RAD51* has recently been designated as a Fanconi anemia (FA) gene, following the discovery of two patients carrying dominant negative mutations. RAD51 is an indispensable homologous recombination protein, necessary for strand invasion and crossing over. It has been extensively studied in prokaryotes and lower eukaryotes. However, there is a significant lack of knowledge of the role of this protein and its regulation in an *in-vivo* context in vertebrates due to the early embryonic lethality of murine *Rad51* mutants.
Aims: Here we aim to utilize the powerful genetics and translucency of zebrafish to dissect the role of *rad51* in hematopoiesis and to explore the molecular basis of Fanconi anemia pathogenesis.
Methods: Zebrafish carrying homozygous loss of function mutations in *rad51*

generated by ENU mutagenesis were characterized in terms of their hematopoietic and non-hematopoietic phenotypes during embryonic development and adulthood.
Results: The *rad51* mutant fish developed key features of FA, including hypocellular kidney marrow (equivalent to mammalian bone marrow), sensitivity to crosslinking agents and decreased size. Interestingly, although mutants can survive to adulthood, they develop exclusively as sterile males. We show that some of the hematological symptoms stem from both decreased proliferation and increased apoptosis of embryonic hematopoietic stem and progenitor cells. Co-mutation of *p53* was able to rescue the embryonic and adult hematopoietic defects seen in the single mutants, but led to early tumor development in the adult double mutants. We further establish that prolonged inflammatory stress can exacerbate the hematological impairment, leading to an additional decrease in kidney marrow cell numbers linked to excess *p53* expression (Figure 1).

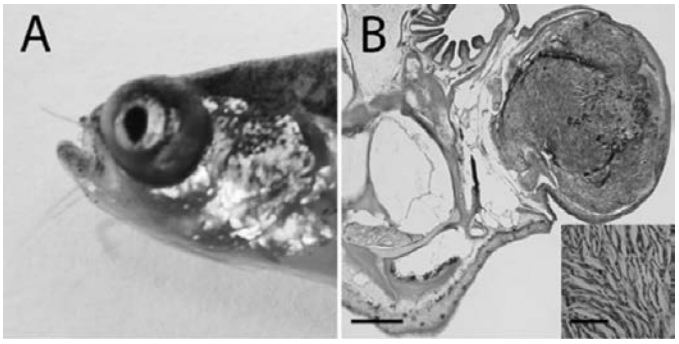

Figure 1. Example image of a p53, rad51 double mutant fish with a tumor behind the eye (A). Histological analysis showed the tumour to be a malignant peripheral nerve sheath tumor (B). The scale bar is 500 and 10µm respectively.

Summary/Conclusions: We demonstrate that zebrafish lacking functional *rad51* are viable and develop symptoms resembling FA. These findings strengthen the assignment of *RAD51* as a Fanconi gene and provide more evidence for the notion that aberrant p53 signaling during embryogenesis leads to the hematological defects seen during later stages of life in FA patients. Further research on this novel zebrafish FA model will lead to a deeper understanding of the molecular basis of bone marrow failure in FA and the cellular role of the RAD51 protein.

P228

A NOVEL TELOMERASE RNA COMPONENT VARIANT IN A FAMILY WITH MACROCYTOSIS AND MILD VARIABLE CYTOPENIAS

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Background: Telomerase RNA component (TERC), encoded by the *TERC* gene, is an essential component of telomerase, a polymerase that adds the telomeric repeat to the 3' lagging strand of DNA during cell replication. *TERC* variants have been causally associated with several haematological disorders, including autosomal dominant dyskeratosis congenita (DKC), aplastic anaemia, myelodysplastic syndrome and acute leukaemia, sometimes accompanied by non-haematological phenotypes. Here we report a likely pathogenic *TERC* variant associated with a haematological phenotype that predominantly affects the red cell lineage.
Aims: To describe the genotypic and phenotypic relationship of a new *TERC* variant.
Methods: Genomic DNA samples were analysed for sequence variants using the Oxford Red Cell Panel, a panel of 33 genes previously associated with human red cell disorders. Sanger sequencing was used to confirm the genetic variant. Telomere lengths were performed at the Laboratory for Molecular Haemato-Oncology (LMH), Rayne Institute, Kings College Hospital.
Results: The index case AM (I.1) was a female who presented at age 56 with fatigue, and was noted to have a longstanding non progressive mild macrocytic anaemia with very minimal thrombocytopenia. Further investigations (Table 1) revealed normal reticulocyte count, LDH, haematinics, thyroid function, liver and renal function. Bone marrow aspirate demonstrated abnormal erythropoiesis with nucleo-cytoplasmic asynchrony, nuclear atypia, ragged cytoplasm, basophilic stippling and bi-nucleate forms. Granulopoiesis and megakaryopoiesis were normal. The two daughters of I.1 also had abnormal blood counts and her paternal grandfather died of "pernicious anaemia". None of the family have somatic features associated with DKC. The elder daughter (age 30) TW (II.1), had isolated lifelong macrocytosis and previous mild neutropenia (Table 1). The younger daughter (age 27) BM (II.2) had macrocytic anaemia, thrombocytopenia (Table 1) and a recent pregnancy complicated by worsening thrombocytopenia, pre-eclampsia, placental dysfunction, liver dysfunction and foetal loss. Following delivery her liver function slowly returned to normal and a fibroscan was within normal limits. All three pedigree members with macrocytosis had a Chr3:169482668 (GRChr37) single nucleotide variant corresponding to a n.181A>C substitution in *TERC* (relative to transcript ENST00000602385.1), within the pseudoknot domain. Residue n.181 is highly conserved across mammalian species. This variant is absent from the gnomAD database of more than 230,000 *TERC* alleles, and the HGMD databases. The variant is within a *TERC* region in which previously reported variants have been associated with haematological phenotypes. In order to determine the pathogenicity of this variant, telomere lengths were assessed and found to be short in both Case I.1 and II.2 (Table 1). There were no other likely pathogenic variants in the Oxford Red Cell Panel genes. Together, these observations suggest that the n.181A>C substitution is causally associated with the macrocytosis phenotype.

Table 1.

Parameter (normal range)	I.1 (index case)	II.1	II.2
Haemoglobin (120-150g/l)	94g/l	120g/l	100g/l
Mean cell volume (83-100fL)	111.6fL	106.0fL	107.4fL
Mean cell haemoglobin (27-32pg)	33.3pg	34.1pg	33.6pg
Total white cell count (4-10x10^9/l)	4.36x10^9/l	6.83x10^9/l	5.07x10^9/l
Neutrophils (1.5-7.5x10^9/l)	2.3x10^9/l	5.4x10^9/l	2.3x10^9/l
Lymphocytes (1-4x10^9/l)	1.51x10^9/l	1.15x10^9/l	2.05x10^9/l
Monocytes (0.52-1x10^9/l)	0.48x10^9/l	0.32x10^9/l	0.52x10^9/l
Eosinophils (<0.51x10^9/l)	0.06x10^9/l	0.11x10^9/l	0.18x10^9/l
Basophils (<0.11x10^9/l)	0.01x10^9/l	0.01x10^9/l	0.02x10^9/l
Platelets (150-400 x10^9/l)	143x10^9/l	187x10^9/l	91x10^9/l
Reticulocytes (50-100x10^9/l)	85x10^9/l	53.7x10^9/l	86.1x10^9/l
Serum erythropoietin (3-18mIU/ml)	-	-	504.9mIU/mL
Blood film appearance	Anisopoikliocytosis, macrocytosis, irregularly contracted cells, polychromasia, large platelets	Macrocytosis only	Anisopoikliocytosis, macrocytosis, large platelets
Telomere length analysis (compared to normal individuals of the same decade of age)	Short telomere length, <1st percentile	-	Short telomere length, <1st percentile

Representative laboratory test results

Summary/Conclusions: This report demonstrates a likely causal association between a newly identified *TERC* variant, short telomere length and a relatively mild haematological phenotype that is largely restricted to red cells. This emphasises the phenotypic heterogeneity associated with *TERC* variants, justifies the rationale of screening multiple genes simultaneously and suggests that *TERC* variant could potentially underlie a broader range of unexplained heritable blood cell abnormalities.

P229

GENERATION OF X-LINKED DYSKERATOSIS CONGENITA-LIKE HUMAN HEMATOPOIETIC STEM CELLS

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Background: X-linked Dyskeratosis congenita (X-DC) is an inherited syndrome caused by mutations in the *DKC1* gene that encodes for the dyskerin nucleolar protein. These mutations reduce the telomerase activity leading to premature telomere length attrition. Several organs can be affected in these patients, although the bone marrow failure (BMF) is the main cause of death in X-DC patients (more than 70% of cases). So far, the only curative treatment for BMF in DC patients is hematopoietic stem cell (HSC) transplantation. However, risks derived from conditioning regimes and the difficulties to find a compatible donor suggest that gene therapy may constitute a promising alternative in treating DC patients.

Aims: Because of the difficulties associated to the use of primary HSCs from DC patients for experimental studies, this study was focused on the generation of X-DC-like human HSCs by means of the down-regulated expression of dyskerin in cord blood HSCs using different anti-*DKC1* short hairpin RNAs (shRNA).
Methods: CD34+ cells were obtained by immunomagnetic purification from healthy human umbilical cord blood samples. These cells were then pre-stimulated and exposed to two cycles of transduction with lentiviral vectors carrying both an anti-*DKC1* shRNA and the puromycin-resistance gene. Transduced samples were then selected for 2 days with puromycin, and cultured *in vitro* or transplanted into immunodeficient NSG mice to evaluate the effects of shRNAs.
Results: Based on the inhibition of *DKC1* gene expression, 3 shRNAs were selected among 7 designed shRNAs. Interfered HSCs showed an inhibited telomerase activity, as well as a reduced clonogenic and hematopoietic reconstitution potential in NSG mice. Additionally, an increase in DNA damage and senescence was observed in *DKC1*-interferred CD34+ cells.
Summary/Conclusions: *In vitro* and *in vivo* data obtained from *DKC1*-interferred CD34+ cells show that these cells mimic the phenotype of primary X-DC-HSCs. The generation of X-DC-like HSCs will facilitate the understanding of the molecular basis of the HSC defects characteristic of X-DC and contribute to the development of new experimental therapies for the treatment of the BMF of X-DC patients.

P230

STUDY OF EXTRACELLULAR VESICLES ROLES IN THE PATHOPHYSIOLOGY OF THROMBOSIS IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS DURING ECULIZUMAB TREATMENT: A PILOT PROSPECTIVE LONGITUDINAL CLINICAL STUDY

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a disease characterized by complement-mediated hemolysis (Brodsky *et al.* Hematology, 2008). Complement can induce the production of extracellular vesicles (EV) (Burnouf *et al.* Transfus Apher Sci, 2015). These EV are cell-derived vesicles whose the size-range is around 50 and 1000nm. They can expose phosphatidylserine (PS-anionic phospholipid) and tissue factor (TF), which explains their involvement in the coagulation cascade (Owens *et al.* Circ Res, 2011). The EV could have a role in the thrombus formation, the leading cause of death in PNH patients (Brodsky *et al.* Hematology, 2008; Simak *et al.* Br J Haematol, 2004; Hugel *et al.* Blood, 1999). Eculizumab, a human anti-C5 monoclonal antibody, used in the treatment of PNH seems to decrease the thrombosis frequency (relative reduction of 85% of thromboembolism event rate with the introduction of the treatment in the patients) (Hillmen *et al.* Blood, 2007; Kelly *et al.* Ther Clin Risk Manag, 2009; Weitz *et al.* Thromb Res, 2012; Al-Jafar *et al.* Hemato Rep, 2015).
Aims: The general purpose of this project is a better understanding about the role of EVs in thrombosis in the context of PNH patients under eculizumab. We assessed the impact of eculizumab on the EV quantification and on their procoagulant activity, in order to check, if the antithrombotic activity of the eculizumab could be in part explained by its interaction with the EVs.
Methods: We conducted a pilot prospective open label longitudinal clinical study with six PNH patients treated with eculizumab. The study was led according to the declaration of Helsinki and approved by the local Ethic Committee. Informed consent was obtained for each patient. The aim was to measure, by flow cytometry, the production of EVs in patient's platelet-free plasma (PFP) before the start of eculizumab, after 4 weeks and after 11 weeks of treatment. We also assessed the procoagulant activity in PFP by STA®-Procoag-PPL assay and by thrombin generation assay (TGA). A more sensitive version of TGA was also performed to study the procoagulant profile induced by the EVs (use of EVs pelleted from PFP). We used mixed-effects linear regression (R 3.1.2 with nlme package) with logarithmic transformation for flow cytometry results. We compared the results after 4 weeks or 11 weeks of treatment against the inclusion value.
Results: We observed a decrease in platelet EVs with the eculizumab treatment ($p<0.05$). STA®-Procoag-PPL assay showed a decrease of the procoagulant profile induced by procoagulant phospholipids (PL) with the treatment. These results were not confirmed by TGA on PFP, due to a lack of sensitivity. By this way, we performed a more sensitive version of TGA that allows to observe variation in the procoagulant profile induced by the EV with the eculizumab ($p<0.05$).
Summary/Conclusions: Eculizumab has an impact on the amount and the procoagulant profile induced by the procoagulant PL and the EVs. The anti-thrombotic performance of the eculizumab can be in part explained by its action on EVs.

P231

TELOMERE LENGTH SCREENING TRIGGERED BY CLINICAL SUSPICION FOR CLASSICAL AND/OR CRYPTIC DYSKERATOSIS CONGENITA – PROSPECTIVE RESULTS FROM THE AACHEN TELOMEROPATHY REGISTRY

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Background: *Classical* Dyskeratosis Congenita (DKC) is a multisystem disorder caused by defective telomere maintenance, mostly due to mutations in genes related to functional activity of telomerase or accessibility of the telomeres themselves. Clinical characteristics are mucocutaneous abnormalities, bone marrow failure, an increased predisposition to cancer and other variable features. While *classical* DKC is typically diagnosed in childhood or adolescence, the incidence of *cryptic* DKC variants typically presenting with a clinically more heterogeneous picture also involving the liver, gut and respiratory system in adults up to 50 years of age is unknown. Accelerated shortening of Telomere length (TL) in peripheral blood leucocytes represents the functional read-out of altered telomere maintenance and thus allows for a screening of patients with suspected DKC. On the basis of TL measured below the 1% percentile of age adjusted healthy controls, next-generation-sequencing (NGS) analysis for underlying mutations was triggered.
Aims: In this study, we report the first results of such a clinical routine screening for telomeropathies carried out within the Aachen Telomeropathy Registry (ATR).
Methods: 184 patients from 52 participating centers (80% academic centers) within Germany, Austria and Switzerland were screened for premature telomere shortening and included with informed consent into the ATR since November 2014. Inclusion criteria and reason for screening was either the clinical suspicion of the treating physician for a telomere maintenance disorder and/or the recommendations of the German Society of Hematology and Oncology (DGHO) published via *Onkopedia*. TL analysis of peripheral blood granulocytes and lymphocytes was carried out using combined fluorescence *in situ* hybridization and flow cytometry (flow-FISH). Mutations in genes suspected to cause telomeropathies (*i.e.* TERT, TERC, DKC1, NOP10, NHP2, USB1, CTC1, RTEL1, TIN2, TCAB1) were analyzed by NGS using customized primer panels and amplicon-based sequencing on a Miseq sequencer (Illumina) in all patients with TL in lymphocytes below the 1% percentile of healthy controls.
Results: Underlying initial diagnosis by the treating physician leading to TL screening were aplastic anemia (AA, n=72, 39% of cases), unexplained cytopenias (UC, n=21, 11%), myelodysplastic syndrome (MDS, n=18, 10%), family members (FM) of known DKC patients (FM-DKC, n=17, 9%), atypical squamous cell cancer of the head-and-neck (SCCHN, n=10, 6%), paroxysmal nocturnal hemoglobinuria (PNH, n=9, 5%), acute myeloid leukemia (AML, n=5, 3%) as well as other disorders (*e.g.* lung fibrosis, Diamond-Blackfan-Anemia, Dubowitz-syndrome, etc., n=32, 17%). Median age was 41.5 y (range from 0.5 to 88 y). TL screening revealed 20% (38/184) patients with lymphocyte TL and 16% (30/184) of patients with granulocyte TL below the 1% percentile. NGS screening identified typical mutations associated with altered telomere maintenance in 15 out of 38 patients (40%) representing 8,2% of the total patient population. Median age of patients with mutations was 45.0 y (range 21 to 68 y). Mutations were detected in RTEL1 (n=3), TERC (n=6), TERT (n=3) and DKC1 (n=3). Mutations were observed in 5% of all AA, 12% of all UC, 50% of all FM-DKC, 13% of all SCCHN, 20% of all screened AML patients.
Summary/Conclusions: We provide the first analysis of a routine TL screening program (within the ATR) for clinically suspected telomeropathy in patients up to the age of 88 y. TL screening is feasible in a routine clinical setting identifying approximately 20% of all samples to reside below the 1% percentile. Genetic testing confirmed the diagnosis of *cryptic* DKC in a variety of initial diagnoses. This study highlights both the diagnostic value of TL screening for *cryptic* DKC as well as its underrated incidence in adults. Proper diagnosis of DKC however is of utmost importance given its significant individual clinical implications towards prognosis, treatment and family counseling.

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TRANSPLANTATION IN PATIENTS WITH ACQUIRED APLASTIC ANEMIA OVER THE AGE OF 40: MORTALITY HAS NOT BEEN REDUCED IN 2010-2015

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Background: Mortality following HSCT in SAA pts over the age of 40 is reported to be in the order of 50%, without taking in to account long term sequelae such as chronic GvHD, known to be more frequent in older patients. This has prompted international guidelines to recommend first line immunosuppressive therapy above 40 years of age. The question is whether this is still true in 2017.

Aims: Assess whether TRM in SAA patients grafted 2010-2015 is reduced, as compared to the era 2001-2009.

Methods: We used the WPSAA EBMT registry, and identified 748 pts aged 40 years or more, with acquired SAA, grafted between 2001 and 2015. We divided pts in 2 transplant eras:2001-2009 (n=327) and 2010-2015 (n=407). In the more recent period (2010-2015) pts were older (53 *vs* 49 year, p<0.01), were more often grafted from alternative donors (ALT) (64% *vs* 43%, p<0.01), with a greater use of BM (54% *vs* 41%, p<0.01), and with a longer interval dx-tx (317 *vs* 258 days , p0.01), and more often received a fludarabine containing regimen (55% *vs* 42%, p<0.01).

Results: The overall survival 5 year survival of pts grafted in 2001-2009 was 57% , compared with 55% for pts grafted 2010-2015 (p-=0.7). In multivariate analysis, including the interval diagnosis transplant, patient's age, donor type, stem cell source and conditioning regimen, the lack of improved survival in 2010-2015 was confirmed (p=0.3).A very strong age effect was shown both in univariate and multivariate analysis: survival of pts aged 40-50 years, 51-60 years and >61 years , was respectively 64%, 54%, 41% (p<0.0001) and this was confirmed in multivariate analysis. The conditioning regimen , also proved to be a significant predictor, with improved survival for ALT transplants receiving FLU containing regimens (56% *vs* 46%, p<0.001). In general pts receiving either CY200 or a FLU containing regimen , did significantly better than pts receiving other preparative regimens (58% *vs* 50%, p=0.02). The use of a sibling donor (SIB) did not prove to predict survival in multivariate analysis. Pts receiving Campath in the conditioning , did significantly better than pts not receiving Campath (65% *vs* 54% p<0.01); similarly survival of patients with ATG was superior 59% *vs* 41% compared to patients not receiving ATG (p<0.01). When pts receiving either Campath or ATG (n=564) were compared to patients not receiving either (n=161), the difference in survival was 61% *vs* 41% (p<0.0001), and this was significant also in multivariate analysis. Combined primary and secondary graft failure was reduced from 16% to 12% in the two time periods (p=0.02), acute GvHD grade II-IV was reduced from 15% to 11% (p=0.1) and chronic GvHD was also reduced from 32% to 26% (p=0.04) Infections remain the leading cause of death in both transplant eras (18% and 22% respectively), followed by GvHD (5% and 4%) and graft failure (5% and 2%), whereas PTLD have been reduced from 3% to 0,5% (Figure 1).

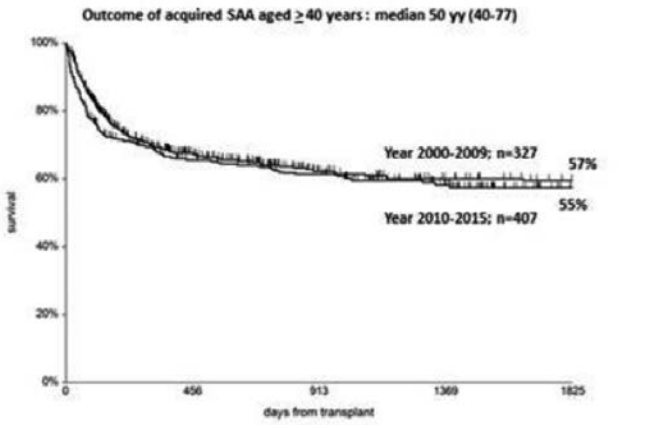

Figure 1.

Summary/Conclusions: HSCT in pts with acquired SAA aged 40 and over, continues to carry a significant risk of TRM also in 2010-2015, ranging from 36% in younger pts (40-50) to 59% in older pts (>60 years). Survival is predicted in multivariate analysis, by two crucial predictors:patients age and the use of either Campath or ATG,the latter giving a 20% survival advantage over no Campath /ATG. ALT and SIB donors produce similar survival. This study gives further support to current guidelines, suggesting first line therapy with ATG+CsA, in pts over the age of 40.

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CLINICAL AND GENETIC DIVERSITY IN DIAMOND-BLACKFAN ANAEMIA: AN UPDATE FROM THE UNITED KINGDOM

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Background: Diamond-Blackfan anaemia (DBA) is an inherited bone marrow failure syndrome (IBMFS) caused by mono-allelic, loss-of-function mutations in ribosomal protein (RP) genes. DBA is rare and has a wide spectrum of clinical manifestations, hence the utility of patient registries.

Aims: We evaluated the clinical and genetic spectrum of DBA in a large cohort of patients in the UK, aiming to identify novel features of the disease.

Methods: We performed a retrospective analysis of data from 103 confirmed cases of DBA, including 4 multiplex families. All living patients had undergone at least one assessment at our specialized centre in the last 5 years. Data were collected from family interviews, patient records and referring clinicians.

Results: The 103 patients with DBA were born in a 48-year period (1967-2015), *i.e.*, an incidence of 3 per million live births. Demographic and clinical characteristics are shown in Table 1. NGS analysis of 80 RP genes plus *GATA-1* identified pathogenic mutations in 71% of cases and 7 putative novel mutations, currently undergoing validation. To date, mutation screening of *both* parents has been performed in 32 families with DBA. Twenty-five mutations are sporadic while 7 are autosomal dominant; in 3 of the latter, the parent is a silent 'carrier' without anaemia. In one case of an affected child, the causative mutation was undetected in the peripheral blood of both parents but was present in 7/22 embryos generated for *in vitro* fertilisation, suggesting germline mosaicism. 80.5% of cases in our cohort presented within the first year of life. For the first time we report a high rate of perinatal problems in DBA. Prematurity +/- intrauterine growth restriction (IUGR) occurred in 31/87 (35.6%) of evaluable patients. Specific abnormalities included: hydrops fetalis (3/87), prematurity (22/87) and IUGR (16/87). In addition to congenital anomalies classically associated with DBA, we identified abnormalities of the spine and axial skeleton in 9.2% of patients. These did not correlate with a particular genotype. Our cohort exhibited multiple comorbidities, including some not previously reported to be associated with DBA: herniae (10.7%), neuropsychiatric (17.4%) and gastrointestinal (GI) disorders (25.7%). These complications were not associated with particular treatment regimens. In terms of the natural history of DBA, a lower proportion of our patients (22%) than previously reported in the literature (40%) were able to maintain a normal Hb on long-term steroids. Three patients failed a metoclopramide trial. In total there were 4 incidents of malignancy (MDS, B-ALL, BCC and cervical intraepithelial neoplasia) in 4 different patients. The lower incidence in our cohort compared with that reported by the North American DBA registry may be explained by differences in the median ages of the 2 cohorts (12y *versus* 18y, respectively) and the shorter follow-up of our patients.

Table 1.

Demographic and clinical characteristics of 103 patients with DBA in the United Kingdom.
Incomplete information was available for some individuals therefore the number of patients for whom data were available (*N=*) is included for each parameter.

Parameter	N (%) or median [range]	Parameter	N (%) or median [range]
Female/male	55/48	Congenital anomalies *N=98*	
		None	19 (19.4)
Ethnicity		Single anomaly	24 (24.5)
Caucasian	83	Multiple anomalies	55 (56.1)
African	3	➢ Cardiac	35 (35.7)
Asian	10	➢ Upper limb	33 (33.7)
Middle Eastern	3	➢ Axial skeleton including spine (confirmed on imaging)	9 (9.2)
Mixed	2	❖ Spina bifida	1
Caribbean	1	❖ Cervical spine fusion	3
Other	1	❖ Abnormal spinal curvature	3
		❖ Cervical ribs	1
Age at inclusion in study (yrs) *N=103*	11.9 [1.2-49.4]	➢ Craniofacial	27 (27.6)
		➢ Genitourinary	5 (5.1)
Birth weight (kg) *N=68*	2.9 [1.25-5.1]		
Age at diagnosis *N=97*	2.4 months [22/40 in utero – 20 years]	Co-morbidities *N=103*	
		Endocrine dysfunction	
Haemoglobin at diagnosis (g/L) *N=81*	43.8 [17-94]	➢ Osteopenia/Osteoporosis + Vitamin D deficiency	15
		➢ Adrenal insufficiency	3
RP gene mutation/deletion by NGS+/-Multiplex Ligation-dependent Probe Amplification *N=100*		➢ Thyroid dysfunction	2
		➢ Growth Hormone dysfunction	2
RPS19	19	➢ Hypogonadism	2
RPS26	14	➢ ≥2 of above	4
RPL5	14	➢ Total	28 (27.2)
RPL11	12	Grommets/hearing aids	20 (19.4)
RPS24	3	Strabismus	7 (6.8)
RPL35A	3	Umbilical or inguinal hernia	11(10.7)
Other RP genes (RPS7, RPS10, RPS17, RPL15, RPS29)	6	Neuropsychiatric disorders	
		➢ Seizures	2
Awaiting validation - novel mutations in known RP genes (n=4) or novel RP genes (n=3)	7	➢ Delayed motor development	4
		➢ Global developmental delay	2
None detected	22	➢ Mood disorder	3
		➢ Learning difficulties	7
Short stature <0.4th centile *N=89*	27 (30.3)	➢ Total	18 (17.4)
		GI disorders	
Treatment status at time of study *N=103*		➢ Severe dairy intolerance	5
Transfusion-dependent; steroid failure	39 (37.9)	➢ Oesophagitis	9
Transfusion-dependent; steroid intolerant	7 (6.8)	➢ Colitis	1
Transfusion- dependent; steroid not yet trialled	3 (2.9)	➢ Chronic diarrhoea/constipation	10
Steroid- responsive	23 (22.3)	➢ ≥ 2 of above	4
Anaemia not requiring treatment	3 (2.9)	➢ Total	29 (25.7)
Remission post-steroids	6 (5.8)		
Spontaneous remission	4 (3.9)		
BMT	16(15.5)		
Deceased	2(1.9)		

Summary/Conclusions: This retrospective analysis of the UK's DBA cohort confirmed several findings from other registries but also revealed novel features, including a high prevalence of i) premature birth and neonatal complications ii) abnormalities of the axial skeleton and iii) neuropsychiatric disorders. Prospective longitudinal studies are warranted to better characterise these co-morbidities and to confirm whether they are intrinsic to DBA or arise as complications of treatment. Above all, the observed clinical heterogeneity in our cohort highlights the need for novel therapies that target the multisystem manifestations of DBA, not just the anaemia.

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BONE MARROW FAILURE SECONDARY TO NOVEL/KNOWN PRIMARY IMMUNODEFICIENCY-RELATED MUTATIONS. A SINGLE CENTER ANALYSIS

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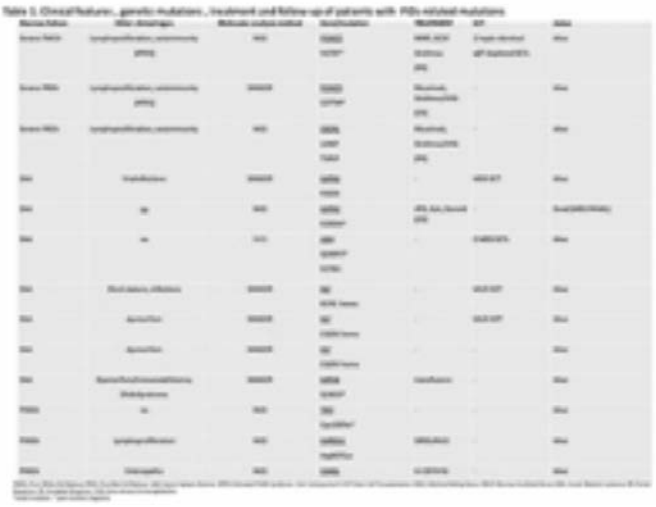
Background: Differential diagnosis between acquired and congenital forms of Marrow Failure (MF), has always represented a crucial point in the diagnostic work-up, since genetic forms do require a different therapeutic approach. It is also known that patients with congenital MF may also show immunodeficiency that, in some cases, can represent the first/ or revalent sign of the disease and therefore can be misinterpreted as a Primary Immunodeficiency (PID). On the other hand, patients with PIDs may also show MF as a result of an immune-mediated attack of marrow precursors thus generating a phenotypic overlap that can impair the correct diagnosis.
Aims: In this report we analyzed all patients with MF evaluated in our Unit with the aim to identify the type and incidence of underlying molecular defects, in particular those related to PIDs.
Methods: We retrospectively evaluated all diagnosis performed in patients with single/multi-lineage MF followed in our Unit. DEB test was used to screen Fanconi Anemia (FA). Other congenital MFs have been searched by Sanger and/or NGS molecular analysis depending on the available tools over the years.
Results: Between 2009-2016, 88 patients have been studied for single-lineage (25) or multilieage (63) MF. 48 (54%) were classified as having an acquired MF, 27 (30%) were diagnosed with a congenital MF (FA 11, Diskeratosis Congenita 5, Severe Congenital Neutropenia 6, Blackfan-Diamond Anemia 3, Congenital Amegakaryiocitic Thrombocytopenia 2), and the remaining 13 patients (14%) were found to have an underlying PID. Table 1 shows clinical characteristics and mutations of patients with PIDs.

Table 1.



Summary/Conclusions: This report shows that patients presenting with single/multi-lineage MF may have an underlying PID in a considerable number of cases. We also show that MF represented the most relevant clinical sign in patients with PI3KCD, TACI, or CD40L mutations, thus widening their clinical phenotype. We conclude that an accurate immunological work-up should be performed in all patients with MF and that PIDs-related genes should be included in the molecular screening of MF in order to identify specific disorders that may potentially receive targeted treatment and/or the appropriate conditioning regimen for SCT.

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COVERSIN, A NOVEL C5 COMPLEMENT INHIBITOR, FOR THE TREATMENT OF PNH: RESULTS OF A PHASE 2 CLINICAL TRIAL

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Background: Paroxysmal nocturnal haemoglobinuria (PNH) leads to episodic haemolysis secondary to an acquired deficiency of PIGA anchor molecules on the surface of erythrocytes which play a critical role in protecting the cells from complement mediated lysis. Until the advent of eculizumab, a monoclonal antibody which prevents the cleavage of C5 to C5a and C5b, PNH was associated with considerable morbidity and a poor long-term prognosis. However, eculizumab needs to be administered by health care professionals by intravenous infusion which may interfere with the life-styles, occupations and personal privacy of patients and the interval dosing has led to concerning breakthrough haemolysis. Coversin is a protein suitable for small-volume subcutaneous (SC) injection which can be self-administered by patients.
Aims: The aim of this study is to investigate the safety and efficacy of the complement C5 inhibitor Coversin in the treatment of PNH.
Methods: A Phase 2 single arm open label trial of Coversin is currently ongoing under which patients, either newly diagnosed with PNH or who have not previously had access to complement inhibitors, are treated for 90 days. Coversin is supplied as a lyophilised powder, reconstituted with water for injection to give a buffered aqueous solution of Coversin 30mg/mL. The trial population consists of up to 10 adult patients with a diagnosis of PNH confirmed by flow cytometry. Treatment commences with an ablating regime (AR) consisting of a fixed dose of 60mg followed by 3 doses of 30mg q12 hours delivered by SC injection. After being suitably instructed patients are encouraged to self-inject the drug. Following the AR, a dose of 15mg q12 hours is given for a further 26 days when, if the patient's disease is well controlled, they switch to 30mg q24 hours for the remainder of the trial. The dose can be increased by two incremental steps according to a pre-specified algorithm for patients not satisfactorily controlled on the basis of serum lactate dehydrogenase (LDH) or clinical grounds at any time during the 90-day period. The primary endpoints are safety and reduction of serum LDH to ≤1.8 X the upper limit of normal (ULN) for the local laboratory. Secondary endpoints include LDH at 28, 60 and 90 days, terminal complement activity assessed by CH50 ELISA (Quidel®), sheep erythrocyte haemolysis assay, PK (free and bound Coversin levels), anti-drug antibodies (ADA) and quality of life.
Results: The trial is still ongoing and has currently enrolled 5 patients, four of whom remain on Coversin. Three patients have required single dose increases during the initial 28-day period, one of whom was later withdrawn when a comorbidity was suspected. Two patients have moved to a single daily dose. Updated results of these and any patients enrolled subsequently will be presented. To date 2 patients have achieved the primary efficacy endpoint, two have not yet reached the 28-day point. There have been no serious or significant adverse events and the drug has been well-tolerated. A few mild injection site reactions have been recorded but these appear to diminish with time. There has been no evidence of the formation of neutralising antibodies.
Summary/Conclusions: It currently appears that treatment with Coversin is safe and effective in controlling hemolysis in PNH and that patients are capable of self-administering the drug. Coversin may be an effective alternative for patients with PNH who prefer the independence of self-administration. The relatively short dose interval may also help to reduce breakthrough events due to trough levels of drugs administered at two weekly intervals or longer.

Chronic lymphocytic leukemia and related disorders - Biology 1

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GERMLINE RARE VARIANT ASSOCIATION ANALYSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: CLL is a highly heritable cancer. Although GWAS have identified ~30 independent SNPs associated with CLL, these are estimated to account for only 19% of the inherited component of CLL.
Aims: We hypothesized that this missing heritability might arise from rare coding variants (MAF <0.01), and sought to identify these through an exome-wide association study comparing rare germline variants between CLL patients and controls.
Methods: We investigated 516 CLL patients of European descent who were compared to 8,920 ethnically matched, non-cancer population controls. CLL cohorts included 235 CLL patients from DFCI (128 previously reported, 107 unpublished exomes), and 281 CLL patients enrolled on the CLL8 trial of the German CLL Study Group (WES data reported previously). An additional 130 CLL patients in an extension cohort included 24 from our published whole-genome sequencing study and 106 from an early publication of the ICGC. Non-cancer controls came from 3 sources: 2,520 from the 1000 Genomes Project; 6,852 from the Exome Sequencing Project; and 7,611 from a study of genetic risk for myocardial infarction. We combined these heterogeneous datasets by: (i) processing sequencing data from all cohorts together and consistently; (ii) jointly calling the variants across all cases and controls; and (iii) analyzing only ethnically matched, unrelated samples over DNA sites with sequencing coverage sufficient to achieve high-confidence genotype calls. This quality control resulted in 8,920 controls available for the association analysis. We further controlled for residual population stratification by correcting for three principal components.
Results: Using an unbiased, gene-based rare variant association analysis comparing cases to controls, we identified two genes significantly enriched for rare coding variants in CLL cases: *CDK1* and *ATM* (OR 5.8, C.I.2.6-13.1, $p=5.8x10^{-7}$ and OR 1.6, C.I. 1.3-2.0, $p=1.4x10^{-6}$, respectively). *CDK1* variants were observed in 8 of 516 CLLs and 24 of 8,920 controls (1.6% *vs* 0.3%, OR=5.8, 95% CI 2.6-13.1). One recurrent missense variant, *CDK1* p.R59C, observed in 5 cases and 10 controls, is predicted to be possibly damaging by the PolyPhen2 prediction tool, and is driving the association. The second significant gene was *ATM*, in which we found a total of 112 cases carrying 52 distinct rare germline variants and 1296 controls carrying 292 rare variants (21.7% *vs* 14.5%; OR=1.6, 95% CI 1.3-2.0). The majority of recurrent rare variants in *ATM* were non-synonymous missense variants, with L2307F one of the most enriched (2.3% cases, OR=10.1, 4.9-20.7). Subsequent validation in 149 independent CLL cases revealed a similar frequency of 2.01% (3 out of 149) of the L2307F variant. We then added 130 CLL cases and performed an expanded joint analysis, which has been shown to improve the statistical power of detecting genetic associations compared to a two-stage replicate analysis. We identified 42 additional patients with rare *ATM* variants, and the significance of *ATM* was greatly increased (q=0.00016, OR 1.79, CI 1.49-2.15). We integrated somatic and germline sequencing data and found that patients with rare germline variants in *ATM* were more likely to harbor an additional *ATM* somatic lesion ($p=9.1x10^{-4}$). Furthermore, 80% of patients with both a rare germline variant in *ATM* and a somatic 11q deletion lost the wild-type *ATM* allele during deletion (p=0.012), suggesting that the germline variants behave as tumor suppressor alleles.
Summary/Conclusions: To our knowledge this analysis represents the first germline association analysis based on exome sequencing data in CLL, and our results implicate rare germline variation in *ATM* in CLL predisposition.

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DIFFERENTIAL ENHANCER TRANSCRIPTION ASSOCIATED WITH RISK ALLELE GENOTYPE IN CLL

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Background: Genome-wide association studies (GWAS) have identified multiple loci that are statistically associated with CLL susceptibility. These single nucleotide polymorphisms (SNPs) are primarily located in non-protein coding genomic regions. Data suggest that these variants are enriched in regulatory elements.
Aims: We tested the hypothesis that CLL risk variants are in or near regulatory elements that influence nearby target genes.
Methods: To investigate SNP allele-specific impacts on gene expression, we selected 15 SNPs from 13 loci that achieved genome wide significance in initial CLL GWAS studies. We investigated either the published GWAS SNP (if present on the Affymetrix 6.0 SNP array) or proxy SNP(s) chosen using the SNP Annotation and Proxy Search (SNAP) software, based on their high linkage disequilibrium (LD) ($r^2>0.68$) with the selected GWAS SNP. Genotypes were determined in tumor (n=143) and saliva (n=79) DNA from CLL patients (who had provided written informed consent); tumor and saliva DNAs were concordant in at least 96% of cases (except rs477184 at 92%). Given the high concordance with saliva, which is likely related to the stable genome of CLL, SNP genotypes from tumor samples were used for the analysis in order to significantly increase our sample size. Allele-specific gene expression was then evaluated in tumor samples (n=143) using Affymetrix U133 Plus 2.0 array gene expression data, focusing on genes within 1 Mb in either direction from a given SNP. In order to elucidate whether these associations were due to functional effects on transcription, we used a novel assay called precision run-on sequencing (PRO-seq). PRO-seq efficiently maps active transcription regulatory elements (TREs) and provides a sensitive, quantitative and directional map of transcriptionally-engaged RNA polymerases. The algorithm, discriminative regulatory-element detection from GRO/PRO-seq (dREG), is then used to predict the presence of TREs from raw PRO-seq data, allowing for identification of functional elements in the vicinity of SNPs and quantification of their allele-specific effect on enhancer activity and gene transcription.
Results: Our gene expression analysis demonstrated 6 significant SNP-gene associations: rs674313 (6p21.3) with *HLA-DQA1* (p <0.0001), rs872071 (6p25.3) with *IRF4* (p=0.01), rs4777184 (15q23; proxy for rs7176508) with *TLE3* (p=0.009), rs783540 (15q25.2) with *CPEB1* (p=0.01), rs305088 (16q24.1; proxy for rs305061) with *COX4NB/EMC8* (p=0.03) and rs4802322 (19q13.32; proxy for rs11083846) with *FKRP* (p <0.0001). Two associations were successfully validated in a completely independent gene expression replication analysis (n=54): rs674313 with *HLA-DQA1* (p<0.0001) and rs4777184 with *TLE3* (p=0.0118). To annotate candidate regulatory elements, we evaluated transcription level at or near all six significant SNPs in the initial gene expression analysis in a cohort of 12 CLL samples. Transcription level at or near 3 SNPs (rs674313, rs4777184, rs305088) correlated with genotype in a dose dependent manner. When we expanded the analysis to the entire region of LD around each SNP, we were able to demonstrate a dose-dependent effect in all SNPs except rs872071.
Summary/Conclusions: We conclude that PRO-seq and dREG analysis identifies evidence of active differential transcription based on genotype in the region of 5 out of 6 GWAS-identified SNPs that we have investigated so far.

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BIALLELIC TP53 GENE MUTATIONS DUE TO COPY-NEUTRAL LOSS OF HETEROZYGOSITY AND MONOALLELIC MUTATIONS IN ABSENCE OF 17P DELETION OCCUR IN CLL WITH COMPARABLE FREQUENCY

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Background: *TP53* gene defects represent an adverse prognostic marker in chronic lymphocytic leukemia (CLL). In the majority of affected cases, *TP53* is inactivated on both alleles due to the concurrent mutation and 17p deletion [del(17p)]. However, in about one third of cases, only *TP53* mutation (*TP53*mut) without deletion is detected. It was reported that in some of these patients, copy-neutral loss of heterozygosity (cn-LOH), also leading to biallelic *TP53* defect, might be present; however the frequency of such event has not been thoroughly investigated.
Aims: We aimed to perform a detailed analysis of the second *TP53* allele status in cases with a *TP53* mutation in the absence of del(17p), and to assess genomic makeup and clinical outcome in these patients.
Methods: We searched for patients with *TP53*mut in absence of del(17p) within the cohort of 200 CLL patients positive for *TP53*mut as determined using FASAY (Functional Analysis of Separated Alleles in Yeast) coupled to direct sequencing;

17p13 deletions were assessed by FISH (MetaSystems). More than a half of the cohort (57%) was also tested using ultra-deep NGS for *TP53* exons 2-11. Genome-wide analysis was performed on CytoScanHD arrays (Affymetrix) and correlated to conventional cytogenetics (CpG/IL-2 stimulation).
Results: Out of the cohort positive for *TP53*mut, 72/200 patients (36%) harbored single dominant *TP53*mut without del(17p). We selected 43 of these cases with variant allele frequency (VAF) >10% for CytoScan analysis to explore the potential presence of 17p cn-LOH. In 42% (18/43) of the cases, cn-LOH in 17p locus was detected in a proportion of CLL clone correspondingly to the *TP53* VAF (median *TP53* VAF 59.4%, range 12.9–99.9%). In 3/43 cases, heterozygous deletion previously undetected by FISH was newly revealed. Thus, the truly monoallelic mutations were confirmed in 51% (22/43) of the cases, where no cytogenetic abnormality in 17p locus was observed (median *TP53* VAF 43.5%, range 10.5–51.3%). Applying a VAF cut-off of 55% indicating fully expanded heterozygous mutation (taking into account the potential unequal representation of forward and reverse strands in NGS data), 7/29 (24%) cases below the cut-off still harbored 17p cn-LOH. These results show that it is not possible to use an arbitrary VAF cut-off (>50%) to identify biallelic mutations due to cn-LOH. When we compared genomic complexity of leukemic clones with monoallelic *vs* biallelic *TP53*mut as determined by the CytoScan array, the latter group exhibited significantly more genomic abnormalities (p=0.0388) and also preference for different recurrent chromosomal abnormalities (p<0.0001; 17p locus excluded from this analysis). However, there was no significant difference in overall survival between the groups (p=0.5856).
Summary/Conclusions: cn-LOH in 17p locus is present in approximately half of the patients with single dominant *TP53*mut and results in biallelic *TP53* gene inactivation despite the absence of del(17p); truly monoallelic *TP53* gene mutations with an intact second allele occur in CLL with comparable frequency. Although 17p cn-LOH is associated with increased genomic instability, it does not have worse impact on clinical outcome than truly monoallelic *TP53*mut.
Supported by the projects AZV-MZCR 15-31834A, 15-30015A, 15-29793A, the EU Horizon2020 project No. 692298, and MEYS CEITEC2020 LQ1601.

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INTERGRATED OLIGO/SNP ARRAY- AND NEXT GENERATION SEQUENCING BASED ANALYSIS IS REQUIRED TO DETERMINE TP53/17P STATUS IN CLL PATIENTS

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Background: B-cell chronic lymphocytic leukemia (CLL) exhibits a highly heterogeneous clinical course, with overall survival rates varying from several months to decades. Mutation status of the *IGHV* genes and specific genomic abnormalities, such as deletion of 11q22 en loss of the 13q14 region provide prognostic information. However, more importantly deletion of 17p and/or the presence of a *TP53* mutation, which are both associated with a poor prognosis identify CLL patients with the highest risk profile. Recently clinical trials with tyrosine kinase inhibitors such as ibrutinib and idelalisib have demonstrated good responses in CLL patients with 17p deletion and/or *TP53* mutations. In many studies interphase fluorescence *in situ* hybridization (FISH) for the detection of 17p deletions and Sanger sequencing of exons 4-10 of the *TP53* gene are applied, resulting in incorrect classification of patients belonging to the highest risk group, due to absence of information regarding the heterozygosity status and low sensitivity of the sequencing technology.
Aims: We have applied an integrated approach to determine the *TP53*/17p status in CLL patients using oligo/SNP-based array which allows a genome-wide detection of copy number alterations (CNAs), down to 100 kb in size, and regions of copy neutral loss of heterozygosity (CNLOH). In addition the presence of *TP53* mutations was evaluated with high sensitivity using next generation sequencing approaches.
Methods: We have studied bone marrow or peripheral blood samples of 179 CLL patients that were referred to our diagnostic genetic center for analysis of 17p deletion, 17p CNLOH and the *TP53* mutation. To determine the *TP53* mutation status (exons 2-11) sequence analysis was performed by next generation sequencing with a sensitivity up to 1% mutant allele frequency. For the determination of the 17p status we have used a high resolution CytoScan HD Array (Affymetrix) platform which allows the detection of copy number alterations (*e.g.* deletions) as well as CNLOH.
Results: Twenty-one of the 179 CLL patients exhibited a loss or CNLOH of the short arm of chromosome 17 as demonstrated by oligo/SNP-based array. Eight of these cases had a CNLOH of 17p and would not have been observed in case interphase FISH had been performed. In addition, by applying *TP53* mutation analysis 26 patients were identified in whom the *TP53* gene was inactivated. In six of these cases the mutant allele frequency was below 20% and would have escaped detection by Sanger sequencing. Therefore ten of the 26 (38%) patients with *TP53*/17p aberration would not have been indentified in case diagnostics was based on FISH and Sanger sequencing. To evaluate whether both alleles of the *TP53* gene were inactivated the data of the 17p deletion, 17p heterozygosity status and *TP53* mutation status were integrated. It appeared that in all 13 patients with a 17p deletion the other *TP53* allele was inactivated due to a mutation. In all patients demonstrating CNLOH of the chromosome 17p region the *TP53* gene was also inactivated due to a mutation. In the other 5 patients with a *TP53* mutation no loss or CNLOH of 17p was demonstrated. However, two of these patients harbored two different *TP53* mutations, suggestive for inactivation of both *TP53* alleles.
Summary/Conclusions: By applying an integrated approach for determination of the *TP53*/17p status a substantial higher amount of patients were categorized to the highest risk group of CLL patients with *TP53*/17p abnormalities.

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CYTOGENETIC CLONAL EVOLUTION OCCURS AT THE TIME OF DISEASE PROGRESSION DURING IBRUTINIB THERAPY IN PATIENTS WITH RELAPSED/REFRACTORY CLL

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Background: Patients (pts) with del(17p) and complex karyotype (CKT) have increased likelihood of disease progression during ibrutinib therapy for CLL. Cytogenetic clonal evolution (CCE) is frequent at the time of disease progression following chemotherapy, particularly emergence of del(17p). Additionally, pts with del(17p) or *TP53* mutations frequently develop additional copy number alterations (CNAs) following chemotherapy.
Aims: To determine whether CCE occurs during ibrutinib therapy and at disease progression.
Methods: We analyzed 336 pts treated on investigational studies with ibrutinib or ibrutinib plus rituximab for CLL. In pts who progressed, we analyzed FISH and cytogenetic results pre-treatment and at progression, to identify CCE. Additionally, we identified a sub-group of 97 relapsed/refractory pts who had serial FISH analysis performed in bone marrow ≥1 year apart, to determine whether there were significant changes in sub-clonal composition of CNAs detected by FISH during treatment in the absence of disease progression.
Results: In total, 37 of 336 pts (11%) progressed during ibrutinib-based therapy. Of these pts, 15 had FISH analysis both pre-treatment and at progression: pre-treatment, 10 had del(17p), 1 had del(11q) and 4 had isolated del(13q). All 4 of the pts with isolated del(13q) pre-treatment had new subclonal FISH abnormalities detected at progression: two pts had del(17p), 1 had del(11q); a 4th pt developed two additional copies of all targeted regions at progression, suggestive of tetraploidy. The pt with del(11q) pre-treatment who progressed developed Richter Transformation (RT) in the bone marrow at progression, without either del(11q) or del(17p) identified by FISH, suggesting that the RT arose from a common ancestral clone without del(11q) or was clonally unrelated. Median FISH%del(17p) pre-treatment in those with del(17p) was 72%; only 1 pt had <50% del(17p) pre-treatment. All these pts had persistence of del(17p), at progression, without significant changes in allelic frequency. Two pts with del(17p) pre-treatment had additional abnormalities detected by FISH at progression: sub-clonal biallelic del(13q) was seen in two pts, one of whom also developed tetrasomy 12. In the absence of disease progression, the only CCE detected was emergence of small subclones with biallelic del(13q) in two patients who initially had monoallelic del(13q). Notably, in responding pts, there was no expansion of high-risk sub-clones. Conventional karyotyping was performed in 10/37 patients who progressed both pre-treatment and at progression. In 4 pts, CCE was identified at progression, including 17 new abnormalities in one pt. All 4 pts had complex karyotype and del(17p) by FISH pre-treatment and 3 of 4 had evidence of multiple, related, complex sub-clones pre-treatment. Figure 1 shows inferred clonal evolution pattern for one pt.

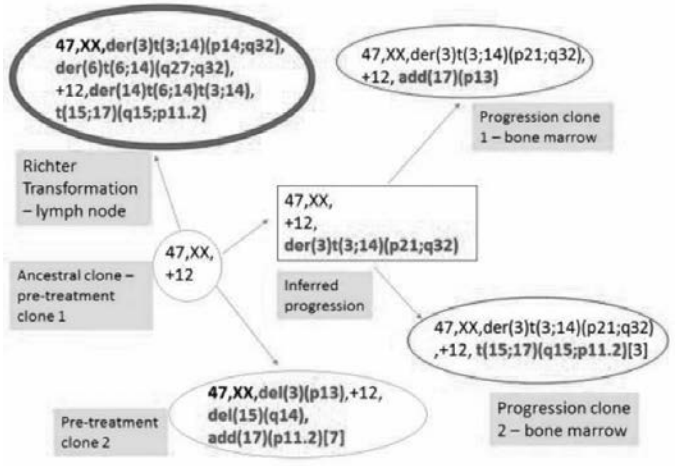

Figure 1.

Summary/Conclusions: Emergence of high-risk clones containing del(17p) and or del(11q) may be seen at disease progression in ibrutinib-treated patients. Analogous to allelic expansion of *TP53* mutations after chemotherapy, we hypothesize that small del(17p) or del(11q) subclones were present prior to therapy in these pts, below the sensitivity of existing FISH techniques and expanded under the selective pressure of ibrutinib treatment. Development of a more sensitive technique to identify small sub-clones with del(11q) or del(17p) may therefore be important. Additionally, complex CCE occurred at progression in several cases, indicating genomic instability and potentially contributing to therapeutic failure.

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LANDSCAPE OF SOMATIC MUTATIONS AND THEIR IMPACT ON RESPONSE AND OUTCOMES FROM LENALIDOMIDE-BASED THERAPIES IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Lenalidomide, either as a single agent or in combination with anti-CD20 monoclonal antibody, is clinically active in CLL and offers durable response in some pts. Predictive and prognostic impact of somatic mutations are not well known in pts with CLL who have received lenalidomide-based therapies.
Aims: Investigate the overall landscape of CLL gene mutations in both previously untreated and relapsed/refractory (R/R) pts. Determine associations between CLL gene mutations and clinical characteristics. Establish predictive and prognostic impact of CLL mutations in the context of lenalidomide-based therapies.
Methods: In the 288 pts with CLL who were treated in one of the lenalidomide-based clinical trials at our institution, we performed targeted gene capture exome sequencing of 295 genes that have been recurrently mutated in hematologic malignancies on pre-treatment samples. This sequencing platform also included more than 1000 cyto SNP position that allowed copy number variation (CNV) estimation. We used Mutect and Pindel algorithms to call high-confidence somatic mutations and used in-house algorithm to detect copy number variations (CNVs) in CLL samples.

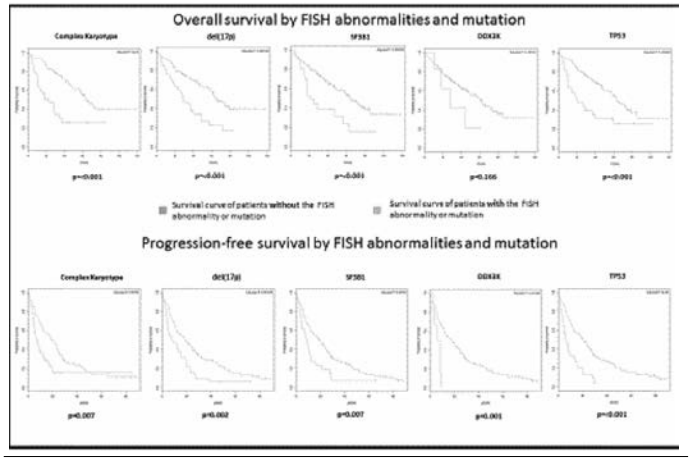

Figure 1.

Results: Among the 288 CLL pts treated with lenalidomide, 102 (35%) were previously untreated and 186 had R/R CLL. Ninety two patients (32%) received lenalidomide as a single agent and 196 patients (68%) received in combination with rituximab or ofatumumab. In total, we detected 470 high-confidence somatic mutations in 61 genes in 281 pts (76%). In addition to the well-known arm-level chromosomal abnormalities like del(13q), del(11q), tri(12), and del(17p), we also detected amp(2p), del(6q), del(8p), amp(8q) and tri(19). The most frequently mutated gene was SF3B1 (15%), followed by NOTCH1 (14%) and TP53 (14%) with 13 gene mutations occurring ≥3%. The number of mutations was similar between untreated and R/R pts (median number of mutations 1 [IQR: 0-2] *vs* 1 [IQR: 1-2], P=0.44) with increased enrichment of complex cytogenetics, TP53 mutation and del(17p) in the R/R cohort (p=0.006, p=0.014 and p=0.031, respectively). The pts with unmutated IGHV status had higher number of mutations compared to mutated IGHV (median 1 [IQR: 1-2] *vs* 1[IQR: 0-2], P=0.002) with MYD88 mutation and del(13q) being significantly enriched in IGHV mutated pts (p=0.005 and p=0.028, respectively) while NOTCH1 and XPO1 mutations were significantly enriched in IGHV unmutated patients (p=0.035 and p=0.047, respectively). Pairwise association showed statistically significant co-occurrence between tri(12) and mutations in KRAS/BCOR (both q<0.05), NOTCH1 mutation and ZMYM3 (q<0.01)/SPEN (q<0.05) mutations, and TP53 mutation and del(17p) (q <0.01)/complex karyotypes (q <0.05). When correlating with clinical response to lenalidomide, worse overall response (OR) in the untreated group was associated with del(17p) (p=0.019) and KRAS mutation (p=0.05), whereas mutation in SF3B1 (p=0.025), MGA (p=0.035), DDX3X (p=0.002), TP53 (p<0.001), complex karyotype (p=0.035) and del(17p) (p=0.031) were associated with worse OR in R/R group. In the untreated group, del(17p) and TP53 were associated with worse progression-free (PFS)(p=0.002 and 0.003, respectively). In R/R cohort, complex karyotype, del(17p) and mutations in SF3B1 and TP53 were associated with worse OS and PFS while DDX3X was associated with worse PFS but not OS (refer to provided Figure 1). In one of the multivariate models, SF3B1 (P=0.005) mutation and having TP53 or del(17p) (p=0.02) were prognostic for survival in R/R cohort.
Summary/Conclusions: Tumor mutational heterogeneity in CLL is due to intrinsic tumor biology and selective drivers from previous treatments, which can then affect response and survival in lenalidomide-based therapies.

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HIGH THROUGHPUT IMMUNOPROFILING OF CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS ASSIGNED TO STEREOTYPED SUBSET #4: NOVEL INSIGHTS INTO THE DEPTH, DIVERSITY AND TEMPORAL DYNAMICS OF CLONAL EVOLUTION

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Background: Chronic lymphocytic leukemia (CLL) clones assigned to stereotyped subset #4 are characterized clinically by a young age at diagnosis and an indolent disease course, and molecularly by B-cell receptor immunoglobulins (BcR IGs) that exhibit distinctive immunogenetic features. More specifically, they are IgG-switched, composed of heavy and light chains encoded by the IGHV4-34 and IGKV2-30 genes, respectively, and their heavy chain complementarity determining region 3 (VH CDR3s) is long and enriched in positively charged residues, reminiscent of pathogenic anti-DNA antibodies. In addition, both the VH and VK domains of subset #4 demonstrate a high impact of somatic hypermutation (SHM), highly indicative of an (auto)antigen selection.
Aims: To obtain comprehensive insights into the ontogeny and evolution of CLL subset #4 using next-generation sequencing (NGS) for in-depth immunoprofiling of the clonotypic BcR IG genes, particularly focusing on analyzing intraclonal diversification (ID) within the IG gene sequences.
Methods: Peripheral blood samples were collected at multiple time-points over a 10-year period from 6 CLL subset #4 patients. The clonotypic IGHV-IGHD-IGHJ and IGKV-IGKJ rearrangements were amplified by PCR using cDNA and sequenced on the MiSeq (Illumina). Our experimental design involved paired-end sequencing, thus allowing sequencing of the CDR3 twice/read, so as to increase the accuracy of results. To maintain stringency, raw NGS reads were subjected to purpose-built, bioinformatics algorithms, which performed: (i) length and quality filtering of raw reads; (ii) merging of filtered-in paired reads via local alignment; and, (iii) length and quality filtering of stitched sequences. No base calls of Q-score<30 were allowed in the 75 nucleotide stretch preceding the GXG motif, further increasing CDR3 sequencing reliability. Data was then analyzed using the IMGT/HighV-QUEST database and clonotype computation was performed using an in-house bioinformatics pipeline.
Results: Overall, 48 samples were analyzed, producing 12,386,554 and 4,506,464 total reads for heavy and light chain, respectively. In addition to filtering out poor quality, incomplete, out-of-frame and unproductive rearrangements, specific parameters were applied regarding V-region identity (>85%), usage of subset #4-specific V- and J-genes, CDR3 length and landmark residues. Applying these strict criteria resulted in 84.1% (median 401,133 reads/sample) and 90.3% (median 141,549.5 reads/sample) of the total sequences obtained for the heavy and light chain, respectively, passing filters. Clonotype computation was solely based on the filtered-in sequences and revealed a median of 1332.5 clonotypes/sample (range: 879-3432) for the heavy chains while a median of 202.5 clonotypes/sample (range: 125-395) was evidenced for the light chains. Overall, our longitudinal analysis revealed: (i) a hierarchical pattern of subclonal evolution showing which SHMs were negatively or positively selected; (ii) distinct clusters of subcloned sequences which at later time-points had often disappeared and hence been selected against; and, (iii) that despite the high intensity of ID, certain residues remained essentially unaltered alluding to strong functional constraints.
Summary/Conclusions: Detailed molecular immunoprofiling by NGS afforded the possibility to gain novel insights into the pathogenesis of CLL subset #4, thus providing conclusive evidence that these patients continue to acquire SHMs within their IG genes; an observation best explained by a clear role for antigen selection in clonal evolution.

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FAILED HYDROXYMETHYLATION CONTRIBUTES TO A CHRONIC LYMPHOCYTIC LEUKEMIA SPECIFIC EPIGENOTYPE

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Background: During normal hematopoiesis, a coordinated epigenetic and transcriptional programming is necessary to achieve lineage development. B cell differentiation is predominantly related to loss of DNA methylation at the enhancers and promoters of B cell-specific genes; *e.g.* transcription factors (TFs). In chronic lymphocytic leukemia (CLL), failure of proper epigenetic programming contributes to deregulation of B cell transcriptional programs and results in CLL phenotypes with highly variable outcomes. The mechanisms leading to failed epigenetic programming and to establishment of a CLL epigenome are not well understood. Genomic sites of failed epigenetic programming coincide with binding sites of key B cell TFs. Active DNA demethylation through TET-dioxygenase mediated conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) and subsequent products is one of the mechanisms involved in physiological epigenetic B cell programming, and deregulation of this process could contribute to establishment of the CLL epigenome.
Aims: Here, we investigated the role of TET2-mediated DNA demethylation through differential 5hmC acquisition in healthy and in CLL B cells. We further studied mechanisms and TFs involved in regulation of 5hmC conversion during CLL pathogenesis.
Methods: Clonal B cell specimens from 122 CLL patients were subjected to DNA methylation profiling using Illumina 450k arrays. 17 CLL and 4 healthy B cell samples (CD19+) were used for DNA methylation profiling using Illumina Epic arrays and for hydroxymethylated DNA immunoprecipitation (hMeDIP) using a monoclonal 5hmC mouse antibody and the NEBNext Ultra DNA Library Prep Kit for analysis on a Illumina HiSeq2000 sequencer. Global 5hmC levels were quantified by dot blots. TET2, and EBF1 mRNA and protein expression was evaluated by qPCR and Western Blot, respectively.
Results: By dot blot, we found decreased 5hmC levels in CLL as compared to CD19+ B lymphocytes. 5hmC was further reduced in IGHV unmutated compared to IGHV mutated CLL patients. To identify distinct regions with gain or loss of 5hmC, we performed genome-wide 5hmC profiling by hMeDIP. We confirmed a significantly lower number of hydroxymethylated peaks in CLL (137114) compared to HBC (249421) which remained stable when separating to good (133234, $p<0.0102$) or bad prognosis CLL (140441; $p<0.0161$) patients (defined by the IGHV mutation status, Rai/Binet stages, CD38 positivity, del(11q) and del(17p)). Differential binding analysis (DBA) revealed 5988 significantly differentially hydroxymethylated reads between CLL and HBC samples (FDR<0.05). Pathway analysis showed that regions which lost hydroxymethylation in CLL were involved in B cell receptor (BCR), Class I PI3K, CXCR-4, c-Mec and IL3 signaling. To further identify mechanisms that are involved in failed hypomethylation and 5hmC loss in CLL, we aimed at profiling sequence characteristics at the respective genomic sites. In our genome-wide DNA methylation data set, we confirmed highly significant enrichment of the EBF1 motif at the respective sites in 122 CLL patients. EBF1 mRNA and protein expression was significantly reduced in the majority of 17 CLL samples compared to HBC. TET2, a potential interaction partner of EBF1, was upregulated in CLL samples on RNA level and expressed to different degree on protein level.
Summary/Conclusions: Here, we demonstrate that 5hmC loss in CLL contributes to a disease specific epigenotype as described earlier. First evidences indicate that alterations of an interaction between the EBF1 and TET2 are mechanistically involved in insufficient hydroxymethylation and consequently failed DNA hypomethylation.

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DNA METHYLATION PROFILING IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS CARRYING STEREOTYPED B-CELL RECEPTORS: A DIFFERENT CELLULAR ORIGIN FOR SUBSET #2?

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Background: Subsets of CLL patients carrying stereotyped B cell receptors (BcRs) display distinct biological and clinical features; however, the DNA methylation landscape for these patients remains largely unexplored.
Aims: To investigate the DNA methylation profiles in three major stereotyped subsets.
Methods: By applying high-resolution 450K methylation arrays, we studied the clinically aggressive subsets #1 (Clan I genes/IGKV(D)1-39, IGHV unmutated, n=37) and #2 (IGHV3-21/IGLV3-21, mixed IGHV mutation status, n=35) and the indolent subset #4 (IGHV4-34/IGKV2-30, IGHV mutated, n=28). In addition, a series of sorted normal subpopulations spanning different stages of B-cell differentiation (*e.g.* naïve, centrocytes, centroblasts, memory) were analyzed.
Results: Unsupervised principal component analysis demonstrated that the investigated subsets formed distinct subgroups and these findings were corroborated by hierarchical clustering analysis. We next explored if and how these subsets match to the recently proposed epigenetic classification of CLL, which subgroups patients into three categories, defined as i) poor-prognostic, naïve like CLL (n-CLL), ii) good-prognostic, memory like CLL (m-CLL), broadly corresponding to IGHV unmutated and mutated CLL, respectively; and iii) a third intermediate CLL subgroup (i-CLL), which have borderline mutated IGHV genes and an intermediate outcome. For this purpose, we utilized the same methylation arrays to study a cohort of CLL cases that did not express stereotyped BcRs ('non-subset', n=325). Comparison of subset *vs* non-subset CLL, grouped based on their epigenetic classification, revealed that subset #1 clustered with n-CLL, subset #4 with m-CLL, while subset #2 clustered separately with i-CLL. We have recently shown that the number of epigenetic changes that a tumor acquired, compared to its cellular origin (*i.e.* 'epigenetic burden'), may be a powerful predictor of clinical aggressiveness (Queiros *et al.*, Cancer Cell 2017). Following this approach in CLL, when comparing specific subsets *vs* their non-subset cases matched by epigenetic subgroup, we noted a significant difference in the epigenetic burden amongst the various groupings; more specifically, in subset #1 *vs* n-CLL (72K *vs* 67K, $p<0.05$) and in subset #2 *vs* i-CLL (76K *vs* 68K, $p=0.001$), while no difference was observed between subset #4 *vs* m-CLL (83K *vs* 82K, p=not significant). This implies that subsets #1 and #2 have a higher epigenetic burden than n-CLL, which is in line with the more aggressive disease seen in these two subsets compared to the broader category of n-CLL patients. Focusing on subset #2, we observed that almost all cases clustered separately from i-CLL in supervised clustering analysis, providing further support that subset #2 forms a distinct subgroup of i-CLL. Subset #2 cases frequently carry del(11q) and harbor *SF3B1* mutations, however, neither the IGHV mutation status nor the presence of del(11q) or *SF3B1* mutations had any impact on the epigenetic burden within subset #2.
Summary/Conclusions: Stereotyped CLL subsets differed significantly in their methylation profiles. That said, subset #1 and #4 clustered at large with n-CLL and m-CLL categories, respectively, implying common cellular origin. In contrast, subset #2 emerged as the first defined member of the i-CLL group, which in turn alludes to a distinct cellular origin for subset #2 and i-CLL patients. Both subsets #1 and #2 displayed a higher epigenetic burden compared to n-CLL and i-CLL, respectively, which is likely reflected in the very poor outcome associated with these two subsets.

Chronic lymphocytic leukemia and related disorders - Clinical

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ADDING OBINUTUZUMAB TO IBRUTINIB ENHANCES DEPLETION OF CLL CELLS IN PERIPHERAL BLOOD AND BONE MARROW AFTER 1 & 6 MONTHS COMBINED THERAPY INITIAL RESULTS FROM THE BLOODWISE TAP ICICLLE EXTENSION STUDY

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Background: A major aim of CLL treatment is to eradicate detectable minimal residual disease (MRD). Ibrutinib is an effective treatment for CLL that results in immediate lymphocytosis persisting in most patients for several months. Obinutuzumab is a second generation anti-CD20 monoclonal antibody which can effect rapid resolution of lymphocytosis and eradication of MRD in some CLL patients. The IcICLLe Extension Study expands on the IcICLLe trial (ISRCTN12695354) to examine the efficacy and safety of the combination treatment of obinutuzumab and ibrutinib.
Aims: The IcICLLe trial was a single-arm, multicentre feasibility study that recruited 40 participants with CLL requiring treatment, 20 treatment-naïve (TN) and 20 relapsed/refractory (RR), to receive continuous ibrutinib therapy until confirmed MRD negative remission (<0.01% residual disease) or disease progression. The IcICLLe Extension Study adds 40 RR participants with CLL requiring treatment to receive continuous ibrutinib therapy from day 0 and 6 cycles of obinutuzumab from day 1. 30 participants have no prior ibrutinib treatment (ibrutinib-naïve), and 10 are pre-treated with ≥12 months of ibrutinib on IcICLLe. The primary outcome for the IcICLLe Extension Study is the proportion of patients achieving MRD-negative remission by IWCLL criteria (depletion of CLL below 0.01% in the peripheral blood and bone marrow) at or before 9 month assessment.
Methods: Adverse Events are collected from registration until 30 days after end of treatment and reported using the Common Terminology Criteria for Adverse Events v4.0. MRD was assessed by multiparameter flow cytometry according to ERIC 2016 guidelines with a detection limit ≤0.004%.
Results: 31 participants (22 ibrutinib-naïve and 9 pre-treated) are evaluable for response assessment after 1 month of combination treatment. There have been no reports of tumour lysis syndrome within the first month of combination treatment. There were 2 separate reports of grade 2 infusion related reactions, both on day 1 of obinutuzumab. In the 22 ibrutinib-naïve cases peripheral blood (PB) CLL counts remained at or below baseline levels in 17/22 cases from week 1 onwards. After 1 month of combination therapy the PB CLL count was a median 31% of baseline levels (range <1%>174%) compared to median 215% (range 29%>3570%) for RR patients on ibrutinib monotherapy. Percentage CLL cells in the bone marrow (BM) aspirate after 1 month of combination therapy reduced from a median 83% (range 23-94%) to a median 47% (range 5-85%, P=0.003, Wilcoxon matched-pairs signed ranks). For RR patients on ibrutinib monotherapy there was no change in BM at 1 month: baseline median 85% (range 11-96%) compared to median 86% (range 50-98%, P=0.96). Changes in BM aspirate CLL percentage were confirmed by morphological assessment of a trephine biopsy with all evaluable patients receiving obinutuzumab showing improvements in the cellularity and/or extent of infiltration. BM assessment at 1 month was not mandated for the 9 pre-treated patients but all showed decreased PB CLL counts with 4/9 achieving <0.01% residual disease within 3 months of starting obinutuzumab. 13 patients have completed 6 months of obinutuzumab treatment with marrow assessment at 9 months showing a further ≥1 log depletion in CLL percentage in 9/13 patients with 4/6 pre-treated patients achieving <0.01% residual disease.
Summary/Conclusions: The data indicate that for RR patients, the addition of obinutuzumab to ibrutinib results in a substantial improvement over ibrutinib monotherapy in the depletion of CLL cells from peripheral blood and bone marrow after 1 month of combination therapy, and continued improvement after 6 months combination therapy, with MRD-negative BM responses for patients who have had >1yr prior ibrutinib monotherapy. Residual disease levels in the BM after the 6 months of combination treatment will be available for 25 participants by June-2017.

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CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS EXPRESSING THE LIGHT CHAIN IGLV3-21 HAVE A POOR PROGNOSIS INDEPENDENTLY OF HEAVY CHAIN IGHV3-21 OR THE IGHV MUTATIONAL STATUS

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Background: The immunoglobulin heavy-chain gene (IgHV) mutational status is currently considered the gold standard of prognostication in Chronic Lymphocytic Leukemia (CLL): unmutated (UM) immunoglobulin heavy chain region (IgHV) is associated with poor prognosis while patients with mutated IgHV (M) have more indolent disease. An exception are patients with IgHV3-21/IgLV3-21 who have poor prognosis irrespectively of the IgHV mutational status. Interestingly, IgHV3-21 is co-expressed with IgLV3-21 in the majority of cases.
Aims: Here we aimed to study the impact of the light chain IgLV3-21 on CLL prognosis. This light chain has never been characterized independently of the heavy chain IgHV3-21.
Methods: Based on 405 CLL patients from 3 independent cohorts (A. an initial cohort of 32 patients with aggressive CLL, and 2 cohorts of CLL patients where samples were obtained at diagnosis (B: n=270 and C: n=103), we analyzed the impact of the presence of IgLV3-21 on treatment-free (TFS) and overall (OS) survival. IgLV3-21 positivity was determined by real-time PCR and confirmed by Sanger sequencing.
Results: Among the 32 patients with aggressive CLL, we found that 9 (28%) patients who had an IgLV3-21 rearrangement, but only 1 patient carried the heavy chain IgHV3-21: IgLV3-21 patients had a median TFS of 17 months compared to 44 months in patients with another light chain (P=0.0270). Similarly, IgLV3-21 patients had a shorter median OS (88 months *vs* >192 months, P=0.0287). We verified these results in 2 independent cohort obtained at diagnosis. In cohort B (n=270), 30 (11%) expressed an IgLV3-21 light chain and 10 (4%) an IgHV3-21 (of which 8/10 also carried the light chain IgLV3-21 rearrangement). Patients with IgLV3-21 had a median TFS/OS of 29/183 months compared to patients without IgLV3-21 who had a median TFS/OS of 88/292 months (P=0.0003/P=0.0142). In cohort C (n=103), 9 (9%) expressed an IgLV3-21 light chain but only 1 (1%) had a heavy chain IgHV3-21. In this cohort, IgLV3-21 patients had a median TFS of 21 months not statistically different from IgHV UM patients (28 months) while IgHV M patients had a median TFS of 93 months (P<0.0001). We then pooled the 3 populations (n=405) in order to increase the under-represented subgroups and analyzed the association of the IgLV3-21 with the IgHV mutational status: patients with either IgHV3-21 or IgLV3-21 (with a M or UM IgHV) displayed a prognosis similar to UM patients: median TFS was 129, 48, 36, 24, 23 months for M, IgLV3-21/M (P=0.0005), UM (P<0.0001), IgLV3-21/UM (P<0.0001) and IgHV3-21 (P<0.0001) patients, respectively (Figure 1A). Similar results were observed for OS with a median OS of 292, 88, 174, 90 and 183 months M, IgLV3-21/M (P<0.0001), UM (P<0.0001), IgLV3-21/UM (P<0.0001) and IgHV3-21 (P=0.0021) patients, respectively (Figure 1B). If all IgLV3-21 (n=48) were considered independently of their heavy chain, IgLV3-21 median TFS (24 months) was similar to UM patients (36 months, P=0.5824) and statistically different from M patients (129 months – P<0.0001, Figure 1C). Similar results were observed for OS (Figure 1D).

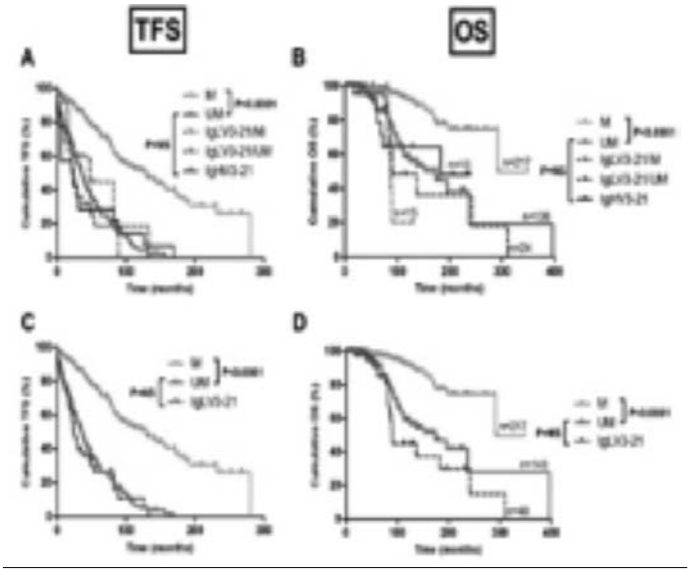

Figure 1.

Summary/Conclusions: Our results highlight for the first time the independent prognostic significance of the light chain IgLV3-21 in CLL: the presence of an IgLV3-21 light chain confers a poor prognosis similar to UM patient irrespectively of concurrent expression of IgHV3-21 heavy chain or IgHV mutational status.

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DURABILITY OF RESPONSES ON CONTINUOUS THERAPY AND FOLLOWING DRUG CESSATION IN DEEP RESPONDERS WITH VENETOCLAX AND RITUXIMAB

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Background: Venetoclax is a potent BCL-2 inhibitor that is approved as monotherapy for certain patients with relapsed or refractory chronic lymphocytic leukemia (CLL) in the United States, the European Union, and other countries.
Aims: Venetoclax combined with rituximab is being assessed in an ongoing Phase 1b study.
Methods: Minimal residual disease (MRD) was assessed in bone marrow using ≥4-color flow cytometry (minimum sensitivity: 0.01%). Patients who achieved complete remission (CR) or MRD-negativity could stop venetoclax and remain on study. Patients who manifested progressive disease while off therapy could re-initiate venetoclax and rituximab.
Results: Forty-nine patients, with a median of 2 (range: 1–5) prior regimens, were enrolled. As of July 2016, the overall response rate was 86%, the CR rate was 51%, and the bone marrow MRD-negativity rate was 57% (28/49) [Seymour *et al* Lancet Oncol 2017]. The 24-month estimate for progression free survival was 78.8% and that for duration of response was 87.8% (100% for patients with MRD-negative CR). Of the 28 patients attaining MRD-negativity, 22 achieved the status at 7 months, which was the first mandatory time point for assessment. The remaining six patients achieved MRD-negativity at the second assessment, which ranged from 12 to 22 months, since the timing of this test was not mandated. Twenty (41%) patients discontinued the study. Eleven had progressive disease while on therapy: five with Richter's transformation between 1–9 months and six with CLL progression after a median of 26.4 months (range: 12–37). The other nine patients: withdrew consent (n=3), failed to report for follow-up evaluations (n=1), discontinued due to adverse events related to venetoclax (n=2; tumor lysis syndrome and worsening of peripheral neuropathy), or discontinued due to adverse events considered not related to therapy (n=3). Seventeen patients continue on therapy: 8 MRD-negative CR, 2 MRD-positive CR, 5 MRD-negative PR, and 2 MRD-positive PR. Median duration of response on therapy is 27.9 months (range: 20.3–40.2). Sixteen patients discontinued venetoclax and remained on study as allowed per protocol following the achievement of a deep response (12 MRD-negative CR, 2 MRD-negative PR, 2 MRD-positive CR) (Figure 1). Their median time on venetoclax is 16.3 months (range: 5–38). Twelve of these patients remain in active follow-up and four discontinued without evidence of progression after achieving MRD-negative CR. Two patients with MRD-positive CR had increasing absolute lymphocyte count (ALC) and asymptomatic progression 24 months after stopping venetoclax. Both re-started venetoclax, 2 and 6 months after ALC >5x10^9/L, and achieved partial remissions. The 10 patients with MRD-negativity in the bone marrow who remain in follow-up have a median duration of ongoing response off venetoclax of 13 months (range: 3–34).

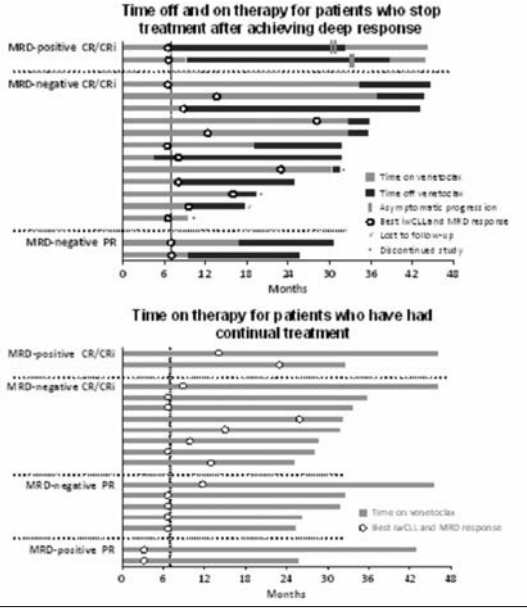

Figure 1.

Summary/Conclusions: Venetoclax with rituximab induces deep and durable responses, with 51% patients achieving CR and 57% achieving marrow MRD-negativity. Patients on continued therapy have durable responses. Additionally, responses are sustained at a median of 13 months among patients who achieve bone marrow MRD-negativity and elected per protocol to stop therapy, demonstrating that it is possible to discontinue venetoclax and maintain prolonged treatment free remission. The 2 patients who progressed at 2 years off therapy responded to the reintroduction of venetoclax.

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PREDICTIVE AND PROGNOSTIC IMPACT OF GENE MUTATIONS IN THE CONTEXT OF FLUDARABINE AND CYCLOPHOSPHAMIDE WITH OR WITHOUT OFATUMUMAB TREATMENT IN PATIENTS WITH REL/REF CLL

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Background: Recurrent mutations in genes such as *TP53*, *SF3B1* and *NOTCH1* are frequent in CLL and have in previous studies been associated with outcome. *SF3B1*^{mut}, *TP53*^{mut}, *BIRC3*^{mut} and *XPO1*^{mut} were adverse prognostic factors in patient cohorts with different therapies, and *NOTCH1*^{mut} associated with poor outcome when rituximab was added to standard chemotherapy. This indicated *NOTCH1*^{mut} as a predictive factor in the context of chemoimmunotherapy.
Aims: We assessed the incidence and clinical associations of mutations in *TP53*, *SF3B1, NOTCH1, ATM, BIRC3, FBXW7, MYD88, EGR2 and XPO1* in the COMPLEMENT-2 trial (relapsed/refractory CLL, FC *vs* FC+ofatumumab (FCO), Robak *et al.*, Leuk Lymphoma, 2017).
Methods: Baseline samples were available from 325 of 365 patients (89%) representative of the full analysis set of the clinical trial. Mutation analyses were performed via custom targeted Next Generation Sequencing (tNGS) for *TP53, ATM, BIRC3, FBXW7, MYD88, EGR2 (*all coding exons), SF3B1 (exon 14-16, 18), NOTCH1 (exon 34) and XPO1 (exon 15, 16). All mutations with a variant allelic fraction >5% were considered significant.

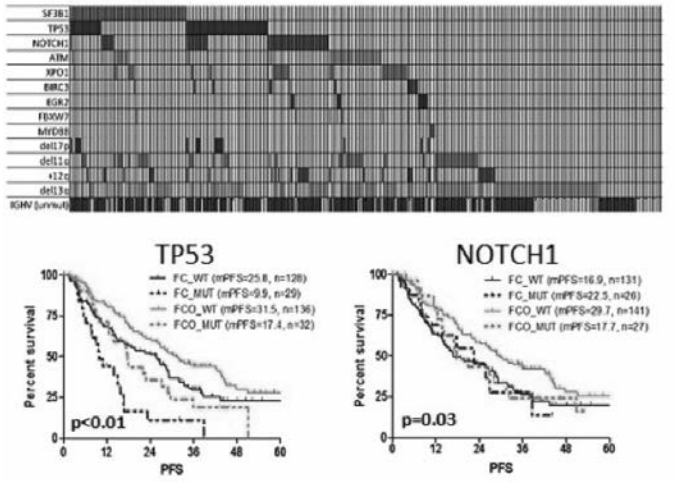

Figure 1.

Results: In total we identified 365 mutations across the 9 genes in 202 of 325 patients (62.2%), with incidences of *SF3B1*^{mut} 19.7%, *TP53*^{mut} 18.8%, *NOTCH1*^{mut} 16.3%, *ATM*^{mut} 13.8%, *XPO1*^{mut} 11.4%, *BIRC3*^{mut} 4%, *EGR2*^{mut} 3.1%, *FBXW7*^{mut} 2.7% and *MYD88*^{mut} 0.9%. We identified a variety of associations of mutational subgroups with genetic, clinical and laboratory parameters, such as *TP53*^{mut} with del17p (p<0.01), *NOTCH1*^{mut}, *FBWX7*^{mut}and *BIRC3*^{mut} with +12q (p<0.01, p=0.01 and p=0.05) and *ATM*^{mut}with del11q (p<0.01). XPO1^{mut} and *ATM*^{mut} associated with unmutated IGHV. CD79B expression on cell surface measured via flow cytometry was lower in *ATM*^{mut} patients, whereas CD20 expression did not differ among the different mutational subgroups. *TP53*^{mut}, *EGR2*^{mut}and*SF3B1*^{mut} patients had worse overall response to therapy (68% p<0.01, 50% p=0.02 and 72% p=0.05 respectively, *vs* 81% overall). Similar to the full analysis set, FCO as compared to FC resulted in significant improved PFS (median 28.1 *vs* 18.8 months, HR=0.67, p<0.01). *TP53*^{mut} and *XPO1*^{mut}were adverse prognostic factors for PFS (HR 1.93 p<0.01 and HR 1.85, p<0.01 respectively), but only *TP53*^{mut} for decreased OS (HR 2.11 p<0.01). All other mutations, in particular *SF3B1*^{mut} and *NOTCH1*^{mut}, did not significantly impact PFS or OS. To identify factors of independent clinical

impact, we performed multivariable Cox regressions for PFS and OS including treatment, IGHV status and all cytogenetic and mutational subgroups. For PFS, the following independent prognostic factors were identified: FCO therapy (HR 0.64 p<0.01), del17p (HR 5.08 p<0.01), unmutated IGHV (HR 2.0 p<0.01), *TP53*^{mut} (HR 1.75 p<0.01) and *XPO1*^{mut}(1.86 p<0.01). Del17p (HR 4.79 p<0.01), unmutated IGHV (HR 1.69 p=0.04) and *TP53*^{mut} (HR 1.76 p=0.03) were identified as independent prognostic factors for OS. With focus on the predictive value of gene mutations, we found a beneficial effect of the addition of ofatumumab to chemotherapy irrespective of *TP53* mutation (HR 0.52 p=0.02 for *TP53*^{mut} and HR 0.68, p=0.02 for *TP53*^{wt}). Regarding *NOTCH1,* ofatumumab was only beneficial in *NOTCH1*^{wt} but not in *NOTCH1*^{mut} patients (HR 0.64, p<0.01 and HR 0.86, p=0.67) (Figure 1).

Summary/Conclusions: In the COMPLEMENT-2 trial evaluating FCO against FC in relapsed/refractory CLL patients, we found *TP53*^{mut} and *XPO1*^{mut} but not *SF3B1*^{mut} or *NOTCH1*^{mut} as independent prognostic factors for PFS. Notably, a benefit of ofatumumab addition to FC treatment was observed among *NOTCH1*^{wt} but not among *NOTCH1*^{mut} patients indicating *NOTCH1* mutation status as a predictive marker in the context of type-1 CD20 antibody addition to chemotherapy.

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RESULTS OF A PHASE II MULTICENTER STUDY OF OBINUTUZUMAB PLUS BENDAMUSTINE IN PTS WITH PREVIOUSLY UNTREATED CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Bendamustine (B) plus rituximab (R; BR) is a commonly used first-line (1L) treatment for chronic lymphocytic leukemia (CLL). The CLL10 study reported an overall response rate (ORR) of 96% and complete response (CR) rate of 31% with BR. Obinutuzumab (GA101; G) is a glycoengineered, type II anti CD20 monoclonal antibody. A randomized Phase III trial in 1L CLL pts showed that G significantly improved progression-free survival (PFS) and CR rate compared with R, when used in combination with chlorambucil (Goede 2014). B plus G (BG) was evaluated in a subgroup of CLL pts in the GREEN study (Stilgenbauer 2015).

Aims: The aim of this Phase II study (NCT02320487) is to evaluate the efficacy and safety of BG as 1L treatment for CLL pts.

Methods: 102 pts with previously untreated CLL received BG, consisting of 6 cycles of G (cycle [C] 1: 100mg day (D) 1, 900mg D2, 1000mg D8 and D15; C2–6: 1000mg D1) and B (90mg/m^2: C1, D2 and D3; C2–6, D1 and D2). Each cycle was 28 days. The primary endpoint was CR assessed using iwCLL criteria. Secondary endpoints included ORR, PFS, overall survival, and minimal residual disease (MRD). Median follow-up at the time of analysis was 11.0 months.

Results: Median pt age was 61 yrs (range 35–90); 68.6% were male; 44.1% had Rai stage 3–4. For evaluated pts, IgVH status was 32.9% mutated and 67.1% unmutated. The incidences of trisomy 12, normal cytogenetics, and deletions of 13q, 11q, and 17p were 23.4%, 37.5%, 17.2%, 15.6%, and 6.3%, respectively. Investigator-assessed CR rate was 49.0% (95% CI 39.0–59.1) and ORR was 89.2% (95% CI 81.5–94.5) after 6 cycles. MRD negativity in blood, as measured by 4-color flow cytometry, was achieved in 42.7% of pts at the end of induction response assessment and in 75.5% of pts at any time following treatment. MRD negativity in bone marrow (BM) was 60.8% in pts with BM samples. The most common adverse events (all grades [Gr]) were infusion reactions (72.5%), nausea (52.0%), pyrexia (36.3%), neutropenia (34.3%), fatigue (34.3%), constipation (26.5%), and rash (26.5%). The most common Gr 3–4 adverse event was neutropenia (26.5%). Incidence of Gr 3–4 infections was 11.8%. Incidence of tumor lysis syndrome was 4.9% (all Gr 3). Three pts died; none were deemed related to study treatment or CLL by investigators.

Summary/Conclusions: BG is an effective regimen for 1L treatment of CLL pts, inducing a high CR rate after 6 cycles of therapy. No unexpected safety signals were observed.

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RELATIVE SURVIVAL REACHES A PLATEAU IN HAIRY CELL LEUKEMIA: A POPULATION-BASED STUDY ON INCIDENCE, PRIMARY TREATMENT AND SURVIVAL AMONG 1,427 PATIENTS DIAGNOSED IN THE NETHERLANDS, 1989-2014

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Background: The introduction of cladribine and pentostatin has revolutionized the management of HCL as from the late 80s. As a result of that revolution, HCL patients (pts) are rarely included in clinical trials. Population-based studies can inform on issues related to outcomes of HCL pts managed in daily practice. At present, however, population-based studies that assess patterns of incidence, treatment and survival in HCL are very scarce.

Aims: The aim of this comprehensive nationwide population-based study was to assess trends in incidence, primary treatment and survival among HCL pts diagnosed in the Netherlands.

Methods: We selected all adult (≥18 years) pts diagnosed with classic HCL in the Netherlands between 1989-2014 from the nationwide Netherlands Cancer Registry with survival follow-up through February, 2016. Age-standardized incidence rates (ASR) were calculated per 1,000,000 person-years and standardized according to the European standard population. Data on primary treatment (*i.e.* no therapy, chemotherapy [CT] and immumotherapy [IT]) were available for individual pts. Pts were categorized into 2 periods (1989-2000 and 2001-2014) and 3 age groups (18-59, 60-69 and ≥70 years). We calculated relative survival (RS) and the relative excess risk of mortality as measures of disease-specific survival.

Results: We included a total of 1,427 newly diagnosed HCL pts in the study (median age, 59 years; age range, 22-95 years; 77% males). The annual ASR of HCL remained quite stable over time and was 3.1 and 3.3 in the first and last period, respectively. Men had a higher overall incidence than women (5.3 *v* 1.3 in 2001-2014). The age-specific incidence rates for males were 5.5, 15.0 and 15.3 in 2001-2014 for the three age groups. The corresponding rates for females were 1.2, 3.1 and 5.5. The application of CT increased over time for all age groups. The proportions of CT for the three age groups were 56, 51 and 34% in 1989-2000, as compared with 81, 73 and 53% in 2001-2014. The corresponding proportions for IT were 21, 13 and 17% in 1989-2000, as compared with 2, 1 and 4% in 2001-2014. Lastly, the corresponding proportions for pts who did not receive therapy were 23, 36 and 49% in 1989-2000, as compared with 17, 26 and 42% in 2001-2014. Overall, when corrected for age and sex, pts diagnosed in 2001-2014 had 49% lower excess mortality during the first 10 years after HCL diagnosis, as compared with pts diagnosed in 1989-2000 (*P*=.005). Ten-year RS (95% confidence intervals) was impressive for pts age 18-59, namely 92% (88% - 96%) and 98% (94% - 100%; *P*=.176) in the first and last period, respectively (Figure 1a). Most of the significant improvement was observed in pts age ≥60. More specifically, 10-year RS for pts age 60-69 increased from 82% (71% - 92%) to 99% (89% - 106%; *P*=.009; Figure 1b), and for pts age ≥70 from 67% (49% - 86%) to 85% (68% - 102%; *P*=.366; Figure 1c) between the first and last periods. In addition, older age (*P*<.001), but not sex (*P*=.058), was associated with higher excess mortality.

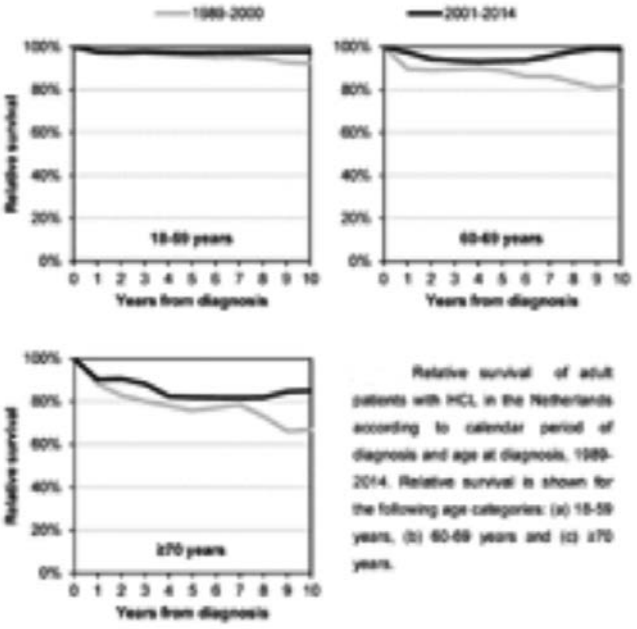

Figure 1.

Summary/Conclusions: The incidence of HCL remained stable during a 26-year period in the Netherlands. RS for pts diagnosed in the period 2001-2014 eventually reached a plateau, indicating that by then their survival is comparable to that of the general population. Survival was already excellent for younger patients throughout the entire study period. Survival improvement was most pronounced for pts age ≥60, although it was not statistically significant for pts age ≥70. This could be explained by the increased use CT over time. Population-based cancer registries are useful instruments to assess outcomes of pts rarely included in clinical trials.

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CUMULATIVE ILLNESS RATING SCALE PROVIDES PROGNOSTIC INFORMATION BEYOND THE INTERNATIONAL PROGNOSTIC INDEX FOR CHRONIC LYMPHOCYTIC LEUKEMIA: AN ACROSS-TRIAL ANALYSIS BY THE GCLLSG

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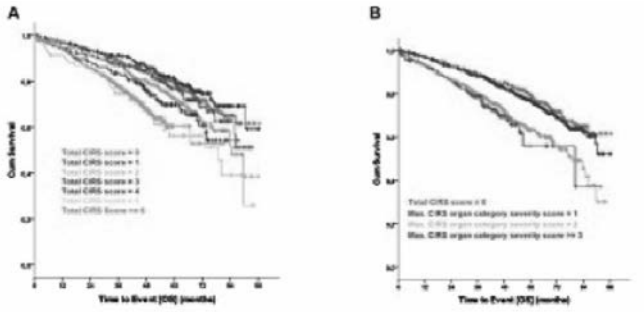
Background: CLL-IPI is a prognostication tool to stratify patients with chronic lymphocytic leukemia (CLL) for low, intermediate, high, or very high risk. CLL-IPI uses age, Binet stage, beta-2-microglobulin, 17p deletion / *TP53* mutation, IGHV mutational status, but not comorbidity as weighted factors to model prognosis. CIRS is a tool which allows assessing and quantifying burden of comorbidity in individual patients.
Aims: To validate CIRS in CLL and to assess whether CIRS is of further value when estimating prognosis by CLL-IPI in CLL.
Methods: This is a comprehensive evaluation of CIRS in 2158 patients pooled from the CLL8, CLL10, and CLL11 trials of the German CLL Study Group (GCLLSG). Median observation time was 55 months. All patients had CIRS data prospectively assessed prior to study treatment (689 FCR, 409 FC, 279 BR, 333 GCLB, 330 RCLB, 118 CLB).



C

COX regression (Overall survival)	Comparison	Hazard ratio	95% Confidence interval: Lower	Upper	p value
All trials (CLL8 / CLL10 / CLL11)					
Total CIRS score (continuous)		1.068	1.029	1.109	<0.001
CLL-IPI risk group					
Intermediate	vs. low	2.069	1.390	3.079	< 0.001
High	vs. low	3.974	2.704	5.840	< 0.001
Very high	vs. low	9.424	6.067	14.591	< 0.001
CLL8 trial (FCR vs. FC)					
Total CIRS score (continuous)		1.193	1.092	1.303	<0.001
CLL-IPI risk group					
Intermediate	vs. low	3.019	1.547	5.891	< 0.001
High	vs. low	5.447	2.803	10.585	< 0.001
Very high	vs. low	17.527	8.774	35.015	< 0.001
CLL10 trial (FCR vs. BR)					
Total CIRS score (continuous)		1.120	0.992	1.263	0.066
CLL-IPI risk group					
Intermediate	vs. low	1.644	0.868	3.114	0.127
High	vs. low	2.919	1.580	5.391	0.001
(No very high risk present in CLL10)					
CLL11 (GCLB vs. RCLB vs. CLB)					
Total CIRS score (continuous)		1.039	0.994	1.086	0.090
CLL-IPI risk group					
Intermediate	vs. low	1.474	0.655	3.313	0.348
High	vs. low	3.029	1.413	6.492	0.004
Very high	vs. low	5.201	2.274	11.896	<0.001

Figure 1.

Results: Median age was 64 years; 69% of patients were males, and 50% had ECOG performance score of 1 or higher. Complete information on age, Binet stage, b_2-microglobulin, 17p deletion and/or *TP53* mutation, and IGHV mutational status was available in 1761 of the 2158 patients. Distribution of CLL-IPI risk groups was as follows: 275 (16%) low risk, 653 (37%) intermediate risk, 712 (40%) high risk, 121 (7%) very high risk. The median total CIRS score was 3 (range 0-22); 81% of the patients had a total CIRS score of at least 1 and 28% of greater than 6. Comorbidities were most frequently captured under the following CIRS organ categories: cardiac, blood pressure, respiratory, musculoskeletal, or endocrine/metabolic. A severity score of >=2 and >=3 in at least one CIRS organ category was present in 46% and 11% of the patients, respectively. There was a positive correlation between total CIRS score and age (r_s=0.5, p<0.001) as well as ECOG performance score (r_s=0.4, p<0.001) and an inverse association between total CIRS score and creatinine clearance (r_s=-0.3, p<0.001). In univariate analysis, increased total CIRS score was associated with shorter overall survival (OS); with poorer OS determined by severity rather than numbers of comorbidities (log-rank: p<0.001, Figure 1A and 1B). In multivariate analysis, total CIRS score was an independent risk factor for OS when used as continuous or categorical variable together with age, gender, Binet stage, ECOG performance score, thymidine kinase, beta-2-microglobulin, IGHV, and 17p deletion (adjusted for treatment intensity). Total CIRS score also remained an independent risk factor for OS when added to the CLL-IPI. Weight of CIRS was highest in the CLL8 and lower in the CLL10 and CLL11 trials as expressed by the hazard ratios (Figure 1C). There was no significant association between total CIRS score and progression-free survival or time-to-next treatment. However, increased total CIRS score was associated with higher risk of grade 3/4 adverse events as well as premature treatment discontinuation during or after treatment with FCR / FC / BR but not GCLB / RCLB / CLB.
Summary/Conclusions: Findings suggest that CIRS provides prognostic information beyond the CLL-IPI. Systematic comorbidity assessment (*e.g.* by CIRS) in addition to the CLL-IPI therefore appears reasonable when estimating overall prognosis and deciding treatment in CLL.

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A PHASE II RANDOMISED STUDY INVESTIGATING THE EFFICACY OF STANDARD OR HIGH-DOSE OFATUMUMAB IN COMBINATION WITH CHEMOTHERAPY IN RELAPSED CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background: The outcome of CLL patients relapsing after chemoimmunotherapy (CIT) has been transformed by targeted therapies, however a proportion of such patients may be successfully retreated with CIT. CD20-monoclonal-antibody-based CIT has been successfully deployed for the retreatment of relapsed patients but the optimum dosage of antibody is unknown.
Aims: COSMIC (Chemotherapy plus Ofatumumab at Standard or Mega dose In CLL) was a phase II randomised study assessing the efficacy of standard (sOf) and high (megaOf) dose ofatumumab in combination with either fludarabine cyclophosphamide (FC) or bendamustine (B). The primary endpoint was complete response (CR/CRi) rate independently assessed 3 months post-therapy. Secondary endpoints were the proportion of participants with undetectable minimal residual disease (MRD); overall response rate; progression-free survival; overall survival; time to MRD relapse; dynamics of MRD relapse; safety and toxicity. Using the A'Hern exact one-stage design with 80% power and 1-sided type 1 error of 5%, 10 CRs were required from 37 recruits in either arm to justify further investigation in a phase III study. Total sample size was intended to be 82 allowing for drop-outs.
Methods: CLL patients relapsing after a minimum of 6 months from the most recent course of chemotherapy, and fit for FC or B, were eligible. Treatment comprised sOf (total 6.3g of ofatumumab: 0.3g day 1 cycle 1, 1.0g day 8 cycle 1, 1.0g day 1 monthly cycles 2-6), or megaOf (total 22.3g of ofatumumab: 0.3g day 1 cycle 1, 2.0g weekly for remainder of monthly cycles 1-2, 2.0g day 1 cycles 3-6) given in combination with FC or B at conventional doses. The choice of FC or B was decided pre-randomisation by the local investigator.
Results: Recruitment was slow with 61 patients randomised and treated, sOf (32: 21 FC and 11 B), and megaOf (29: 17 FC and 12 B). 77% were previously treated with purine analogues and 79% had experienced a remission of greater than 24 months after their most recent therapy. 26% had an 11q deletion and 67% had unmutated VH genes or expressed VH3-21.
With 61 participants, there was 71% power to observe 8 CRs from 28 participants in either arm. In the intention-to-treat (ITT) population 6(19%) sOf and 7(24%) megaOf patients achieved a CR/CRi; 22(69%) sOf and 21(72%) megaOf did not; and 4(12%) sOf and 1(4%) megaOf were unassessable. Rates were similar between FC(26%) and B(19%). Overall responses (CR/CRi+PR) were achieved in 20(63%) sOf and 20(69%) megaOf patients. In the ITT population, 4(13%) sOf and 6(21%) megaOf patients were MRD negative in marrow 3 months post-therapy. Overall, therapy was deliverable with 66% of participants receiving the proscribed 6 cycles of CIT (19(59%) sOf, 21(72%) megaOf). 42 serious adverse reactions were reported (21 sOf, 21 megaOf), 28(67%) being grade 3 or above (13(62%) sOf, 15(71%) megaOf), with the commonest events related to infections (45%) and cytopenias (21%). There was one treatment-related death (sOf-FC).
Summary/Conclusions: The CR rates observed in both treatment arms failed to meet pre-specified levels for the primary endpoint. Response rates observed in both arms are comparable to those obtained in previous studies of CIT in this group and suggest that dose escalation of ofatumumab in relapsed CLL does not lead to a worthwhile improvement in outcomes. However, CIT is deliverable with acceptable toxicity and should still be considered an option, particularly for patients with long-remissions to first line therapy and who do not possess high-risk cytogenetic markers.

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FINAL RESULTS OF THE PHASE IB GALTON TRIAL IN CHRONIC LYMPHOCYTIC LEUKEMIA: DURABLE REMISSIONS WITH FRONTLINE OBINUTUZUMAB (G) PLUS FLUDARABINE/CYCLOPHOSPHAMIDE (G-FC) OR BENDAMUSTINE (G-B)

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Background: GALTON was an open-label, parallel-arm, non-randomized, multicenter, Phase Ib study (NCT01300247) investigating safety and preliminary efficacy of G-FC or G-B in previously untreated CLL.
Aims: We report final results for the planned 36-months' (mo) follow-up (35/41 pts; median observation 40.4 [17.6–43.6] mo); initial results were reported previously (Brown *et al.* 2015).
Methods: Eligible pts met iwCLL 2008 criteria for therapy, were considered fit for chemoimmunotherapy by the investigator, and provided informed consent. Each center selected treatment (G-FC or G-B) for their pts. G was administered intravenously (IV; 100mg day [D] 1, 900mg D2, 1000mg D8 and 15 cycle [C] 1; 1000mg D1 C2–6) with FC (fludarabine 25mg/m^2 IV and cyclophosphamide 250mg/m^2 IV D2–4 C1, D1–3 C2–6) or B (90mg/m^2 IV D2–3 C1, D1–2 C2–6). Each cycle was 28 days. The primary endpoint was safety and tolerability of G-chemotherapy.
Results: 21 pts were enrolled in the G-FC arm and 20 in the G-B arm. Median age was 60 (25–80) years, 78% of pts were male, and around one-third had Rai stage III/IV disease. Median time from diagnosis to therapy was 24 mo (G-FC) and 32 mo (G-B). At data cut-off, 37 pts were alive in follow-up: G-FC (n=18: 2 lost to follow-up) and G-B (n=19). 1 event of progressive disease occurred in each arm, and 1 pt per arm died due to an adverse event (AE; G-B: respiratory failure; G-FC: unknown in the setting of unresolved Grade (Gr) 4 pancytopenia); neither was considered treatment related. Due to the small number of events, median PFS and OS could not be estimated; however, 3-year OS was 95% for each arm (95% CI G-FC, 68–99; G-B, 70–99). Post-treatment, 10/41 pts (24.4%) experienced ≥1 Gr3–5 AE: 2/21 pts (9.5%) in the G-FC arm and 8/20 pts (40.0%) in the G-B arm. 7 serious AEs were reported in 4 pts, all in the G-B arm; these included pneumonitis and respiratory failure (as noted above; both Gr5), Gr4 leukopenia/neutropenia, small cell lung cancer and Gr4 pneumothorax, and melanoma. During follow-up, 6 pts had ≥1 Gr3–4 AE of neutropenia, including 4/20 pts (20.0%) in the G-B arm and 2/21 pts (9.5%) in the G-FC arm. At end of treatment, all pts were B-cell depleted (B-cell count <0.07x10^9/L). Within 6–12 mo of follow-up, very few pts had recovered from B-cell depletion (G-FC: 2/19 pts [10.5%]; G-B: 0/20 pts). At 36 mo follow-up, 9/19 pts (47.3%) in the G-FC arm had recovered, 3/19 (15.8%) were still depleted, and 7/19 did not have data available. In the G-B arm, 6/20 pts (30%) had recovered, 1 was still depleted, and 13/20 had no available data. In a single center exploratory analysis, 9 pts (G-FC) underwent 4-color flow cytometry testing of peripheral blood for minimal residual disease (MRD) 6–14 mo after therapy; all were negative. 8 of these pts (G-FC) who were MRD-negative by 4-color flow cytometry were also tested with the ClonoSEQ immunoglobulin sequencing assay: 4 were MRD-positive and 4 MRD-negative. 4 pts who were MRD-negative with both assays remain in remission, while 2/4 pts who were positive by ClonoSEQ died after follow-up, one of Richter's transformation complicated by pneumonia and the other related to MDS. Another pt who was MRD positive by ClonoSEQ underwent allogeneic stem cell transplantation and remains in remission.
Summary/Conclusions: We conclude that G plus either FC or B results in excellent long-term disease control in previously untreated pts with CLL, and has comparable side-effects to other chemo-immunotherapy regimens.
We thank N Crompton, N Tyson, M Rahman (Roche Products Ltd) and R Morariu-Zamfir (F. Hoffmann-La Roche Ltd) for their support.

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THE PROGNOSTIC SIGNIFICANCE OF CLL-IPI AFTER REDUCED INTENSITY CONDITIONING ALLOGENEIC STEM CELL TRANSPLANT IN CHRONIC LYMPHOCYTIC LEUKEMIA: THE MAYO CLINIC EXPERIENCE

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Background: Allogeneic stem cell transplant (SCT) remains the only potentially curative option for chronic lymphocytic leukemia (CLL) patients. However, up to 40% of patients treated with Reduced Intensity Conditioning (RIC) - SCT relapse after transplantation. Recently the CLL International Prognostic Index (CLL-IPI) was validated as a predictor of 5 year overall survival in CLL patients.
Aims: In this analysis, we aimed to elucidate the factors that may predict the outcomesfollowing RIC SCT, including the CLL-IPI.
Methods: This is a retrospective analysis of all CLL patients who underwent RIC-SCT at Mayo Clinic between 2006-2013. The study was approved by the Institutional Review Board. The prognostic value of several CLL, patient and transplant related variables were analyzed. Continuous variables were reported as mean and compared using the T-test. Dichotomous outcomes were compared using the chi-square test. Survival was estimated and compared using the Kaplan Meier and Log Rank tests.
Results: Between 2006 and 2013, 50 patients with a median age of 56 years old underwent RIC-SCT for the treatment of CLL. The median time from diagnosis to RIC-SCT was 4.7 (0.6-22.9) years. Fourteen (28%) patients had 17p deletion at time of transplantation. CLL-IPI prognostic score calculated prior to transplant was intermediate in 30%, high in 42% and very high in 28% of patients. Disease status at the time of transplant was partial or complete remission in the majority of patients (39 patients, 78%). The overall transplant related mortality (TRM) was 6% and the 5-year non-relapse mortality was 14%. Relapse rates at 5 years were 54%. Acute graft *versus* host disease (GVHD) developed in 30 (60%) of patients and chronic GVHD was noted in 32 patients (64%).We evaluated the impact of CLL characteristics, disease status, and patient and transplant characteristics on clinical outcomes. Development of chronic GVHD post-transplant was the dominant predictor of both disease-free survival (DFS) (HR 0.29, 95% CI=0.10-0.69, P=0.006) and OS (HR 0.04, 95% CI=0.01-0.19, P<0.0001, Figure 1A). Very high CLL-IPI risk category (28% of patients) was associated with high relapse rates (82%) post RIC-SCT. DFS was also different between different CLL-PI categories (18.2% in very high 52.9% in high *vs* 66.7% in intermediate, p=0.04, Figure 1B). However, there was no significant difference in overall survival suggesting potential benefits from novel therapies in relapsed patients.Given that development of chronic GVHD was the most significant predictor for OS, we evaluated what pre-treatment patient, disease (including CLL-IPI), and transplant characteristics predicted for subsequent development of chronic GVHD. ZAP70 over expression (OR 0.09 [95% CI 0.01-0.79], p=0.03), disease status at transplant (progression *versus* remission OR 0.22 [95% CI 0.05-0.92], p=0.038), and alemtuzumab exposure within 3 months of transplantation were associated with lower rates of chronic GVHD (OR 0.08 [95% CI 0.01-0.79], p=0.03). CLL-IPI was not a significant predictor for the development of chronic GVHD in our analysis.

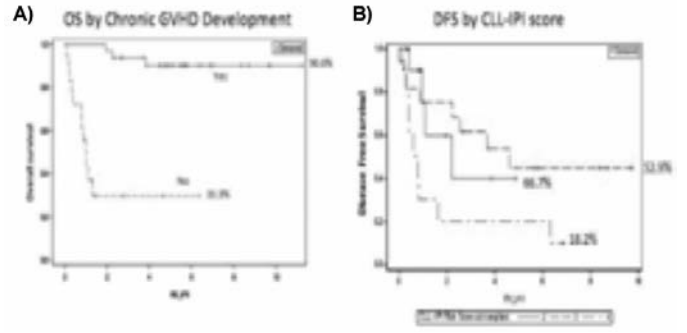

Figure 1.

Summary/Conclusions: This study found that the development of chronic GVHD post-transplant is themost significant predictor for both OS and DFS in surviving patients after RIC-SCT in CLL. Interestingly, 82% of patients with very high risk CLL-IPI relapsed after RIC-SCT. This is the first report to evaluate the prognostic significance of CLL-IPI for stratifying post-transplant outcomes and to identify high relapse rates in the very high risk CLL-IPI category.

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IMPACT OF ABCG2, OCT1 AND ABCB1 (MDR1) ON TREATMENT FREE REMISSION IN AN EUROSKI SUBTRIAL

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Background: Several studies showed that tyrosine kinase inhibitors (TKIs) can safely be discontinued in patients with sustained deep molecular response. So far, deep molecular response (DMR) and treatment duration were predictive for successful treatment-free remission (TFR) whereas age, risk scores, gender and molecular response level before stopping were without influence (Mahon FX. *et al.* and Pfirrmann M. *et al.*, ASH 2016). In addition, biomarkers like NK-cells and CD86+ cells (Ilander M. *et al.* and Schütz C. *et al.*, Leukemia 2017) seem to be of impact. *ABCG2, OCT1* and *ABCB1* are known to play a crucial role in acquired pharmacokinetic drug resistance and DMR in the context of nilotinib, imatinib and dasatinib. The influence of these mechanisms have not yet been analyzed for their correlation with TFR.

Aims: In a substudy of the EUROSKI trial, expression levels of the influx transporter *OCT1* and the efflux transporters *ABCG2* and *ABCB1* (*MDR1*) have been quantified in order to investigate their impact on TFR. As all patients are in DMR, we investigate whether these transporters confer a constitutional disposition for TFR.

Methods: The expression levels of *OCT1, ABCG2* and *ABCB1* have been determined by an absolute transcript quantification method in the peripheral blood of patients, enrolled in the EUROSKI trial and screened in our center. Minimal inclusion criteria were three years TKI treatment and one year MR^4 duration (*BCR-ABL*^{IS} <0.01%). Plasmid standards have been designed including the genes *OCT1, ABCG2, ABCB1* together with *GUS* as reference gene. Expression measurements were performed by qRT-PCR on baseline (day of stopping TKI treatment) samples. Cutoff levels were determined by the minimal p-value approach and adjusted for multiple testing by the Bonferroni method. The predictive significance of the efflux and influx channel transcript levels was quantified by a multivariate Cox's regression model. Relapse has been defined as loss of major molecular response at one time point.

Results: In our cohort, 132 chronic phase CML patients discontinued TKI treatment (87% imatinib 1st line treatment), showing a relapse rate of 46%. Median MR^4 and TKI treatment duration was 4.3 and 7.6 years respectively. The majority of patients were positive for the e14a2 transcript (e14a2+: 63%, e13a2+: 28%, e13a2+/e14a2+: 9%). The mean expression of *OCT1* and *ABCB1* between 'relapse' and 'no-relapse' patients showed no significant difference (p=0.99 and p=0.66), whereas *ABCG2* showed a weak differential expression (1.1% *vs* 0.8%, p=0.065). Cutoff analyses showed a significant risk stratification only for the *ABCG2* efflux transporter at a distinct cutoff value of 4.5‰ (p=0.04). Patients with an *ABCG2/GUS* transcript level above 4.5‰ (n=93) had a 30-months TFR of 47%, whereas patients with low *ABCG2* expression (<4.5‰, n=39) showed a 12-months TFR of 67%. The hazard ratio and predictive significance of the *ABCG2* transcript levels were investigated by a multivariate Cox's regression model. Only *ABCG2* expression was retained as independent covariate in this model (p=0.033). Thus, patients with an *ABCG2/GUS* transcript level above 4.5‰ showed an up to two-time higher risk of relapse after treatment discontinuation (HR=2.1, 95% CI: 1.06-4.05).

Summary/Conclusions: Here we investigated for the first time the impact of pharmacokinetics in the context of a CML discontinuation trial. *ABCG2* but not *OCT1* and *ABCB1 (MDR1)* predicted treatment-free remission after TKI discontinuation. High expression of the *ABCG2* efflux transporter correlated with a two-time higher risk of relapse in multivariate analysis. Further prospective validation is warranted.

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HLA-G MOLECULES AND CLINICAL OUTCOME IN CHRONIC MYELOID LEUKEMIA

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Background: The human leukocyte antigen-G (HLA-G) gene encodes a tolerogenic protein known to promote tumor immune-escape mechanisms.

Aims: We investigated the potential role of HLA-G polymorphisms and soluble HLA-G molecules in susceptibility to chronic myeloid leukemia (CML), as well as in achievement and maintenance of deep molecular remission ($MR^{4.5}$) in 68 patients treated with tyrosine kinase inhibitors (TKIs).

Methods: The entire HLA-G gene was amplified by long-range PCR and sequenced using next-generation sequencing (NGS) with Illumina's Nextera® technology and a 300 bp paired-end read protocol. The BioVendor sHLA-G ELISA (RD194070100R sHLA-G ELISA - EXBIO Praha a.s. BioVendor) immunoassay was used for the quantitative measurement of HLA-G1 and HLA-G5 soluble forms in EDTA-plasma samples

Results: The frequency of the G*01:03 allele was significantly associated to CML (10,29% *vs* 4,46; p=0.001). Patients carrying the G*01:01:01 or G*01:01:02 allele had a significantly higher mean value of soluble HLA-G compared to patients carrying G*01:01:03 (109.2±39.5 *vs* 39.9±8.8 units/ml; p=0.03), and showed significantly lower EFS compared to patients with other allelic combinations (62.3% *vs* 90.0%; p=0.05). Moreover patients carrying the G*01:01:03 allele had significantly higher rates of $MR^{4.5}$ (100% *vs* 65%), with earlier achievement of deep $MR^{4.5}$ (median of 8 *vs* 58 months, p=0.001). TKIs were discontinued in 24 patients after 2 years of confirmed $MR^{4.5}$. Treatment free remission (TFR) was 57.7%. None of the patients homozygous for the G*01:01:01 or G*01:01:02 allele remained in TFR (0% *vs* 68.4%, p=0.023) (Figure 1). All patients carrying the G*01:01:03 allele remained in TFR.

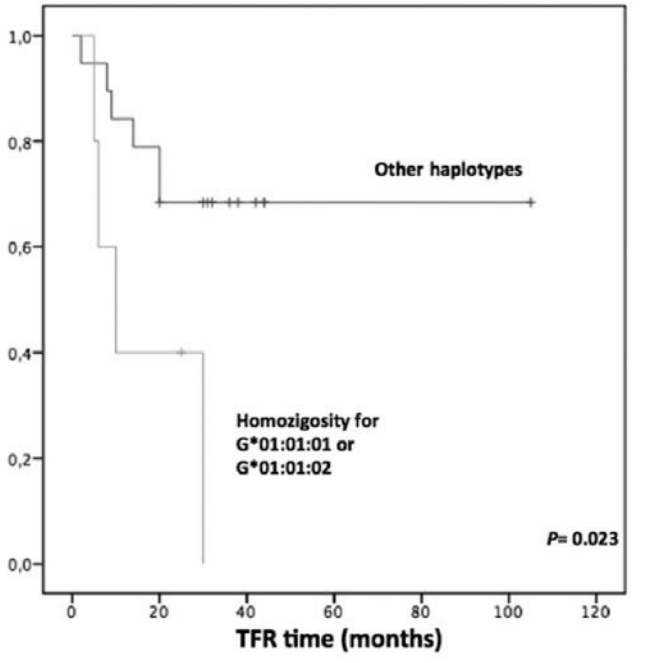

Figure 1.

Summary/Conclusions: HLA-G alleles with higher secretion of soluble HLA-

G would seem to be associated with lower EFS and TFR, possibly because of a stronger inhibitory effect on the immune system in favor of tumor escape mechanisms. Conversely, the allele associated to lower levels of sHLA-G promoted achievement of $MR^{4.5}$ and TFR, suggesting increased cooperation of the host immune system in CML cell clearance.

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DURABLE TREATMENT-FREE REMISSION AFTER STOPPING SECOND-LINE NILOTINIB IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE: ENESTOP 96-WK UPDATE

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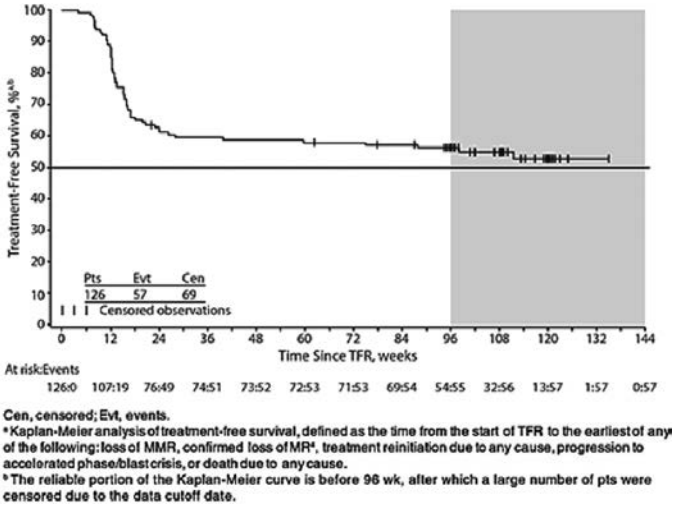
Background: ENESTop (NCT01698905) is evaluating the ability to stop treatment and remain in TFR in pts with CML-CP who achieved a sustained deep molecular response (MR) after switching from imatinib (IM) to NIL. In the primary analysis, 57.9% of pts (73/126) who stopped treatment remained in TFR (no loss of major MR [MMR; *BCR-ABL1* ≤0.1% on the International Scale (IS)], no confirmed loss of MR^4 [*BCR-ABL1*^{IS} ≤0.01%], and no treatment reinitiation) at 48 wk.

Aims: To evaluate the proportion of pts remaining in TFR at 96 wk after stopping second-line NIL in ENESTop.

Methods: Eligible pts had ≥3 y of prior tyrosine kinase inhibitor treatment (>4 wk IM, then ≥2 y NIL) and achieved $MR^{4.5}$ (*BCR-ABL1*^{IS} ≤0.0032%) after switching to NIL. All pts provided informed consent. Enrolled pts continued NIL for 1 y in the consolidation phase (MR assessed every 12 wk). Pts without confirmed loss of $MR^{4.5}$ during consolidation were eligible to enter the TFR phase (MR assessed every 4 wk for the first 48 wk, every 6 wk for the second 48 wk, then every 12 wk). Pts with loss of MMR or confirmed loss of MR^4 reinitiated NIL. This analysis was conducted when all pts who entered the TFR phase had completed 96 wk of TFR, reinitiated treatment, or discontinued from the study (data cutoff, 7 Nov 2016).

Results: At 96 wk of the TFR phase, 67 of the 126 pts (53.2% [95% CI, 44.1% - 62.1%]) who entered the TFR phase remained in TFR. Four pts who were in TFR at 48 wk reinitiated NIL due to confirmed loss of MR^4 at 60, 72, 90, and 96 wk, respectively. Two other pts discontinued from the study between 48 and 96 wk due to pregnancy (last *BCR-ABL1*^{IS} of 0.0035% at 60 wk) and pt decision (maintained $MR^{4.5}$ through 90 wk), respectively. Based on Kaplan-Meier analysis, the median duration of treatment-free survival has not been reached and the curve appeared to plateau (Figure 1). Of 56 pts who reinitiated NIL by the data cutoff, 52 (92.9%) regained MR^4 and $MR^{4.5}$, and the time by which 50% regained MR^4 and $MR^{4.5}$ was 12.0 and 13.1 wk, respectively. The time by which 50% of pts regained $MR^{4.5}$ was shorter for pts who reinitiated NIL due to confirmed loss of MR^4 (n=22; 11.0 wk) *vs* loss of MMR (n=34; 16.0 wk). Two of the 4 re-treated pts who did not regain MR^4 were ongoing in the treatment reinitiation phase (duration, 87.9 and 6.9 wk, respectively); the other 2 discontinued from the study before 48 wk, 1 due to not regaining MMR (retreatment duration, 20 wk) and 1 due to an adverse event (AE) after regaining MMR. Two deaths occurred after the first 48 wk of TFR, both in post-treatment follow-up: 1 due to cardiopulmonary failure 111 days after pt discontinued retreatment due to an AE, and 1 due to adenocarcinoma 77 days after pt discontinued retreatment due to initiation of chemotherapy for secondary malignancy. Among pts who remained in TFR for >48 wk (n=73), rates of all-grade AEs were 82.2% and 63.0% during the first and second 48 wk of TFR, respectively, *vs* 79.5% during the consolidation phase. Rates of musculoskeletal pain-related AEs were 47.9% and 15.1% during the first and second 48 wk of TFR, respectively, *vs* 13.7% during the consolidation phase.

Summary/Conclusions: Updated 96-wk analyses from ENESTop showed stability of the TFR rate, with few pts reinitiating treatment between 48 and 96 wk after stopping second-line NIL. Rates of overall and musculoskeletal pain-related AEs decreased in the second 48 wk of TFR *vs* the first 48 wk. Overall, these results demonstrate the durability of TFR after stopping NIL in pts who achieved a sustained deep MR after switching from IM to NIL.



Cen, censored; Evt, events.
[a]Kaplan-Meier analysis of treatment-free survival, defined as the time from the start of TFR to the earliest of any of the following: loss of MMR, confirmed loss of MR^4, treatment reinitiation due to any cause, progression to accelerated phase/blast crisis, or death due to any cause.
[b]The reliable portion of the Kaplan-Meier curve is before 96 wk, after which a large number of pts were censored due to the data cutoff date.

Figure 1.

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NILOTINIB-INDUCED METABOLIC DYSFUNCTION: INSIGHTS FROM A TRANSLATIONAL PILOT STUDY USING *IN VITRO* ADIPOCYTE MODELS AND PATIENT COHORTS

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Background: Impaired glucose and lipid metabolism is an adverse effect associated with nilotinib (NILO), a tyrosine kinase inhibitor (TKI) used in the treatment of chronic myeloid leukaemia (CML). Indeed the 5-year safety analysis of the ENESTnd trial observed elevations in blood glucose and lipid levels in the NILO arms; importantly NILO-treated patients also showed an increased incidence of arterial occlusive events. Adipose tissue is a key regulator of lipid and glucose homeostasis; dysregulation of adipogenesis, altered adipocyte lipid accumulation and reduced insulin sensitivity are implicated in the pathogenesis of metabolic disease. We investigated the effect of NILO on adipose tissue to explain the mechanisms behind NILO-associated metabolic adverse effects.

Aims: i) To study the effect of NILO and imatinib (IMA) on adipocyte function and adipokine secretion using an *in vitro* adipocyte model; ii) To utilise the *in vitro* model to explore potential therapeutic strategies to reverse NILO-mediated effects, and iii) To validate the *in vitro* results in a pilot patient cohort.

Methods: Differentiating 3T3-F442A murine adipocytes were incubated with clinically relevant concentrations of NILO (1-20μM) and IMA (5μM), in the presence or absence of telmisartan (1-10μM), an angiotensin receptor blocker with potential beneficial effects on insulin sensitivity and lipid homeostasis. Cytotoxicity and adipogenesis were assessed by MTT assay and Oil Red O staining, respectively. Expression of adipogenic genes, peroxisome proliferator-activated receptor gamma (*PPARγ*), lipin1 (*LPIN1*), sterol regulatory element-binding protein 1 (*SREBP1*) and glucose transporter 4 (*GLUT4*) were investigated by quantitative PCR and secreted adiponectin was measured by ELISA. Plasma samples were collected from 30 CML patients on either NILO (first line, n=6; second line, n=9) or IMA (first line, n=15) at baseline and at 3 and 12 months of therapy, and adiponectin was measured by ELISA. Data are presented as mean ± SD for 20μM incubations but full concentration response relationships were measured.

Results: Neither NILO nor IMA were cytotoxic to the adipocytes at clinically relevant concentrations. A dose dependent reduction in lipid accumulation was observed for NILO (for 20μM, 0.76 ± 0.005 absorbance units; $p<0.01$) but not IMA (0.98± 0.007), compared to vehicle control. NILO, but not IMA, dose dependently downregulated the mRNA expression of *PPARγ* (52% downregulation), *LPIN1* (28% downregulation) and *SREBP1* (54% downregulation). Both NILO and IMA resulted in significant downregulation of GLUT4 mRNA (NILO, 93%; IMA, 79%; $p<0.01$) and of secreted adiponectin (NILO, 5.99ng/ml; IMA, 31ng/ml; both $p<0.01$ in comparison to vehicle control, 79.2ng/ml). Co-incubation with telmisartan resulted in significant reversal of NILO-mediated effects on lipid accumulation, adipogenic gene expression and adiponectin secretion. In the patient cohort, IMA resulted in a significant increase in adiponectin levels at 3 (38.4±7.1mg/l; $p<0.01$) and 12 (36.7±7.2mg/l; $p<0.01$) month time points compared to baseline (27.3±5.7mg/l). In contrast, second line NILO showed a trend for *reduction* in adiponectin at both 3 (15.2±1.8mg/l; p=NS) and 12

(14.6±1.7mg/l; p=NS) months compared to baseline (21.3±3.4); however this was not evident in the first line NILO-treated group.
Summary/Conclusions: NILO-induced detrimental effects on adipocyte lipid accumulation and adiponectin secretion could be the mechanistic basis for NILO-mediated metabolic dysfunction. This was reversed by telmisartan, a PPARg partial agonist. A larger sample size is required to fully characterise the effect of TKIs on metabolic parameters in the patient population.

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EARLY PREDICTION OF THE MOLECULAR RESPONSE TO BCR-ABL1 TYROSINE KINASE INHIBITORS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background: A *BCR-ABL1* transcript level at 3 months after the initiation of imatinib therapy has shown to predict the long-term clinical outcomes in patients with chronic myeloid leukemia in chronic phase (CP-CML). The levels obtained earlier than 3 months may also have a similar prognostic significance.
Aims: To assess the prognostic value of the *BCR-ABL1* transcript levels at baseline, and 1 and 3 months after the initiation of a tyrosine kinase inhibitor (TKI) in predicting the major molecular response (MMR) achievement by 12 months, and to compare the patterns of molecular response (MR) to a TKI therapy between good and poor responders using a nonlinear model.
Methods: The clinical data were collected from the 178 patients with newly diagnosed CP-CML who were treated with a TKI at Seoul St. Mary's Hospital. *BCR-ABL1* transcript levels were obtained at baseline, and 1, 3, 6 and 12 months after the initiation of a TKI. The levels were reported as the percent ratio relative to the control gene *ABL1* in accordance with the International Scale (*BCR-ABL1/ABL1*^{IS}[%]). A confirmed MMR was defined as a *BCR-ABL1/ABL1*^{IS}≤0.1% on two consecutive occasions. The predictability of the levels at baseline, and 1 and 3 months post TKI therapy for the achievement of a confirmed MMR by 12 months was evaluated using a logistic regression method with a receiver operating characteristic (ROC) analysis. The areas under the ROC curve (AUCs) were calculated to quantify the predictability. In addition, the patterns of molecular responses over time were described by a nonlinear model to compare the model-derived parameters between the patients who achieved a confirmed MMR by 12 months ("good responders") and who did not achieve the MMR ("poor responders").
Results: Of 178 patients, 67 achieved a confirmed MMR by 12 months but 111 did not. At baseline, the transcript level was not useful to predict the achievement of a confirmed MMR by 12 months. At 1 month post therapy, the levels measured at 1 month significantly (p <0.0001) predicted the MMR with an AUC of 0.77. The patients with the level of 38% or less at 1 month had a better chance to achieve the MMR. By 3 months post therapy, the transcript level measured at 3 months (p <0.0001) accurately predicted the MMR with the AUC of 0.87. The patients with the level of 0.48% or less at 3 months had a better chance to achieve the MMR. A nonlinear sigmoid model was used to fit the transcript data from 149 patients as follows: $MR=MR0\ [1 - t^{\gamma} / (t_{50}{}^{\gamma}+t^{\gamma})]$; where MR0 is the predicted molecular response at baseline; t, time post TKI initiation; γ, slope factor; t_{50}, time required to achieve 50% reduction in MR. Statistically significant differences were observed between the good and poor responders in the median values for the model-derived parameters of MR0 (73.3% *vs* 82.2%; p=0.003), γ (4.98 *vs* 3.32; p <0.0001) and t_{50} (0.95 month *vs* 1.12 month; p=0.002).
Summary/Conclusions: A *BCR-ABL1* transcript level measured at 1 month after the initiation of a TKI may be used as an early indicator to reliably predict the MMR achievement by 12 months in patients with CP-CML. The level obtained at 3 months appears to accurately predict the MMR. Further studies are needed to evaluate the association between the transcript level at 1 month and long-term clinical outcomes.

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Abstract withdrawn.

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A HIGH SENSITIVITY HIGH SPECIFICITY DIGITAL PCR ASSAY FOR BCR-ABL

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Background: Digital PCR (dPCR) generates an absolute read out that is largely robust to variations in PCR efficiency and should reduce the requirement for standardisation by laboratory-specific conversion factors. DPCR is an appealing technology for quantitative detection of specific mutations with simultaneous measurement of the reference gene and is particularly suitable for minimal residual disease (MRD) diagnostics in chronic myeloid leukaemia (CML). However, a limitation of the dPCR assays compared to standard quantitative PCR (qPCR) is the background (termed lower limit of blank, LoB) of 1 or 2 positive droplets for BCR-ABL (Franke *et al.*, ASH 2015, Cross *et al.*, Leukemia 2016). The resulting false positive rate (FPR) limits the sensitivity and the ability to detect deep molecular remissions. This has hindered the definition of the lower limit of detection (LoD). We report here on the LoB and and the LoD of a newly developed assay.
Aims: Determination of LoB, LoD, linearity and precision of an optimized BCR-ABL digital PCR assay.
Methods: The assay was developed by Bio-Rad laboratories and consisted of a reverse transcriptase reaction followed by a duplex PCR detecting ABL and both the b2/a2 and b3/a2 transcripts of BCR-ABL. Digital droplet PCR was performed using the Bio-Rad QX200 system.
LoB and cross-hybridisation were assessed in non-template controls (NTC), BCR-ABL negative cell lines and healthy wild-type donor samples. LoD, precision and linearity were measured in serial dilutions of patient's cDNA in healthy donor's cDNA to simulate MMR, MR^4, $MR^{4.5}$, MR^5 and $MR^{5.5}$. Finally, the assay was tested on a certified CAP $MR^{4.7}$ sample.
Results: The specificity was >95% for both BCR-ABL and ABL in NTC and wild type samples. Extensive NTC analysis yielded no false positives for BCR-ABL PCR (n=0/176; LoB=0) and 1% false positives in ABL PCR (n=2/176; 1-2 positive droplets, LoB=0). The BCR-ABL assays of healthy donors were positive in 2% (n=5/234) with 1 positive droplet/positive sample. For 2 wells analysis, the detection rate for BCR-ABL for $MR^{4.5}$ and MR^5 was 100 and 88%, resulting in a LoD between 6 and 3 BCR-ABL copies/2 wells. Although the ABL copy number was only 200000 for 2 wells, the $MR^{5.5}$detection rate was 42%. However, in a merged 4 wells analysis, the detection rate was 100% for MR5 and 67% for $MR^{5.5}$ with an average of 6 and 2 BCR-ABL copies/4 wells (see Table 1). The $MR^{4.7}$sample was analyzed repeatedly, with 59 of 60 replicates evaluable. A total of 89 BCR-ABL copies (1.51/replicate) and 4,329,846 ABL copies (73387/replicate) were detected, resulting in a ratio of 0.0021 ($MR^{4.69}$). The false negative rate was below 3% when combining any pair of replicates, indicating an LoD of $MR^{4.7}$ for assays carried out in duplicate.

Table 1.

	2-well Analysis			4 well analysis		
Theoretical value	BCR-ABL Copies	ABL Copies	Detection rate	BCR-ABL Copies	ABL Copies	Detection rate
MR3	285	200750	24/24 (100%)	571	401500	12/12 (100%)
MR4	26	200375	24/24 (100%)	52	400750	12/12 (100%)
MR4.5	6	198992	24/24 (100%)	11	397983	12/12 (100%)
MR5	3	200558	21/24 (88%)	6	401117	12/12 (100%)
MR5.5	1	199350	10/24 (42%)	2	398700	8/12 (67%)

Summary/Conclusions: We report here on an optimized digital PCR assay with a LoB of zero and a LoD of 3 copies/2 wells. This highly sensitive and specific assay allows accurate detection of MRD in BCR-ABL positive diseases with a detection rate of 100% for MR5 and 67% for MR5.5 in a 4 wells analysis.

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VALIDATION OF THE EUTOS LONG TERM SURVIVAL SCORE IN DUTCH CML-PATIENTS

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Background: Risk scores in chronic myeloid leukemia (CML) use baseline characteristics of CML patients in chronic phase to predict outcome and can be used to make decisions regarding first line TKI choice and monitoring frequencies. Until recently, risk stratification of CML patients was used based on scores developed in the pre-imatinib era (Sokal and Hasford risk score) with overall survival as the end point of interest. After the introduction of imatinib, the EUTOS score was established to predict the chance of achieving CCyR at 18 months, as a proxy for survival. However, since the major causes of death of CML patients are no longer CML-related, the need for baseline risk prediction has shifted from overall survival towards disease specific mortality. Therefore, recently the EUTOS long-term survival (ELTS) score was introduced to predict the risk of dying of CML in patients treated with first line imatinib.
Aims: The primary objective of this study was to perform a validation of the ELTS score in an independent cohort of "real-world" population-based CML patients.
Methods: Data from chronic phase CML patients were derived from the PHAROS-CML population based registry and Hemobase. Patients were stratified into a low, intermediate and high risk group according to the ELTS score. Data on "death due to CML" were provided by the Netherlands comprehensive cancer organization (IKNL) in combination with details from the patient records and a competing risk analysis was performed, to take death due to other causes into account.
Results: In total 349 patients were eligible for analysis; 273 patients (78%) were treated with first line imatinib and 76 patients (22%) were treated with a first line second generation TKI (2GTKI). Sokal, Hasford and EUTOS risk scores all did not predict differences in risk of "death due to CML". The ELTS score identified 163 patients as low risk (47%), 127 patients as intermediate risk (36%) and 59 patients as high risk (17%) at diagnosis. The 5 year cumulative incidence of "death due to CML" was indeed significantly higher in the high risk group (11%) compared to both the intermediate risk group (2%, p=0.02) and the low risk group (1%, p=<0.001). Between the intermediate and low risk group no statistically significant difference in risk of dying from CML was observed. A subgroup analysis of only imatinib treated patients showed similar results.
Summary/Conclusions: In the current study based on a "real-world" population-based CML patient cohort, we were able to validate the predictive value of ELTS high risk stratification for "death due to CML" in the current TKI era. Therefore, the ELTS score should be preferred over Sokal, Hasford and EUTOS scores in clinical practice.

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FINAL STUDY RESULTS OF DISCONTINUATION OF DASATINIB IN PATIENTS WITH CML WHO MAINTAINED DEEP MOLECULAR RESPONSE FOR LONGER THAN ONE YEAR (DADI TRIAL) AFTER THREE YEARS OF FOLLOW-UP

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Background: A second-generation tyrosine kinase inhibitor (TKI), dasatinib, is more potent in inhibiting BCR-ABLkinase activity than imatinib. We had previously reported an interim analysis of 63 patients with CML-CP who had discontinued dasatinib treatment after maintaining a deep molecular response (DMR) for more than a year (Lancet Haematology, 2015; 2 (12):e528-35) and demonstrated that dasatinib could be safely discontinued in patients with a DMR of at least 12 months. However, longer follow-up results would clinically be more critical in the treatment of CML.
Aims: In this trial, the total follow-up duration was set as 36 months after the discontinuation. The aim of the current follow-up study was to investigate whether those patients were able to discontinue dasatinib treatment for a longer follow-up period without relapse.
Methods: The eligibility criteria for pre-registration included CML-CP patients, 15 years or older, receiving dasatinib treatment as the second-line or subsequent therapy after imatinib. All participants gave written informed consent.
In this trial, DMR was defined as "no detectable BCR-ABL1 transcript determined using the international scale-based RQ-PCR at a single central laboratory (BML Inc., Tokyo, Japan; the cutoff corresponded to BCR-ABL1 0.0069% IS or molecular response (MR) 4.0)." Patients who showed a sustained DMR for 1 year (1-year consolidation phase) were subsequently included in the dasatinib-discontinuation stage. RQ-PCR was performed monthly for the first 12 months, and then every 3 months for the second year, and every 6 months for the third year, after discontinuing dasatinib. Relapse was defined as any positivity of BCR-ABL1 transcript by RQ-PCR even at one analysis point. In the present study, we assessed the estimated overall treatment-free remission (TFR) after discontinuing dasatinib, with 3 years of follow-up.
In addition, we also evaluated the impact of immunological profiles, including the cell counts of T and NK cell subsets in the peripheral blood throughout the 1-year consolidation phase, on TFR.
Results: Sixty-three patients were included in the dasatinib-discontinuation stage. The median follow-up was 44.0 months (IQR 40.5–48.0). After the 1-year-data cutoff, two additional molecular relapses were detected at 18 and 21 months after dasatinib discontinuation. After molecular relapse, 1 patient showed fluctuating BCR-ABL1 transcript values below MR 4.0 without restarting dasatinib therapy. A total of 35 out of 63 patients showed molecular relapse. No patient showed disease progression. The estimated overall TFR rate was 44.4% (95% confidence interval [CI], 32.0-56.2) at 36 months. A high count of two NK-cell phenotypes (CD3-CD56+ cells ≥539 cells/μl and CD16+CD56+ cells ≥506 cells/μl) and a low count of gd+ T-cells (<120 cells/μl) were detected to be significant factors affecting molecular relapse in the interim analysis; these showed sustained significance as predictors of a favorable TFR (P=0.0475, 0.0202, and 0.0093, respectively).
Summary/Conclusions: As the overall provability of TFR was relatively stable even for a longer follow-up period, our findings provided more compelling evidence supporting dasatinib discontinuation after a DMR for more than 1 year; this is feasible especially in patients with imatinib intolerance. We also reconfirmed that the counts of NK cells and functionally specific T-cells in the peripheral blood during dasatinib treatment might affect the TFR following dasatinib discontinuation.

Hematopoiesis, stem cells and microenvironment

P264

ACUTE MYELOID LEUKEMIA ALTERS THE PERMEABILITY OF THE BONE MARROW VASCULAR MICROENVIRONMENT, FOSTERING DISEASE PROGRESSION AND DRUG RESISTANCE

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Background: The biological and clinical behavior of hematological malignancies is not only determined by the properties of the leukemic cells themselves, but it is also highly affected by the interaction with the microenvironment, pointing to the existence of an active crosstalk between the two compartments. Previous studies showed that acute myeloid leukemia (AML) actively modify endothelial cells *ex vivo* via several pathways, mainly mediated by VEGF. However, anti-VEGF therapies haven't produced successful results in clinical trials.
Aims: Our aim is to perform an extensive study of the vascular niche in the bone marrow (BM) of AML xenografts to provide a global picture of the vasculature in AML disease and design new therapeutic strategies.
Methods: We combined the use of mouse models of AML, human AML derived xenografts (PDX) and direct analysis of patients derived samples to study the vascular niche in AML disease. We used two-photon confocal microscopy as a powerful tool to functionally image the BM vasculature *in vivo*. We used RNA-sequencing to study the AML-associated transcriptomic profile in vascular endothelial cells.
Results: We found several abnormalities in the vascular architecture and function in PDX, such as increased number of endothelial cells, increased microvascular density (MVD), loss of normal sinusoidal architecture and increased hypoxia. Moreover, vascular permeability was increased as measured via two-photon imaging. Interestingly, induction chemotherapy failed to normalize the vascular permeability in the BM, although it significantly reduced the AML engraftment. Via high-throughput transcriptomic analysis, we showed that AML-induced hypoxic environment altered the molecular signature of vascular endothelial cells, activating pro-angiogenic pathways and positively regulating the response to hypoxia. We identified increased nitric oxide (NO) as a major mediator of the AML-induced vascular leakiness in the BM. Notably, increased NO levels were found also in BM aspirates of patients at diagnosis compared to healthy donors, and failure in reducing NO levels after chemotherapy appeared to be associated with a higher incidence of unsuccessful treatment. Strikingly, inhibition of NO production in mouse models of AML and in AML-derived PDX reduced vascular permeability, preserved normal HSC function and significantly improved treatment response (Figure 1).

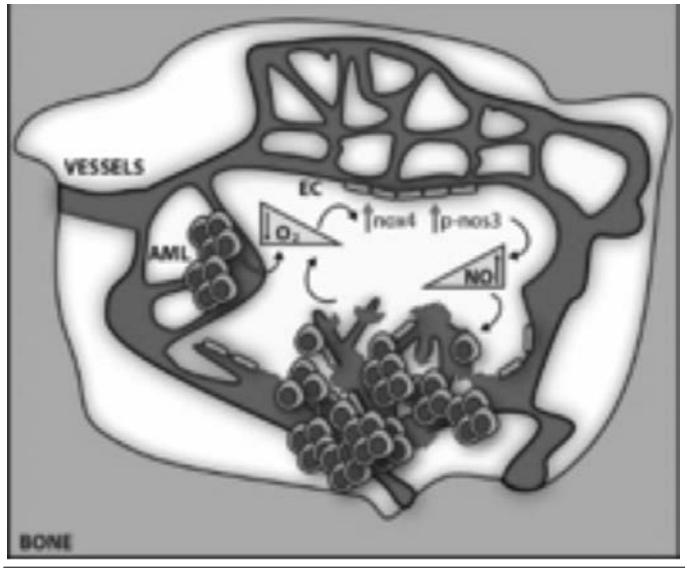

Figure 1.

Summary/Conclusions: We have shown an altered highly permeable vascular niche in the BM of AML PDX, mainly caused by increased NO production by the endothelial niche, contributing to disease progression and treatment failure. Our data call for clinical trials incorporating NOS inhibitors during the remission phase, to target the abnormal vascular niche and improve AML treatment response.

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BUILDING HUMAN BONE MARROW-LIKE MODELS TO STUDY NICHE INTERACTIONS

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Background: Previously, we have reported that our human bone marrow-like scaffold (huBM-sc) xenograft model allows the engraftment and outgrowth of normal and malignant hematopoiesis (*e.g.* multiple myeloma (MM) and acute lymphoblastic leukemia (ALL) (Groen *et al.* Blood 2012; Gutierrez *et al.* JCI 2014) and more recently acute myeloid leukemia (AML; Antonelli *et al.* Blood 2016). These studies show that i) engraftment is not correlated with prognostic risk-groups, ii) there is preferential outgrowth in humanized scaffolds compared to the murine BM, iii) the huBM-sc environment results in better maintenance of self-renewal potential and less clonal drift of the leukemic cells. Although the presence of human osteoblasts and bone mimics a human BM niche more closely than the murine BM in standard xenotransplant models (*e.g.* NOD-SCID/NSG mice), still some essential components of the human BM niche, *i.e.* human blood vessels, are missing.
Aims: To implement human vasculature in the huBM-sc xenograft model in order to create a multi-tissue compartment that "maximally humanizes" the BM-like niche of our scaffolds.
Methods: Towards successful implementation of a human vascular system we compared: i) scaffold material composition (biphasic calcium phosphate (BCP) *vs* tricalcium phosphate (TCP)); ii) scaffold shape (particles *vs* tubes); iii) different types of matrigel for cord blood-derived endothelial progenitor cells (CB-EPCs) embedding.
Results: Histological analysis of these fully humanized scaffolds showed a large number of functional human CD31-positive blood vessels, and additional CD44, CD146, LEPR and nestin-positive stromal niche cells. Comparison of the composition and the shapes of the scaffolds indicated superiority of TCP and tube-shaped scaffolds in supporting the formation of vessels. Engraftment of BM-derived CD34+ cells in the CB-EPC embedded huBM-sc resulted in increased multilineage hematopoietic engraftment, as compared to huBM-sc without CB-EPCs. Moreover, we observed that incorporation of CB-EPCs provides faster kinetics of engraftment of both patient-derived MM and AML cells, and proved to be essential for the engraftment of blast cells from myelofibrosis patients.
Summary/Conclusions: Thus, with the addition of human CB-EPCs and BM stromal cells, our scaffold systems now simulate both endosteal and vascular niches of the BM, thereby more closely recapitulating the human hematopoietic microenvironment.

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MULTISCALE IMAGE-BASED QUANTITATIVE ANALYSIS OF BONE MARROW STROMAL NETWORK TOPOLOGY REVEALS STRICT SPATIAL CONSTRAINTS FOR HEMATOPOIETIC-STROMAL CELLULAR INTERACTIONS

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Background: Adult bone marrow (BM) cavities host continuous, demand adapted and high throughput blood cell production, which is maintained by a rare population of self-renewing, multipotent hematopoietic stem cells (HSCs). Aside from its diverse hematopoietic content, the BM is populated by a heterogeneous fraction of mesenchymal, endothelial and neural stromal cells, which provide the necessary tissue infrastructure for hematopoiesis to unfold while playing fundamental regulatory roles in hematopoietic development. Recent evidence suggests that tissue regions around BM venous microvessels (termed sinusoids), which are enriched for mesenchymal CXCL12-abundant reticular cells (CARc), serve as the principal regulatory niches for HSCs as well as other hematopoietic progenitor populations. Despite this proposed role as putative specific niche-restricted components, comprehensive data on the frequency, global spatial distribution and topology of sinusoidal endothelial and CAR cell networks is largely lacking to date.
Aims: The principal aim of our work is to employ state of the art imaging techniques to perform a detailed 3D quantitative and structural analysis of the BM stromal infrastructure, with a special focus on sinusoidal microvasculature and the CAR cell mesenchymal component, both of which are essential regulators of HSC maintenance.

Methods: We have developed i) advanced microscopy techniques allowing multiscale 3D visualization of entire bone marrow cavities with cellular and subcellular detail ii) customized computational tools enabling the detection and quantification of discrete cell subsets/structures in 3D images of the BM in an unbiased fashion, as well as a rigorous spatial statistical analysis of cellular interactions.
Results: Using 3D-quantitative microscopy (3D-QM) we uncover that BM stromal cells are in fact 15-20 fold more abundant than previously reported. The massive underestimation of these relevant cell subsets results from the highly inefficient isolation of these cellular types with currently employed flow cytometry protocols. Our image-based analyses further reveals that sinusoidal and CAR cell stromal networks occupy a disproportionately large fraction of the BM space, consequently constraining the tissue volume available for hematopoietic cell distribution. In fact, the vast majority of BM resident hematopoietic cells are unavoidably in direct contact with the CAR cellular projections and in close proximity (<25µm) to the extraluminal surface of sinusoidal endothelium.
Summary/Conclusions: Collectively, our quantitative description of stromal microarchitecture, challenges current models of cell type-specific niche interactions in the BM, which are based in largely inaccurate estimations of cell frequency and spatial confinement of stromal cells in this organ.

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TEMPLATED V(D)J INSERTIONS ARE A NOVEL BIOLOGIC MECHANISM FOR B-CELL RECEPTOR REPERTOIRE DIVERSIFICATION

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Background: Recently, large LAIR1 insertions at the V-D junction were described as a novel mechanism to generate antibodies against *P. falciparum* RIFIN antigens on infected erythrocytes (Tan *et al.*, Nature 2016). These templated insertions potentially add a novel biological mechanism used by the immune system to generate B-cell receptor repertoire diversity.
Aims: We investigated whether templated insertions occur in the B-cell repertoire of healthy donors and whether such insertions could be functionally expressed to explore their biological function.
Methods: We obtained >52,000 unique full-length VDJ sequences of IgM, IgG, IgA, and IgE isotypes by unbiased ARTISAN PCR (Koning *et al.*, BJH 2016) from 6 healthy donors. Abnormally long sequences and junctions were searched for templated insertions by BLAST. Identified VDJ carrying templated insertions were co-expressed with a panel of 172 light chains on multiple myeloma cell lines and assessed for surface expression of transgenic immunoglobulin. The VDJ described by Tan *et al.* were included as controls.
Results: Six unique VDJ sequences, all from the same donor, carried a templated insertion in-frame ($E=10^{-37}$ – 0). These sequences represented all VDJ sequences with a CDR3 region >150 bp. Exonic sequences from RPLP0, ZNF316, and an inverted IGHV-IGHD sequence were identified as insertions in unmutated IgM VDJ transcripts. The LAIR1 exon described by Tan *et al.* and an intergenic region adjacent to IGHD3-22 were identified as insertions in IgG VDJ transcripts. One IgA VDJ contained two intergenic sequences positioned closely together on chromosome 22. Somatic hypermutation burden correlated strongly between the the IGHV segment and the templated insertions ($r=0.9944$; $p<0.001$). All templated insertions harboured cryptic RSS sites at their termini. All three IgM VDJ carrying templated insertions and the IgG rearrangement with the IGHD locus templated insertion gave rise to detectable surface immunoglobulin after coexpression with at least one light chain in the panel. The IgG VDJ carrying the LAIR1 templated insertion produced no detectable surface immunoglobulin. In contrast, the VDJ sequences carrying LAIR1 templated insertions as described by Tan *et al.* could be expressed with the majority of the light chains. The IgA rearrangement remains to be tested in this system.
Summary/Conclusions: Templated insertions represent a novel antibody diversification mechanism. Their presence in naïve B-cells, their exclusive positioning in VDJ junctions, and the universal presence of cryptic RSS sites point to primary VDJ recombination or secondary V gene editing as the generating mechanism. Certain loci (*e.g.* LAIR1) and certain individuals appear to have increased susceptibility. The available data suggest RAG to be involved in these insertions. We propose that templated insertions represent inserted signal sequences from aberrantly rearranged chromosomal sequences with cryptic RSS sites.

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TARGETING THE CASPASE / NOX2 AXIS TO MODULATE MACROPHAGE POLARIZATION

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Background: Caspases, which are key effectors of apoptosis, have demonstrated non-apoptotic functions. One of these functions is the differentiation into macrophages of peripheral blood monocytes exposed to Colony-Stimulating Factor-1 (CSF1). Conversely, GM-CSF induces the differentiation of monocytes into macrophages in a caspase-independent manner. Macrophages generated by CSF1 and GM-CSF have distinct polarity.
Aims: Macrophage polarization plays an important role in the pathogenesis of diverse human diseases as cancer, leading us to explore if caspase inhibition would affect macrophage polarization.
Methods: To explore the role of caspases in CSF1 differentiation, we used human monocytes sorted from buffy coats or from blood of NOX2-deficient patients treated by cytokines, and we generated monocyte-restricted caspase-8 knockout and caspase-3/caspases-7 double knockout mice, which were treated with bleomycin to induce pulmonary fibrosis.
Results: Caspase activation is involved in the generation of M2 polarized macrophages. Caspase inhibition delays the *ex vivo* differentiation of peripheral blood monocytes exposed to CSF1 and modifies the phenotype of generated macrophages, *e.g.* cell shape, surface markers and cytokine secretion. In mice, caspase knock-out also modified the phenotype of monocytes induced to differentiate into macrophages. Caspase activation appeared to be prominent at the mitochondria level and responsible for the NOX2-dependent generation of cytosolic radical oxygen species (ROS). Activation of the NOX2 complex is associated with p47phox cleavage by caspases. Mice treated with bleomycin typically develop a pulmonary fibrosis. Bleomycin-induced lung fibrosis was delayed in monocyte-restricted caspase-8 knockout mice and prevented by treatment with a caspase inhibitory molecule, including zVAD-fmk and the clinically developed IDN6556. This effect was associated with a change in the polarization of lung-infiltrating macrophages.
Summary/Conclusions: Caspase inhibition in monocytes prevent the development of bleomycin-induced lung fibrosis by modifying macrophage polarization, suggesting that caspase inhibitory molecules may be an exciting therapeutic strategy to modulate macrophage polarization with diverse applications including cancer treatment.

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MULTIPLE MYELOMA-POLARIZED M2C MACROPHAGES PROMOTE A TUMOR-SUPPORTIVE OSTEOLYTIC MICROENVIRONMENT VIA CXCL13

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Background: Previous studies including our work revealed a role of MM-educated M2-like macrophages (MΦ) in MM survival and drug resistance. However, the mechanism by which neoplastic plasma cells shape BM microenvironment and affect MΦ polarization is still poorly defined.
Aims: To investigate tumor-promoting changes in the myeloid compartment of the BM niche that are initiated by MM cells.
Methods: We utilized our *in vivo* xenograft model of BM-disseminated human myeloma. The CRISPR/Cas9 technology was used to knockdown CXCL13 expression in MM cell lines.
Results: BM analysis of mice inoculated with human CXCR4-expressing RPMI8226 cells revealed a significant increase in M2 MΦ in comparison to non-injected controls ($p<0.01$). Characterization of MM-associated changes in the BM milieu revealed murine chemoattractant CXCL13 being one of the most profoundly increased factors upon MM development. Elevated CXCL13 was also detected in blood of MM-bearing animals comparing to healthy controls. IHC staining identified myeloid cells as the main source of increased murine CXCL13 detected in MM-occupied BM. Murine BM CXCL13 mRNA expression strongly correlated with human β2-microgllobulin mRNA levels ($p<0.0001$ $R^2=0.64$), indicating the interrelation between tumor burden and CXCL13 induction. Reduction of MM load upon the treatment with anti-myeloma agents bortezomib and panobinostat resulted in corresponding correlated decrease in murine CXCL13, both in BM and blood, suggesting the possible utilization of CXCL13 levels as surrogate marker of anti-MM treatments response.
CXCL13 expression by MΦ is known to represent specific anti-inflammatory M2c phenotype acquisition. Indeed, we observed a significant up-regulation of additional M2c markers, such as MERTK and MRC1 in the BM occupied with MM in strong correlation with CXCL13 expression ($p<0.001$ $R^2=0.65$). In vitro studies confirmed the ability of MM cell lines (n=6) to induce CXCL13 and concurrent expression of M2c markers (MERTK, CD206, CD163) in co-cultured human monocytes and MΦ. Interaction with MΦ reciprocally induced CXCL13 expression in MM cell lines and primary human CD138+ cells. Mechanistically, TGFβ signaling was involved in CXCL13 induction in both MM cells and MΦ, as TGFβ receptor inhibitor SB431542 interfered with CXCL13 induction. Osteo-

clastogenic assays were used to elucidate the down-stream effects of the elevated CXCL13. Recombinant CXCL13 as well as medium produced by co-cultured MM-MΦ increased RANKL expression and induced TRAP+ osteoclast (OC) formation *in vitro*, while CXCL13 neutralization blocked these activities. We next abrogated CXCL13 expression in MM cell lines using the CRISPR/Cas9 technology. The loss of CXCL13 had no effect on MM *in vitro* growth or drug sensitivity. However, mice inoculated with CXCL13-silenced MM cells developed significantly weaker BM disease compared to mice receiving the non-manipulated cells. Reduced tumor load correlated with decreased numbers of M2c MΦ in BM, decreased bone disease, and lower expression of OC-associated genes. Finally, the presence of CXCL13 in primary MM samples was evaluated. Elevated levels of CXCL13 transcript and protein were detected in BM aspirates from MM patients (n=24) in comparison to normal BM (n=5) and were in correlation with gene expression signature associated with OC activation and M2c MΦ phenotype (Figure 1).

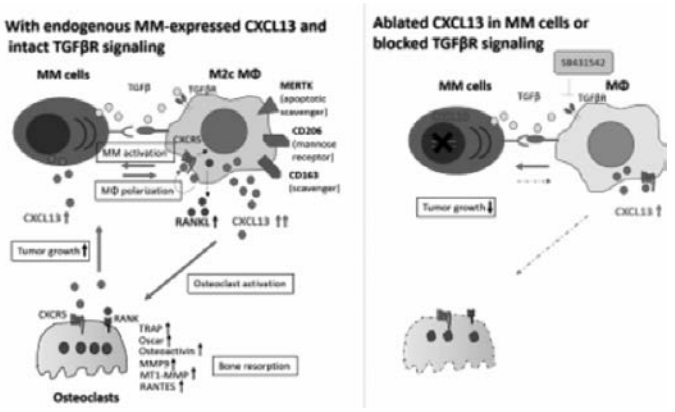

Figure 1.

Summary/Conclusions: Our findings suggest that bidirectional interactions of MΦ with MM tumor cells result in M2c MΦ polarization, CXCL13 induction and subsequent OC activation, enhancing their ability to support bone resorption and MM progression. CXCL13 may thus serve as potential novel target for the diagnosis and treatment of MM.

P270

RE-ORDERING THE B CELL DEVELOPMENT HIERARCHY IN HUMAN FETAL BONE MARROW: CHARACTERISATION OF A NOVEL HUMAN FETAL B PROGENITOR

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Background: The cellular hierarchy of normal human fetal B-lymphopoiesis remains poorly defined. We have previously identified a novel population of PreProB progenitors (CD34+CD19+CD10-) in fetal liver (FL)[1] that is further expanded in fetal bone marrow (FBM)[2], and co-exists with adult-type CD34+CD19+CD10+ ProB progenitors. Increasing evidence indicates that infant ALL and many cases of childhood ALL arise in fetal life, suggesting that ontogeny-related changes in B-cell development may be important for *in utero* leukaemia initiation. Therefore, understanding the human fetal B cell hierarchy, especially the differences between PreProB and ProB progenitors may be key to understanding the origins of infant and childhood leukaemia.
Aims: To determine B cell developmental pathways in human second trimester FBM, with a view to establishing the fetal B cell hierarchy.
Methods: The characteristics of the haematopoietic stem cell (HSC), lymphomyeloid multipotent progenitor (LMPP), early lymphoid progenitor (ELP) and committed B-progenitor compartments of FBM samples were analysed by multiparameter flow cytometry, differentiation and clonogenic assays, transcriptome analysis and single cell RQ-PCR.
Results: All stages of B cell development were demonstrable in human FBM up to transitional B cells with a rapid expansion of B progenitor numbers from the LMPP stage. FBM HSC, progenitors (MPP, LMPP, ELP, PreProB and ProB-progenitors) and B-cells were FACS-sorted for functional/ molecular assays. *Functional assays*: B cell differentiation assays demonstrated expected multilineage output from HSC, MPP and LMPP, but a pure B cell output from PreProB and ProB progenitors. While PreProB progenitors gave rise to ProB progenitor cells *in vitro*, the converse was not true; thereby placing PreProB upstream of ProB in the B cell hierarchy. Myeloid colony assays gave expected multilineage output from HSC/MPP; and GM colonies from LMPP; while ELP, PreProB and ProB progenitors generated no colonies, confirming their lymphoid commitment. *Transcriptome analysis and single cell RQ-PCR*: Fetal HSPC populations were flow sorted and analysed by RNASeq of bulk populations as well as single cell RQ-PCR using a customised 96-gene panel. Stem cell populations (HSC, MPP and LMPP) showed good spatial segregation from lymphoid progenitors (ELP, PreProB and ProB) by principal component analysis both at bulk and single cell level. Single cell analysis demonstrated a differentiation trajectory from HSC to mature B cells with PreProB progenitors clustering between LMPP and ProB. There was sequential upregulation of B lymphoid specific genes from HSC to ProB progenitors and the level of expression was always lower in PreProB compared to ProB, suggesting they are upstream of ProB. PreProB progenitors demonstrated a distinct gene expression profile compared to ProB progenitors. 739 genes were significantly differentially expressed (FDR<0.1) between these two populations with 538 of these being upregulated in PreProB, including some myeloid and leukaemia-associated genes. Genes overexpressed in ProB included B cell specific genes.
Summary/Conclusions: Detailed immunophenotypic, functional and molecular studies allow us to propose a human fetal B cell developmental hierarchy for the first time in which the unique PreProB progenitors are distinct from and lie upstream of the ProB progenitors. These results may have important implications in understanding the pathogenesis of infant and childhood leukaemias.
References: [1] Roy, A. *et al.*, PNAS, 2012. [2] Roy, A. *et al.* Blood 2014. 124:4331.

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HUNDREDS OF EMBRYONIC HEMATOPOIETIC PRECURSORS CONTRIBUTE TO LIFE-LONG HEMATOPOIESIS

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Background: Prior studies of the frequency of emerging hematopoietic stem cells (HSCs) and their precursors during mammalian ontogeny have all required embryo disruption, transplantation assays and concluded that very few HSC emerge from endothelial precursors during embryogenesis to establish life-long hematopoiesis. An alternative approach independent of embryo disruption or transplantation would more accurately reflect the true dynamics of HSC emergence during mammalian development.
Aims: To determine the frequency of emerging HSCs and their precursors throughout mammalian ontogeny.
Methods: Here, we employed the *Confetti* allele, in which a cassette targeted to the *ROSA26* locus randomly and permanently marks cellular progeny with GFP, YFP, RFP or CFP when exposed to *Cre recombinase*. We determined empirically that sample-to-sample variance in the distribution of *Confetti* colors in the blood of the following adult mice inversely correlated with the number of hematopoietic precursors specified during the indicated window of *Cre recombinase* activity: *ROSA26(Confetti/+) Flk1(+/*Cre*)* (mesodermal precursors, E7), *ROSA26(Confetti/+) VE-cadherin(+/*Cre*)* (mid-gestation endothelial precursors, E8.5-E10.5), and *ROSA26(Confetti/+) Vav1(+/*Cre*)* (hematopoietic progenitors E11.5-E14.5). This correlation was used to estimate the number of hematopoietic precursors emerging during each stage of development.
Results: An inverse correlation of sample-to-sample variance in the distribution of *Confetti* colors and numbers of labeled initiating events was established *in vitro* by plating limiting dilution replicates of immortalized *Confetti+* fibroblasts and assessing the resulting sample-to-sample *Confetti* variance of each cell dose plated. We thus derived a linear formula to estimate numbers of initiating events using the sample-to-sample Confetti color variance in a particular tissue (*e.g.* peripheral blood (PB)). We tested this formula *in vivo* via limiting dilution transplantation with *Confetti+* bone marrow. Classic limiting dilution analysis of transplanted mice revealed about 1/12,000 repopulating units in the transplanted BM. The sample-to-sample variance in the distribution of *Confetti* colors in the PB of recipients yielded a similar estimate of the frequency of repopulating events, validating our empirically-derived formula. We further validated our approach *in vivo* by calculating 222 repopulating units in transplant recipients of dissociated cultured E11.5 aorta-gonad-mesonephros (AGMs) explants. This finding correlates very well with previous reports that E11.5 cultured AGM explants contain about 150 transplantable HSCs. Analyses of the sample-to-sample variance of the *Confetti* colors in the blood of cohorts of *ROSA26(Confetti/+) Flk1(+/*Cre*)*, *ROSA26(Confetti/+) VE-cadherin(+/*Cre*)* and *ROSA26 (Confetti/+) Vav1(+/*Cre*)* mice revealed that about 719 mesodermal precursors, 633 endothelial precursors and 545 fetal liver hematopoietic precursors contribute to the emerging hematopoietic system. Our findings are in sharp contrast to previous reports that very few precursors and emerging hematopoietic progenitors establish the hematopoietic system. Our data further suggest that a "developmental bottleneck" exists after the fetal liver stage of hematopoietic ontogeny that restricts the numbers of precursors that ultimately contribute to life-long hematopoiesis.
Summary/Conclusions: We report here a novel approach to examine the complexity of the emerging hematopoietic system without perturbing the developing embryo. This represents a new platform to identify biologically relevant processes that govern the dynamics of different populations during embryonic

development. We thereby report for the first time that the clonal origin of blood is much more complex than previously thought, with hundreds of precursors contributing to the establishment of the mammalian blood system at multiple stages of ontogeny.

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A20 RESTRAINS THYMIC REGULATORY T CELL DEVELOPMENT

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Background: Maintaining immune tolerance requires the production of Foxp3 expressing T regulatory (Treg) cells in the thymus. Activation of NF-κB transcription factors is critically required for Treg cell development, partly via initiating Foxp3 expression. NF-κB activation is controlled by a negative feedback regulation through the ubiquitin editing enzyme A20, which reduces pro-inflammatory signaling in myeloid cells and B cells. In naive $CD4^+$ T cells, A20 prevents necroptosis and promotes inflammation.
Aims: This study is aimed at analyzing the role of the NF-κB regulator A20 in Treg cell development and function.
Methods: We used $A20^{F/F}$ CD4Cre mice, which specifically lack A20 in T cells, to analyze the Treg cell compartment *in vivo.* We characterized expansion and differentiation of A20-deficient Treg cells *in vitro*. We performed competitive bone marrow engraftment between WT and A20-deficient bonemarrow *in vivo* to analyze whether one bone marrow compartment would outperform another or would favor development of certain T cell or other immune cell subsets. We performed alloegeneic hematopoietic stem cell transplantation with WT BM+T cells ± WT *vs* A20-deficient Treg cells to analyze whether A20-deficient T reg cells would reduce GVHD to the same extent as WT Treg cells.
Results: Using mice deficient for A20 in T lineage cells, we show that thymic and peripheral Treg cell compartments are quantitatively enlarged due to a cell-intrinsic developmental advantage of A20-deficient Treg cells. $A20^{-/-}$ Treg cells efficiently suppressed effector T cell mediated graft-*versus*-host disease after allogeneic hematopoietic stem cell transplantation, demonstrating normal suppressive functionality. Holding thymic production of natural Treg cells in check, A20 thus integrates reduced regulatory T cell activity and increased effector T cell survival into an efficient $CD4^+$ T cell response.
Summary/Conclusions: In light of the largely anti-inflammatory effects that have been attributed to A20 in many cell types, this proinflammatory aspect of A20 physiology in effector and regulatory $CD4^+$ T cells is particularly important since it may contribute to a change of perception of the functions of A20 as a negative regulator of NF-κB in the context of inflammation. Whether targeted modulation of A20 activity allows the induction of Treg cell mediated immune tolerance or, alternatively, boosting of favorable T cell immunity is a question of translational relevance that needs to be addressed in the future. .

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THE TRANSCRIPTION FACTOR C/EBPG REGULATES MAST CELL DEVELOPMENT AND FUNCTION

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Background: Mast cells are key effector cells involved in protection against infection and allergic responses. Defects in mast cells are related to immunological disorders, and therefore it is critical to fully understand the transcriptional network that controls their generation and activity. Differentiation of progenitors to mature mast cells is promoted by several transcription factors, such as GATA1, GATA2, STAT5, and MITF, and requires downregulation of C/EBPα. Recently, we identified another member of the C/EBP family of transcription factors, C/EBPγ, as a direct C/EBPα target gene. However, the role of C/EBPγ in mast cells remains so far elusive.
Aims: In this study we aim to determine the role of the transcription factor C/EBPγ in mast cell development and function. Next, we investigate the mechanisms by which C/EBPγ is controlling these processes.
Methods: In order to determine the role of C/EBPγ in murine mast cells, we generated *Cebpg* conditional knockout mice, which allow excision of *Cebpg* in the hematopoietic system from the early embryogenesis. We employed $Cebpg^{flox/flox}$ Vav-1Cre- and $Cebpg^{flox/flox}$ Vav-1Cre$^+$ mice, referred here as WT and *Cebpg* KO, respectively. Excision of *Cebpg* was assessed by RT-PCR and western blot analysis in bone marrow and spleen cells. Using flow cytometry, we enumerated mast cell counts in the peritoneal cavity of healthy WT and *Cebpg* KO mice. To elucidate whether C/EBPγ plays role in mast cell response to bacterial infection, we challenged these mice intraperitoneally with lipopolysaccharide (LPS). Finally, we used intraperitoneal injection of distilled water to eradicate peritoneal mast cells and then monitored repopulation of peritoneum over time. To further explore the role of C/EBPg in mast cells *in vitro*, we established bone marrow derived mast cells (BMMCs) and determined their growth (cell numbers), morphology (toluidine blue staining), and transcription factors expression (RT-PCR) at different time points. Degranulation potential of BMMCs was specified by measuring the percentage of b-glucuronidase released to the supernatant upon anti-TNP IgE sensitization and TNP-BSA activation. To investigate the effects of absence of *Cebpg* during mast cell migration, we employed transwell migration assays.
Results: We verified efficient ablation of *Cebpg* on mRNA and protein level in bone marrow and spleen of *Cebpg* KO mice. Analysis of peritoneal cavity of WT and *Cebpg* KO mice showed similar frequency and numbers of mast cells in steady state conditions. However, *Cebpg* deficient mice exhibit increased number of peritoneal mast cells after LPS stimulation in comparison to WT control littermates. Surprisingly, mice lacking *Cebpg* presented defective peritoneal mast cell repopulation. Since mast cells are scarce and difficult to isolate from *in vivo* models, we employed BMMCs to investigate the effects of *Cebpg* ablation in mast cell development and function. We observed that bone marrow from *Cebpg* KO mice generated reduced number of BMMCs in comparison to WT controls. Functionally, we demonstrated that deletion of *Cebpg* reduced mast cell migration towards antigen, SCF or PGE, and impaired degranulation upon FcεRI-mediated activation. Further, BMMCs exhibit increased expression of C/EBPα in the absence of C/EBPγ.
Summary/Conclusions: In summary, we revealed C/EBPγ as important transcription factor which suppresses C/EBPα expression, thereby favoring mast cell development and function. Our data identifies a new component of the mast cell transcriptional network and provides a better understanding of mast cells in normal physiological conditions and disease.

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TRANSCRIPTIONAL DIVERSITY AND DEVELOPMENTAL POTENTIAL OF EARLY HEMATOPOIETIC PROGENITORS REVEALED BY CELLULAR BARCODING AND TRANSCRIPTOME-WIDE PROFILING

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Background: One of the main complications of anti-cancer therapies or bone marrow transplantation protocols is their deleterious effect on the blood system, leading to prolonged neutropenia and increased risk for infections. Manipulating hematopoietic stem cells differentiation pathways to favor production of specific lineage-committed progenitors might optimize blood recovery.
Aims: In this study we aimed 1) to determine and quantify the contribution of medullary progenitor populations (MPP) to the repopulation of the T cell pathway using the barcode cellular labelling strategy that we have previously developed and 2) to decipher the heterogeneity of these MPP at the transcriptional level.
Methods: Three different MPP subsets, of the following phenotype: VCAM1+Flt3 (MPP1); VCAM1-Flt3+ (MPP2) and VCAM-1Flt3+ILR7 (CLP), were tagged with different barcodes carried by a collection of lentivirus and transplanted in mice. Barcoded cells in recipient mice were analyzed by flow cytometry. For whole transcriptome strand-specific sequencing, three biological replicates, per cell population, were sequenced at high depth of coverage (2 x 120 million reads).
Results: The results allowed the *in vivo* dynamic tracking of the progeny of the barcoded progenitors in transplanted recipients. Moreover, transcriptome-wide profiling allowed to identify, by cluster analysis of RNAseq profiles together with gene ontology annotation, unique co-expressed markers for the prospective isolation of these populations. Unsupervised classification correctly classified reference surface markers, currently used to purify progenitors, which validate our bioinformatic methodology. Transcriptional regulation of these cell surface markers was further assess by searching for co-expressed transcription factors and enriched binding sites in their promoters. Their grouping enabled to establish undescribed regulatory networks, specific to each progenitor cell.
Summary/Conclusions: Collectively, the cellular barcoding tool and the molecular changes observed at RNA and functional levels as they occur *in vivo* in the context of physiologic commitment processes, highlighted data that contribute to a deeper understanding of the dynamic of T-lineage differentiation and the lineage restriction process.

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LONG-TERM OUTCOME OF PATIENTS WITH NODULAR LYMPHOCYTE-PREDOMINANT HODGKIN LYMPHOMA TREATED WITHIN THE RANDOMIZED HD7-HD15 TRIALS: AN ANALYSIS FROM THE GERMAN HODGKIN STUDY GROUP

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Background: Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) is a rare entity accounting for approximately 5% of all Hodgkin lymphoma (HL) cases. Pathological and clinical features differ from classical HL (cHL). Pathologically, the malignant lymphocyte predominant (LP) cells stain consistently positive for CD20 and are negative for CD30. Clinically, NLPHL often has a rather indolent course. Despite of these differences, the first-line treatment of NLPHL is mostly very similar to cHL. However, analyses on the long-term course of patients with NLPHL who were treated identically to cHL are scarce.
Aims: To shed more light on characteristics and outcome of NLPHL patients treated identically to cHL, we performed an analysis using the database of the German Hodgkin Study Group (GHSG).
Methods: A total of 471 patients with NLPHL who had received first-line treatment within the randomized GHSG HD7-HD15 trials for newly diagnosed HL were identified. The studies were conducted between 1993 and 2009. Patients at all stages (early favorable: HD7, HD10, HD13; early unfavorable: HD8, HD11, HD14; advanced: HD9, HD12, HD15) were included.
Results: Among the 471 NLPHL patients, the median age was 39 years; 76% of patients were male; 53% of patients had early favorable, 16% had early unfavorable and 31% had advanced-stage disease. Study treatment consisted of ABVD- or BEACOPP-based chemotherapy alone, radiotherapy (RT) alone or combined-modality treatment (CMT). After a median observation of 9.2 years, the 8-year progression-free survival (PFS) rate for the whole patient group was 81.3% (83.2% for early favorable, 85.2% for early unfavorable, 76.2 for advanced stages). 80 of 471 patients (17%) had refractory disease or relapsed during the course of follow-up (primary disease progression: 8 patients; early relapse: 6 patients; late relapse: 66 patients). Second malignancies including histological transformation into aggressive B-cell non-Hodgkin lymphoma (NHL) occurred in 48/471 patients (10%) (solid tumor: 25 patients; leukemia: 7 patients; NHL: 13 patients; unspecified malignancy: 4 patients). For all 471 patients included in the present analysis, the 8-year overall survival (OS) rate was 93.3% (95.1% for early favorable, 98.6% for early unfavorable, 87.4% for advanced stages). A total of 43 deaths were observed during follow-up resulting in a death rate of 9%. However, only a minority of these deaths was NLPHL-related (n=10). In contrast, most patients died from second malignancies (n=20) or due to other causes (n=13) such as heart failure and lung disease.
Summary/Conclusions: Taken together, the results from this large analysis on NLPHL patients prospectively treated and followed within randomized clinical studies for newly diagnosed HL indicate an excellent lymphoma-specific outcome. Nonetheless, further treatment optimization is necessary as the majority of the observed deaths were not NLPHL-related but due to second malignancies or other treatment-related late effects. Thus, future clinical trials including NLPHL patients should evaluate whether it is possible to reduce the treatment intensity without compromising efficacy. This goal may be achieved by the partial replacement of conventional chemotherapy by targeted drugs such as anti-CD20 antibodies as well as the reduction of RT fields and doses.

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ADVANCED HODGKIN LYMPHOMA IN THE EAST OF ENGLAND CANCER NETWORK: A 10-YEAR COMPARATIVE ANALYSIS OF OUTCOMES FOR ABVD AND ESCALATED-BEACOPP TREATED PATIENTS AGED 16–59

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Background: The majority of young patients with advanced-stage Hodgkin lymphoma (HL) in the UK are managed with ABVD. However, following publication of the HD9 trial results in 2009, escalated-BEACOPP (escB) was introduced by some UK centres to improve disease control in poor-risk patients.
Aims: We present a 10-year retrospective multicentre analysis for advanced-stage HL patients, aged 16–59, diagnosed between 2004–2014 in the East of England Cancer Network and treated predominantly outside of clinical trials. Our study period includes the 5 years before, and after, the introduction of escB. We estimated the 5-year progression-free survival (PFS) and overall survival (OS) rates for the whole cohort, and treatment subgroups, to assess the impact of escB on survival outcomes.
Methods: We collected data retrospectively from 8 hospitals in the East of England using electronic medical records and cancer registry data. Only patients with a minimum follow-up of 18 months from diagnosis were included. The 5-year PFS and OS were estimated using the Kaplan-Meier method, and subgroups were compared using the standard log-rank test.
Results: We identified 250 patients (stage IIA bulk–IVB) treated in the East of England Cancer Network over a 10-year period from a referral population of 2.64 million (incidence: 0.95 cases per 100,000). Six of the 8 centres introduced escB for poor-risk patients, as determined by physician and patient choice; 44 patients were treated with escB, 202 with ABVD, 3 with alternative regimens, and 1 died pre-treatment. The median age at diagnosis was 35 years (16–59) and the median follow-up was 72 months (19–139). The 5-year PFS for all patients was 82% and 5-year OS was 92%. There was evidence of a physician–patient preference to treat poor-risk patients with escB, as a greater proportion of escB patients had a high international prognostic score (IPS 3+) than in ABVD patients (escB 75% *vs* ABVD 38%, p<0.0001). For the whole cohort, PFS was better for patients treated with escB compared with ABVD (5-year PFS 95% *vs* 80%; HR 4.3 (95% CI:1.97–9.7), p=0.0261), but there was no difference in OS (5-year OS 97% *vs* 92%; HR 2.6 (95% CI:0.69–10.4), p=0.312). However, patients with IPS 3+ had both a PFS and OS advantage when treated with escB compared with ABVD (5-year PFS 96% *vs* 74%; HR 9.24 (95% CI:3.43–24.89), p=0.012: 5-year OS 100% *vs* 84%; p=0.0325). Twenty-nine ABVD patients and 3 escB patients had at least 1 subsequent stem cell transplant (including 6 allografts post-ABVD and 3 allografts post-escB), and there was equal use of consolidation radiotherapy between regimens (11% of both ABVD and escB patients). Treatment-related infertility is an important consideration for escB patients. In our patient population, of the 20 pre-menopausal women treated with escB, 11 of the 14 (78.6%) aged <30 years at diagnosis regained menstrual periods during follow-up, 5 (45.5%) of whom subsequently conceived (including 6 live births, 1 miscarriage, and 1 termination). Only 1 of the 6 (16.7%) pre-menopausal women aged >30 years at diagnosis regained menstrual periods, which were not sustained beyond 3 years' follow-up.
Summary/Conclusions: Our data reflect clinical trials results which indicate a first-remission PFS but not OS advantage for unselected young advanced-stage HL patients treated with escB compared with ABVD. However, our data strongly suggest that patients with a poor IPS score derive a PFS and OS benefit from treatment with escB compared with ABVD.

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IMPACT ON SURVIVAL OF EARLY DETECTION OF RECURRENCE IN THE FOLLOW-UP OF HIGH RISK HODGKIN LYMPHOMA IN FIRST COMPLETE REMISSION

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Background: Despite the high complete response (CR) rate to anthracycline-including first-line therapy, approximately one-third of patients with advanced-stage Hodgkin lymphoma (HL) relapses. Many relapses (30–50%) are clinically asymptomatic, without any physical and/or laboratory signs. For patients at high-risk of relapse, a close monitoring, based on imaging procedures is justified if an early detection of recurrence would allow a timely administration of salvage therapy and a survival improvement.
Aims: The purpose of this study was to evaluate the response rate to salvage therapy of relapsed HL by comparing patients who received surveillance with conventional clinical assessments *versus* patients who received surveillance with imaging procedures. The primary end-point was to assess the rate of CR to salvage therapy at first relapse (confirmed by FDG PET/TC performed before ASCT). Secondary endpoints were: the overall rate of recurrence after first CR, the stage and extranodal involvement at relapse, and the disease-free survival (DFS), from the end of salvage treatment.
Methods: Between June 2001 and December 2009, we analyzed 306 patients with high-risk HL in CR after anthracycline-including induction treatment. In this case-control study, the first cases (n=156) consisted of patients who received a conventional follow-up program including symptom assessment, blood tests and physical examination; in these patients imaging procedures were performed only in case of suspected relapse (Historical group). Subsequent patients (n=150) received routine imaging procedures comprising ultrasonography (US) for the evaluation of superficial, anterosuperior mediastinal, abdominal, and pelvic lymph nodes (SMAP US), and chest radiography (CXR), as integrated part of the follow-up strategy (Imaging group). Follow-up procedures were performed at 4, 8, 12, 16, 20, 24, 30, 36, 48, 60, 84, and 108

months after treatment discontinuation in both groups. Relapses were documented by histologic examination in both groups. When relapse was documented all patients received salvage therapy with high dose chemotherapy (DHAP), for at least two courses, followed, in case of CR, by ASCT.
Results: After a median 62-months observation (range, 4–108), 83 patients, evenly distributed in the two groups, had a relapse of disease. Of these, 29 of 43 patients (67.4%) of the historical cohort *vs* 17 of 40 patients (42.5%) of the imaging cohort, showed a larger spread of disease at restaging, *i.e.* stage superior to IIB, and a more frequent extranodal involvement, 10/43 (23.3%) patients in the historical group *vs* 3/40 (7.5%) patients in the imaging group (p=0.01). Furthermore, if we considered only asymptomatic patients, one recurrence was detected in 26 of 43 patients in the imaging group and 17 of 40 patients in the historical group, p=0.02. CR rate with second line therapy were higher in the imaging group (27, 67.5%) compared with the historical group (19, 44.2%; p=0.032). The 3-years DFS was 75% in the imaging group and 36% in the historical group, p=0.02.
Summary/Conclusions: This is the first prospective case-control study using SMAP-US plus CXR to monitor patients with advanced stage HL. We show that SMAP-US plus CXR is a valuable tool to improve follow-up in patients with a high risk of recurrence. Our data indicate that the early detection of HL recurrence allows to begin rescue therapy in patients with a more limited disease and, consequently, increase its effectiveness in terms of probability to response and DFS.

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LATER LINE DRUG TREATMENT PATTERNS OF CLASSICAL HODGKIN'S LYMPHOMA PATIENTS IN CANADA, FRANCE, GERMANY AND THE UNITED KINGDOM

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Background: Whilst cHL is seeing increasing 'cure' rates, a cohort of patients remain who, due to multiple relapses, require 3rd (3L) or 4th (4L) lines of drug treatment. Real world treatment patterns for RRHL patients are currently less understood.
Aims: To understand the drug treatment patterns of cHL patients in 3rd or later lines.
Methods: Real-world data were collected through a cross-sectional survey administered to physicians in Canada (Ca), France (Fr), Germany (Ge), and the UK between May and Sep 2016. Physicians provided data on the last 8 cHL patients receiving 3rd or 4th line drug treatment. Data captured included demographics, disease history and treatment patterns. Auto/allo stem cell transplants (auto/alloSCT) were not classified as a treatment line and limited data was available to determine when a SCT was received. Summary statistics were reported and differences between sub-groups assessed using chi-square tests.
Results: In total 116 physicians (Ca, 16; Fr, 31; Ge, 44; and UK, 25) provided information on 959 cHL patients (Ca, 128; Fr, 243; Ge, 351; and UK, 237) on 3rd or later lines of drug treatment. Data for 954 cHL patients on 3rd line drug treatment was captured. Patients had a mean age of 54.0 years (SD: 16.79) at the point of data capture. 57% were male, 43% female. 30% had bulky disease. 84% of patients had been tested for the Epstein Barr virus (EBV), 36% confirmed as positive. The most commonly prescribed 3rd line drug treatment was a brentuximab-vedotin (BV) based regimen (35%). BV use was significantly different across the markets; Canada (34%), France (35%), Germany (30%) and the UK (44%) (p=0.010). The next most commonly prescribed 3rd line treatments were DHAP (8%), BEAM (7%) and bendamustine (7%). 4% of 3rd line patients received a PD-1 inhibitor. Of 3rd line BV patients the majority received ABVD (69%) or BEACOPP (19%) at 1st line. Their most common 2nd line drug treatments were DHAP (21%), ICE (10%), ESHAP (9%) and BEACOPP (9%). 59% of all 3rd line BV patients had undergone an auto/alloSCT at some point during their treatment history. Of 3rd line patients receiving non BV-based regimens 6% had been treated with BV previously (1st / 2nd line). Of 3rd line patients treated with a PD-1 inhibitor 7% had been previously treated with BV. Data for 453 cHL patients on 4th line drug treatment was captured. 4th line patients had a mean age of 55.5 years (SD: 16.79) at the point of data capture. 56% were male, 44% female. 83% had been tested for EBV, 38% confirmed positive. 30% of 4th line patients received a BV based regimen – BV use across markets was significantly different; Canada (20%), France (38%), Germany (23%) and the UK (36%) (p=0.007). At 3rd line this cohort had most commonly received DHAP (16%), BEAM (15%) or ICE (11%). 5% of 4th line BV patients also received a BV based regimen at 3rd line. 129 4th line patients had received a BV regimen at 3rd line. At 4th line 38% of this cohort received a PD-1 inhibitor, 19% bendamustine and 9% gemcitabine.
Summary/Conclusions: Real-world data indicates an unmet medical need for cHL patients with multiple relapses, reinforced by the use of PD-1 inhibitors in those relapsing post BV based regimen at 3rd line. There also appears to be no clear standard of care at 3rd line, again highlighted by use of a range of regimens and PD-1's.
This study was sponsored by Bristol-Myers Squibb.

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CHEMOTHERAPY AND RADIATION IMPROVE SURVIVAL IN EARLY STAGE CLASSICAL HODGKIN LYMPHOMA, A STATEWIDE CANCER REGISTRY ANALYSIS.

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Background: Early stage classical Hodgkin Lymphoma (cHL) has been shown to have an excellent outcome. Recent studies have therefore focused on decreasing the toxicity that results from the addition of radiation therapy to chemotherapy. However, it remains unclear whether omitting radiation as part of the initial therapy of cHL is associated with a similar survival
Aims: The primary aim of this study is thus to investigate the outcomes observed in a statewide cancer registry for cHL patients treated with chemotherapy alone *vs* patients treated with both chemotherapy and radiation.
Methods: All adult patients (older than 18) diagnosed with cHL in Kentucky Cancer Registry (KCR) from 2005-2014 were retrospectively reviewed. Baseline characteristics including age at diagnosis, gender, histology, stage, B symptoms, extranodal involvement, and the site involved were collected. First line treatment modalities as well as overall survival outcomes were reviewed. Stage I and II patients without B symptoms were considered favorable, while those with B symptoms were considered unfavorable. Patients with stage III and IV disease were given an advanced stage designation. To adjust for selection bias, patient deaths during the first 6 months of diagnosis were censored for overall survival analysis.
Results: A total of 961 patients were identified. Median age was 41 (range 18-91) and 60.9% (n=585) were younger than 50. The group included a mild predominance of males (55.5%). Only 1.7% (n=16) had extranodal involvement at presentation. Of those with known histology (78.6%), the most common was nodular sclerosis (71.2%), followed by mixed cellularity (22.8%), lymphocyte rich (3.8%) and lymphocyte depleted (1.9%). Median follow up time was 45 months (range 0-136). The 10-year overall survival for the favorable group (n=329) was 77% (95% CI: 71.1-88.8) *versus* 68% for the unfavorable group (n=144) and 42% for the advanced group (372) (p<0.001). There was no statistical difference in survival between stage I (n=170), and stage II (n=385) disease (p=0.99). Treatment modalities were then compared for the favorable risk group alone. Those who received chemotherapy alone (n=145) were compared to those who received combined chemotherapy and radiation (n=148) as their primary therapy. The 10-year overall survival for the cohort receiving chemotherapy and radiation was 87% compared to 75% for those receiving only chemotherapy (p<0.001) (Figure 1). When adjusted by multivariate analysis for risk factors affecting 10 year survival of the favorable cohort, only age <50 and the treatment modality were independently associated with a statistically significant difference in overall survival (HR of 0.11 (p<0.001) and 3.94 (p=0.001), respectively).

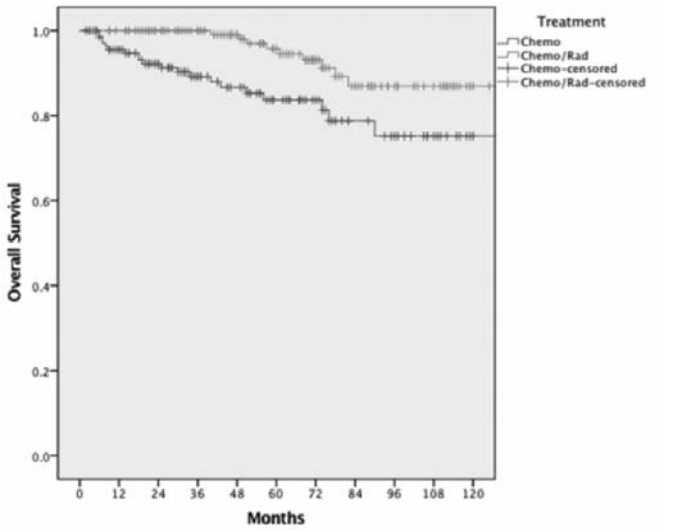

Figure 1.

Summary/Conclusions: Our large data cohort shows the presence of B symptoms was more prognostic than the number of nodal regions involved for early stage disease. Although the use of radiation as part of initial therapy for early stage disease might have increase long term toxicity, it continued to provide superior survival at 10 years.

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THE IMPACT OF TREATMENT WITH BRENTUXIMAB VEDOTIN ON OVERALL SURVIVAL OF PATIENTS WITH HODGKIN LYMPHOMA RELAPSED AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION. A NATIONWIDE POPULATION BASED ANALYSIS

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Background: Patients with Hodgkin Lymphoma (HL) who relapse after autologous Stem Cell Transplantation (auto-SCT) have a dismal prognosis. Advanced disease stage, presence of B-symptoms, extranodal involvement at the time of relapse and duration of remission of less than 12 months are parameters associated with decreased overall survival (OS). Brentuximab Vedotin (BV), an anti-CD30 monoclonal antibody conjugated to a microtubule-disrupting agent, has shown clinical efficacy in HL. Although in the setting of post-auto-SCT relapse, BV produces an overall response rate of approximately 75% with a median progression free survival (PFS) of 9 months, the impact of BV on OS has not been addressed in previously published studies

Aims: To examine the impact of treatment with BV on OS of patients with HL relapsed after auto-SCT.

Methods: Data for patients with HL who underwent auto-SCT in Greece during the last 20 years were collected. Study group consisted of 214 patients who experienced post auto-SCT relapse. In order to examine the impact of BV on OS, patients were divided in 2 cohorts depending of the date of BV availability in Greece (January/2013). Cohort 1 consisted of 178 patients who relapsed before January/2013, while Cohort 2 consisted of 36 patients relapsed after BV became available. Patient's characteristics are shown in Table 1.

Table 1. Patients characteristics.

Parameter	Cohort 1 Before BV (no=178)	Cohort 2 After BV (no=36)	Significance
Age, median	29, (18-65)	32, (19-68)	n.s
Sex, M/F	106/72	22/14	n.s
B-symptoms, (yes v.s no)	60/118	10/26	n.s
Stage (I-II v.s III-IV)	71/107	14/22	n.s
Extranodal (yes v.s no)	76/102	15/21	n.s
Time from SCT to relapse (≤12 v.s >12 months)	137/41	27/9	n.s
Response to 1st salvage (yes v.s no)	105/73	18/18	n.s
Allo-SCT after relapse	28/178 (16%)	8/36 (22%)	n.s
BV after relapse	32/178 (18%)	36/36 (100%)	p<0.001
BV as 1st salvage after relapse	4/32 (12%)	20/36 (56%)	p<0.001

The following variables were included in a multivariate Cox proportional hazard regression analysis model: 1) age of patient, 2) Sex, 3) B-symptoms (yes *vs* no), 4) Stage of disease (I-II *vs* III-IV), 5) extranodal disease, 6) time from auto-SCT to relapse (≤12 *vs* >12 months), 7) Relapse before or after BV availability (Cohort 1 *vs* Cohort 2). In order to exclude any confounding effect of subsequent treatments, analysis was performed by censoring patients at the time of allogeneic SCT or treatment with immune checkpoint inhibitors (IC-inhibitors).

Results: In multivariate analysis the following variables were statistically associated with OS: 1) The presence of B-symptoms [HR=2.07, (95% CI, 1.39-3.07), p<0.001] and 2) Relapse in less than 12 months after auto-SCT [HR=2.02, (95% CI, 1.22- 3.35), p= 0.005] were associated with decreased OS, while 3) Response after 1st salvage [HR=0.46, (955 CI, 0.31- 0.68), p<0.001], and 4) BV availability [HR=0.36, (95% CI, 0.16-0.79), p= 0.011] were associated with increased OS (Figure 1). Similar results were obtained when analysis was performed without censoring patients at the time of allo-SCT or treatment with IC-inhibitors (data not shown).

Summary/Conclusions: Patients in Cohort 2 survived longer even when censored for allo-SCT or treatment with IC-inhibitors. All patients in Cohort 2 treated with BV while only 18% of patients in Cohort 1 received treatment with BV. The results of our study strongly suggest that BV improves OS in patients with HL relapsed after auto-SCT. To our knowledge this is the first study showing an OS advantage of treatment with BV.

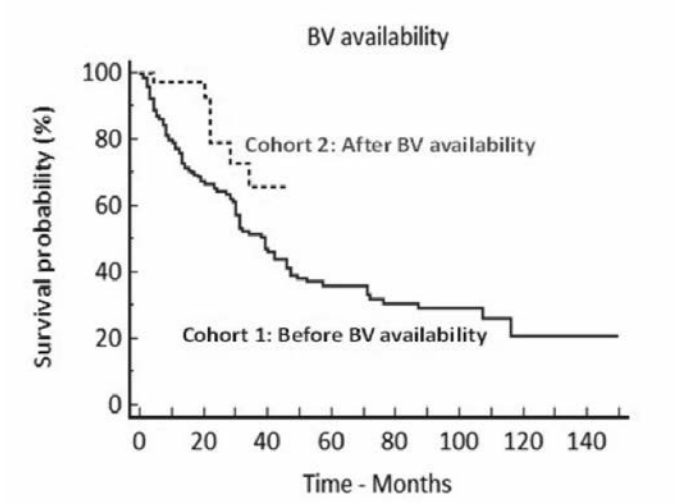

Figure 1.

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NIVOLUMAB FOR RELAPSED OR REFRACTORY HODGKIN LYMPHOMA: EXPERIENCE IN TURKEY

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Background: The programmed death-1 (PD-1) inhibitors have been approved by FDA for patients who relapse following autologous stem cell transplantation and brentuximab vedotin (BV) therapy.

Aims: This retrospective multicenter study aimed to provide information about the efficacy and safety of nivolumab in the "real-life" setting in Turkey.

Methods: 23 centers from Turkey participated in this study. Eligible patients were required those treated with at least 1 course of nivolumab and with available radiological response evaluation. The decision about inclusion of patients with organ dysfunction was made by the attending physician. Patients received nivolumab via a named-patient program, and there was no restriction for BV- and/or transplantation-naïve cases. Nivolumab was adminstered at a dose of 3 mg/kg iv infusion over 60 min q2wk in outpatient setting until death of any cause, unacceptable toxicity, withdrawal of consent, or primary physician's decision. The study was approved by the local ethical commitee. The primary endpoint was the overall response rate (ORR); secondary endpoints were overall survival (OS), PFS, and safety. The response was assessed by positron-emission tomography/computed tomography or CT. Early radiological evalua-

tion was defined as imaging at or before week 12 of treatment, whereas late radiological evaluation was performed at or after week 16. Response evaluation was performed according to the Lugano Classification and its update regarding immunomodulatory therapy.

Results: Between 06/2015-11/2016, 87 patients were enrolled in a name-based program in Turkey. Two, 19, and 3 patients who had not yet received nivolumab, had not reach the time for early radiological evaluation, and who died before any radiological evaluation were excluded from the analysis. Thus, 63 patients from 23 centers were retrospectively analyzed. Median follow-up was 6 months, median age was 29 (18-75) and patients had a median 5 (2-11) previous lines of therapy. 44 patients (70%) had been treated by stem cell transplantation (SCT) and 48 (76%) patients had been treated by BV. The ORR was 68% with 15 CR (95%CI 0.020-0.28; CR 26%, PR 42%, SD 12%, PD 20%) among 59 patients evaluated in 12 weeks of nivolumab treatment. The ORR was 67% with 9 (24%) patients with CR after 16 weeks of treatment (95%CI 0.004-0.26; CR 24%,PR 43%, SD 6%,PD 27%). Estimated OS was 95% (95%CI 0.80-0.98) and estimated PFS was 71% (95%CI 0.55-0.82) at 12-months. Median OS was not reached, while, according to the late response rates, the median PFS was 14 months. However, it was only 3 months in patients with PD at the late radiological evaluation. Regarding responses to last treatment prior to nivolumab, we detected that 28 (67%) of 42 PD cases had objective early responses and 70% of PD cases had ORR in the late response evaluation (CR in 4, PR in 12 pts). 8 patients underwent transplantation following nivolumab. Among 5 patients who had been treated by allo-SCT, 4 had CR at the time of transplantation and they are alive with ongoing response. Safety profile was acceptable and only two patients required cessation of nivolumab due to serious adverse events: one due to autoimmune encephalitis and one due to aggravation of graft *versus* host disease. At the time of analysis, 40 cases were still on nivolumab treatment (64%). Among the 40 cases with early objective responses to nivolumab, 35 (88%) showed ongoing objective responses. All 24 cases with objective responses in the late evaluation had ongoing responses at the time of analysis (Figure 1).

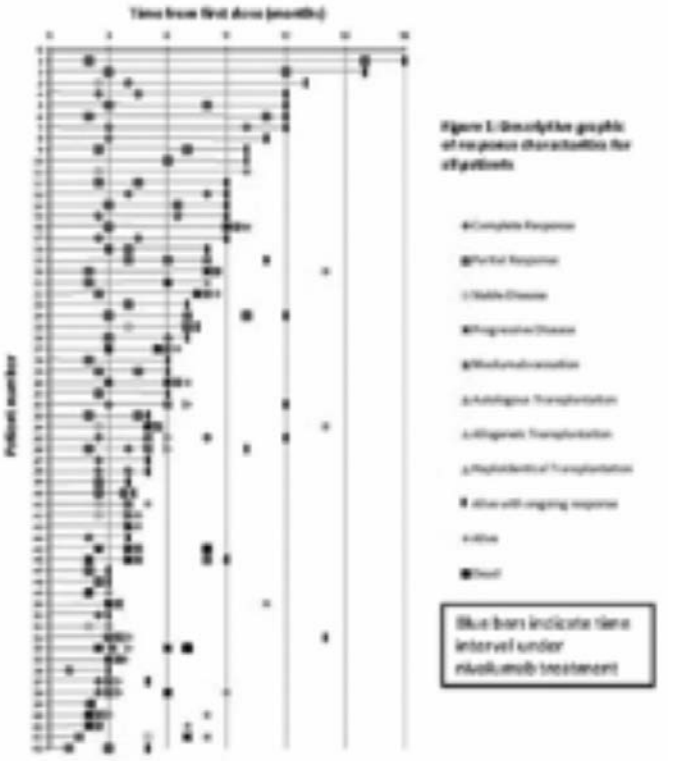

Figure 1.

Summary/Conclusions: In conclusion PD-1 blockers are new options to meet the unmet need in patients with cHL refractory to BV treatment, and possibly a bridge for these patients before transplantation.

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GENOTYPING OF HODGKIN LYMPHOMA ON THE LIQUID BIOPSY

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Background: In classical Hodgkin lymphoma (cHL) the low representation (1-5%) of Reed-Sternberg cells (RS) challenged tumor genotyping on the diagnostic tissue biopsy. Consistently, the mutational profile of newly diagnosed cHL is poorly characterized, and the genetics of refractory disease is completely unknown. Cell free DNA (cfDNA) is shed into the blood by tumor cells undergoing apoptosis and can be used as source of tumor DNA for the identification of somatic mutations. In addition cfDNA is representative of the entire tumor heterogeneity, thus allowing the identification of mutations from tumor cells residing in non-biopsied sites.

Aims: This study aims: i) at providing the evidence that the mutational profile of cHL can be tracked by using plasma cfDNA; and ii) at characterizing the genetics of newly diagnosed cHL and, for comparative purposes, of refractory cHL.

Methods: The study incudes 28 newly diagnosed cHL and 9 chemorefractory cHL. All cases were provided with cfDNA from plasma collected at baseline, before treatment start, and paired DNA from granulocytes as source of germline DNA to filter out polymorphisms and sequencing noise. Paired genomic DNA from formalin fixed paraffin embedded (FFPE) tumor tissue biopsies was available for 17 patients, including 3 cases for which RS enriched areas were macrodissected. A targeted resequencing panel optimized to include the coding exons and splice sites of 77 genes (192Kb) that are recurrently mutated in B-cell lymphomas was used for genotyping. Libraries were prepared from plasma cfDNA, germline gDNA and tumor gDNA according to the CAPP-seq targeted enrichment strategy (Nimblegen) and subjected to ultra-deep-next generation sequencing (NGS) on the MiSeq platform (Illumina). The sequencing was tailored to obtain a depth of coverage >2000x in >80% of the target region in all samples, which allowed a sensitivity of $3x10^{-3}$. The somatic function of VarScan2 was used to call non-synonymous somatic mutations, and a stringent bioinformatic pipeline was applied to suppress tha background noise and to filter out sequencing errors.

Results: In newly diagnosed cHL patients, genotyping of plasma cfDNA identified non-synonymous somatic mutations in *STAT6* (43%), *TNFAIP3* (43%), *ITPKB* (32%) *B2M* (21%), *GNA13* (14%), *CIITA* (7%), *XPO1* (7%) and *CD58* (4%) among the most recurrently affected genes (Figure 1A-B). In refractory cHL patients, genotyping of plasma cfDNA identified non-synonymous somatic mutations in *ITPKB* (44%), *TNFAIP3* (33%), *KMT2D* (33%), *B2M* (33%), *GNA13* (33%), *XPO1* (22%), *TET2* (22%), *IKBKB* (22%), *BIRC3* (22%) and *STAT6* (22%) among the most recurrently affected genes. Mutations of *KMT2D* (33%) and *TET2* (22%) were enriched in refractory cHL patients compared to newly diagnosed cases, suggesting that they contributed to the chemorefractory phenotype (Figure 1C-D). By using highly sensitivity techniques, most of the mutations discovered in cfDNA were also identified in pair tumor DNA from the tissue biopsy and/or macrodissected RS cells, thus confirming their tumor origin (Figure 1F). By pathway analysis, the mutational profile pointed to the involvement of PI3K/AKT signaling, cytokines signaling, NF-kB signaling and the immune escape in cHL. *ITPKB* (a negative regulator of the PI3K/AKT signaling pathway) was specifically mutated in cHL across aggressive B cell lymphomas.

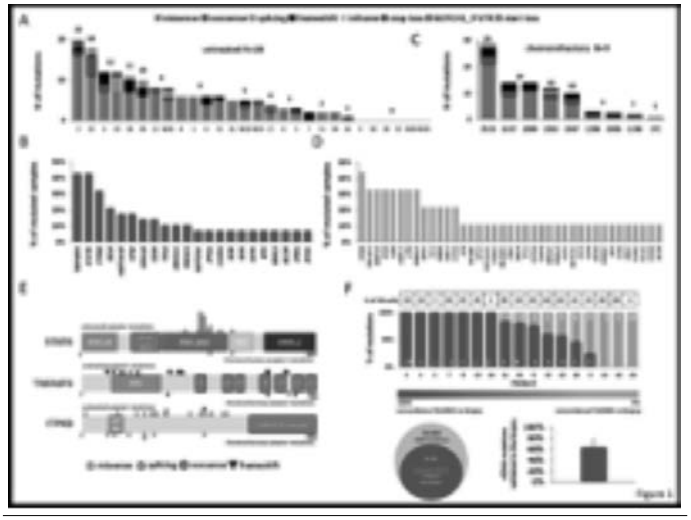

Figure 1.

Summary/Conclusions: This study provides the evidence that cHL can be genotyped using plasma cfDNA as source of tumor DNA, pointed to a non overlapping genotype between newly diagnosed and refractory cases, and identified *ITPKB* as a new gene specifically involved in ~30-50% of cHL patients.

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FDG PET-CT MAYBE A USEFUL TOOL TO IDENTIFY DOXORUBICIN INDUCED CARDIOTOXICITY IN HODGKIN LYMPHOMA

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Background: Doxorubicin (DXR) induced cardiotoxicity is related to several mechanisms, including interference of mitochondrial respiratory chain and acceleration of glycolysis.We previously reported that this treatment may enhance myocardial FDG uptake.
Aims: The present study aimed to verify whether this metabolic response on serial PET/CT imaging can predict myocardial function, non-invasively evaluated by follow-up echocardiography (ECHO).
Methods: 18F-FDG PET/CT of 25 patients affected by Hodgkin Disease (HD), treated following ABVD scheme were analyzed. Inclusion criteria were: 1) availability of 4 consecutive PET/CT scan for staging (PET1), interim (PET2), post-therapy (PET3) and six months follow-up evaluation (PET4); 2) full remission after two ABVD cycles; 3) normal baseline EKG and ECHO findings and 4) no concurrent treatment with external thoracic radiotherapy. A volume of interest was manually drawn on the left ventricular myocardium. Average standardized uptake value within this region was normalized for the corresponding blood pool index measured in the inferior vena cava to obtain LV-SUV. All patients underwent a 12 months cardiological follow-up assessment encompassing clinical evaluation, EKG and ECHO. A cardiologist unaware of PET findings performed this procedure
Results: LV-SUV progressively increased from PET1 to PET4 in 6 patients (24%, 2 females, mean age 39±17, termed "increasers") being 1.34±0.9, 3.34±2.6, 4.32±2.8 and 4.43±1.5 respectively. In the remaining 19 patients (76%, 7 females, 36±14), FDG uptake showed a largely variable response without any progressive increase. Accordingly, the ratio between PET4 and PET1 LV-SUV in the two subgroups was 3.85±0.8 and 1.06±0.4, respectively ($p<0.001$). Up to six months after therapy discontinuation, none of the 25 patients showed signs or symptoms potentially related to DXR cardiotoxicity. However, late follow-up ECHO detected the appearance of first-degree diastolic impairment with respect to baseline in 9 of the 25 examined patients (36%, 4 females, mean age 36 ±18). This finding occurred in 5/6 "increasers" (83%) and in only 4/19 non-increasers (21%) ($p<0.001$).
Summary/Conclusions: The present data indicate that DXR related myocardial damage can be preceded by an enhanced glucose uptake. 18F-FDG PET/CT imaging might represent a useful tool to identify high-risk patients and to implement personalized program to monitor and prevent DXR-induced cardiotoxicity.

Iron metabolism, deficiency and overload

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ELEVATED SYSTEMIC HEME AND IRON LEVELS AS RISK FACTORS FOR VASCULAR DYSFUNCTION AND ATHEROSCLEROSIS: EVIDENCE FROM B-THALASSEMIA AND HEMOCHROMATOSIS COHORT STUDIES

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Background: Increasing evidence from animal studies suggests that free heme exerts vasculotoxic, pro-inflammatory and pro-atherogenic effects due to its ability to trigger endothelial and immune cells activation. Moreover, we recently demonstrated a role for iron in the pathogenesis of atherosclerosis, analyzing a mouse model of type IV hereditary hemochromatosis, hallmarked by severe iron overload. We also showed that iron-deficient diet and chelation therapy prevent atherosclerosis progression in those mice.
Aims: Here we aimed at evaluating the clinical relevance of these findings and whether parameters of vascular status correlate with iron levels and suggest a predisposition to vascular dysfunction and atherogenesis in iron-overloaded individuals.
Methods: To this purpose we examined serum samples from a cohort of patients with β-thalassemia major and intermedia, who received recurrent blood transfusions but inconsistent chelation therapy, and a cohort of patients with hereditary hemochromatosis (HFE ^{C282Y} homozygous mutation), treated with phlebotomy.
Results: β-thalassemia patients show high systemic heme and iron levels, which correlate with a severe drop in the plasma scavengers for hemoglobin and heme, Haptoglobin and Hemopexin, respectively. Hemochromatotic patients show high systemic iron levels and reduced hepcidin levels. Consistently, in the two cohorts, transferrin saturation, non-transferrin bound iron (NTBI) and serum ferritin are elevated. Interestingly, both thalassemic and hemochromatotic patients present with high systemic levels of soluble adhesion molecules (sVCAM-1, sICAM-1, sE-Selectin, sP-Selectin) and reduced nitrotyrosine levels, hallmarks of endothelial activation and vascular dysfunction. In addition, they show increased serum lipid peroxidation, elevated circulating oxidized LDLs and high pro-inflammatory cytokines, which are known to promote atherosclerosis. All parameters significantly correlate with increased systemic heme and iron indices, including NTBI, as well as decreased scavenger levels.
Summary/Conclusions: These results emphasize the involvement of serum hemoglobin, heme and iron in the pathogenesis of vascular dysfunction in β-thalassemia and hemochromatosis and suggest a pro-atherosclerotic role for these molecules. These findings are relevant, on one side, for cardiovascular diseases and vasculopathy, when iron parameters are altered, and on the other, for iron overload disorders, where premature atherosclerosis might develop. Finally, our data highlight the key protective role of heme/iron scavengers and support the potential therapeutic benefit of chelation therapy to counteract heme-/iron-driven vascular toxicity and atherosclerosis in hemolytic and iron-overload conditions.

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REAL-WORLD ADHERENCE TO IRON CHELATION THERAPY: COMPARING A FILM-COATED TABLET VERSUS DISPERSIBLE TABLET OF DEFERASIROX

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Background: Iron chelation therapy (ICT) is effective in removing excess iron and preventing iron overload-related complications in patients (pts) with transfusion-related iron overload. However, adherence to ICT has historically been suboptimal. While deferasirox (DFX) dispersible tablet (DT) has shown better adherence than other oral and non-oral ICT agents, adherence could be further improved. The recently approved DFX film-coated tablet (FCT) provides a simpler method of oral ICT administration, and was found to have improved patient adherence as compared to DFX DT in the recent ECLIPSE clinical trial (NCT02125877). Real-world evidence outside of a trial is lacking.
Aims: To assess and compare real-world adherence and persistence to ICT in pts switching from DFX DT to DFX FCT.

Methods: A retrospective pre-post cohort study was conducted in pts switching from DFX DT to FCT using pharmacy and medical claims (06/2014 - 05/2016) from the Symphony Health Solutions' Integrated Dataverse (IDV®) database. Eligible pts were ≥2 years old, had a diagnosis of an inherited or acquired hematological disorder requiring transfusions (*e.g.*, sickle cell disease, myelodysplastic syndrome), ≥2 DFX FCT claims (1st claim=index date), ≥2 DFX DT claims pre-index, and ≥6 months of continuous clinical activity (baseline period) pre-index. Medication possession ratio (MPR) (percentage of time with access to medication) was computed for DFX DT during the "DFX DT period" (from earliest DFX DT claim to index date) and for DFX FCT during the "DFX FCT period" (from index date to end of data availability/ICT switch). Proportion of days covered (PDC) and persistence (without a gap ≥30 or 60 days between claims) were assessed in the DFX DT and DFX FCT periods over fixed intervals of 3 and 6 months, which started from the index date in the DFX FCT period, or dispensing date of the most recent DFX DT claim prior to the beginning of a 3- or 6- month interval in the DFX DT period. Comparisons between the two periods were made using the Wilcoxon sign-rank test for continuous data and McNemar's test for dichotomized data.
Results: Of the 606 eligible pts, 56% were female, 64% were <35 years old, and 42% had transfusions during the baseline period. The median durations of the DFX DT and DFX FCT periods were 356.5 days and 290.0 days, respectively. Compared with adherence to DFX DT, adherence to DFX FCT was significantly improved across all measures. Mean MPR of DFX FCT *vs* DFX DT was 0.80 *vs* 0.76 (p<0.001); 60.9% pts had a mean MPR ≥0.8 during the DFX FCT period compared to 54.3% during the DFX DT period (p<0.01). Mean 3-month PDC of DFX FCT *vs* DT was 0.83 *vs* 0.71 (p<0.001); 50.0% pts had mean 3-month PDC ≥0.8 during the DFX FCT period compared to 34.5% during the DFX DT period (p<0.001). The proportion of pts with 3-month persistence to DFX FCT *vs* DFX DT (without a gap ≥30 days) was 87.2% *vs* 63.4% (p<0.01). Similarly consistent and significant results for PDC and persistence were observed using a 6-month time interval and/or a 60-day gap between claims.
Summary/Conclusions: Adherence and persistence to ICT was significantly improved in pts who switched from DFX DT to DFX FCT. Reasons for switching, which may contribute to improved adherence, were not examined in this study. Nevertheless, since the majority of pts were already adherent to DFX DT, the improvement with DFX-FCT suggests that ICT can be further augmented with this formulation. This real-world study complements the ECLIPSE trial results and supports previous evidence of improved adherence to DFX FCT.

P286

MEDIATION BY PATIENT-REPORTED OUTCOMES ON THE ASSOCIATION BETWEEN FILM-COATED VERSUS DISPERSIBLE FORMULATIONS OF DEFERASIROX AND SERUM FERRITIN REDUCTION: A POST HOC ANALYSIS OF THE ECLIPSE TRIAL

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Background: The ECLIPSE clinical trial (NCT02125877) demonstrated that a new film-coated tablet (FCT) formulation of deferasirox (DFX) had a similar safety profile, with fewer patients (pts) experiencing severe gastrointestinal (GI)-related adverse events (AEs), and more favorable patient-reported outcomes (PROs) compared to the dispersible tablet (DT). PROs of FCT pts showed better adherence, satisfaction, palatability and fewer concerns about iron chelation therapy (ICT). FCT pts had a higher median absolute reduction in serum ferritin (SF) from baseline to the end of treatment (tx) (-350.0 *vs* -85.5 ng/mL), despite tx groups receiving similar mean actual ICT doses, suggesting better efficacy of FCT (Taher AT, *et al.* Am J Hematol. 2017). Since DFX FCT has the same active ingredient as DT and was developed to enhance pt adherence, it is hypothesized that the observed difference in efficacy may be due to differences in adherence. PROs may be a useful surrogate marker of actual pt adherence to ICT.
Aims: To estimate the proportion of the association between tx with DFX FCT *versus* DT and SF reduction from baseline that is mediated through PROs, in a *post hoc* analysis.
Methods: The ECLIPSE trial was a phase II, randomized open-label study in which 173 pts with transfusion-dependent thalassemia or myelodysplastic syndrome were randomized to receive DFX FCT (N=87) or DT (N=86) at average planned doses of 21.5 mg/kg/day (DT equivalent dose=30.7 mg/kg/day due to differing bioavailability) and 28.7 mg/kg/day, respectively. PROs were assessed at weeks 2, 3, 13, and 24 (end of tx) using the Palatability and modified Satisfaction with Iron Chelation Therapy (mSICT) questionnaires, the latter assessing 3 domain scores: adherence, satisfaction, and concerns. Frequency of GI-related AEs was assessed during the tx period. Mediation analysis, *i.e.*, comparing a model adjusted for hypothesized mediators to an unadjusted model (Lin DY, *et al.* Stat Med. 1997), was used to compute proportion mediated (PM). PM quantifies how much of the association between tx with DFX FCT *versus* DT and SF reduction from baseline is operationalized through pt-reported adherence score, other PRO scores, and frequency of GI-related AEs during tx. The analysis was adjusted for confounders including age, sex, race, underlying hematological disease, prior use of DFX DT, baseline level of iron overload severity, average planned dose, and number of blood transfusions on tx. Sensitivity analyses were conducted in subgroups of pts who had prior use of DFX DT (DT non-naïve), had thalassemia, and were DT non-naïve pts with thalassemia.
Results: The association between tx with DFX FCT *versus* DT and SF reduction was substantially mediated by pt-reported adherence (PM=66.6%, p=0.01). Pt-reported adherence, along with pt-reported satisfaction, concerns, and palatability scores, and frequency of GI-related AEs together mediated 76.7% of the association (p=0.02). The proportion mediated was increased in the DT non-naïve subgroup (PM=92.7%, p=0.03). Similarly, an increased PM was seen in the subgroup of DT non-naïve pts with thalassemia compared to all thalassemia pts (PM=70.1%, p=0.08 *vs* PM=56.5%, p=0.12, respectively).
Summary/Conclusions: Better PROs, especially increased pt-reported adherence, due to improved attributes associated with DFX FCT are significant intermediates of the association between tx with DFX FCT *versus* DT and SF reduction from baseline. The proportion mediated was increased in pts with prior DT exposure, suggesting their enhanced appreciation for DFX FCT over DT.

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ASSESSMENT OF THE PERFORMANCE OF A WIDELY AVAILABLE T2*/R2* LIVER IRON CONCENTRATION METHOD USED IN CLINICAL PRACTICE IN A POPULATION OF THALASSEMIA PATIENTS

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Background: Measurements of liver iron concentration (LIC) by magnetic resonance imaging (MRI) have become established and validated in several research intensive centers. While the validity of spin density projection assisted (SDPA) R2-MRI together with a core laboratory service has been validated in routine clinical practice settings, methods relying on in-house establishment of data acquisition protocols and data analysis have not yet been validated in this way.
Aims: To determine the limits of agreement between measurements of LIC by a widely available T2*/R2* MRI method and a reference standard SDPA R2-MRI method in a routine clinical practice setting.
Methods: Thalassemia patients (N=100) referred by the National Institute of Haematology and Blood Transfusion, Hanoi, Vietnam for routine LIC measurement by MRI were prospectively recruited with informed consent. Patients were randomised to be scanned in either a Philips Ingenia or a Siemens Avanto 1.5T scanner. The LIC of each patient was measured twice, once by a T2*/R2* technique using freely available software and protocols (Iron Health Calculator: http://www.ironcalculator.com) and once by SDPA R2-MRI using a quality controlled core laboratory data analysis service (FerriScan®). Analysts using the T2*/R2* data analysis method were blinded from the SDPA R2-MRI results and vice versa. Reported data were analysed using the statistical methods of Bland and Altman.
Results: A plot of the T2*/R2* LIC against the SDPA R2-MRI LIC (Figure 1) shows the vast majority of the data falling below the line of equivalence indicating that the T2*/R2* method is underestimating the LIC relative to the SDPA R2-MRI validated reference standard. The geometric mean ratio of T2*/R2* LIC to SDPA R2-MRI LIC was 0.44 (95% CI 0.36 –0.55) indicating severe underestimation of LIC by the T2*/R2* method. The geometric mean ratios of the two LIC measurements were significantly different for the two scanners (0.28 for Philips and 0.68 for Siemens, p <0.0001) indicating that the bias of the T2*/R2* method against the reference standard is not universal but is dependent on both/either scanner type and/or data acquisition method. Bland Altman analysis indicates that 95% of pairs of measurements are predicted to have ratios between 3.73 and 0.05 indicating a very large random variability between the T2*/R2* method and the reference standard. The performance of the T2*/R2* method in predicting SDPA R2-MRI LIC values above the clinically relevant thresholds of 7 and 15 mg Fe/g dw is characterized in the Table 1 showing positive predictive values (PPVs) and negative predictive values (NPVs) together with their 95% CIs.

Table 1.

LIC Threshold	Positive Predictive Value	Negative Predictive Value
>7 mg Fe/g dw	1.00 (0.95 – 1.00)	0.23 (0.09 – 0.44)
>15 mg Fe/g dw	0.98 (0.89 – 1.00)	0.40 (0.26 – 0.54)

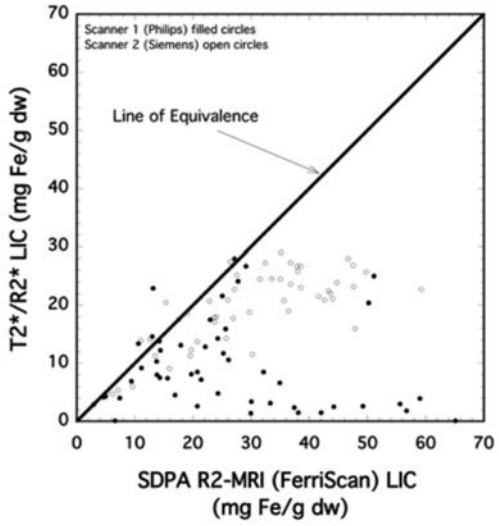

Figure 1.

Summary/Conclusions: The data indicate that the T2*/R2* method of measurement of LIC is not safe for routine clinical measurement of LIC because of the extremely poor NPVs which could result in inappropriate clinical decision making. The severe discrepancies of the T2*/R2* method from the reference standard are likely caused by several factors including non-optimal curve fitting algorithms, lack of a method to identify non-analysable data, and the use of a calibration curve from the literature generated from data acquisition and analysis methods different from those used locally. These or similar pitfalls are likely to be encountered in many MR centres using non-regulated MR methods of LIC measurement.

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SIMILAR TRENDS IN RENAL FUNCTION AS MEASURED BY SERUM CREATININE DURING LONG-TERM IRON CHELATION TREATMENT WITH OR WITHOUT DEFERASIROX IN PATIENTS WITH TRANSFUSIONAL HEMOSIDEROSIS

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Background: Regular transfusion and iron chelation therapy (ICT) are often indicated for patients with β thalassemia, sickle cell disease (SCD) and other anemias, and can be lifelong requirements. As most patients now survive into adulthood and many experience prolonged exposure to ICT, there is increased risk of age-, disease- or drug-related complications, including changes in renal function. Evidence suggests that some patients receiving ICT experience changes in markers of renal function, mostly within normal limits, non-progressive and reversible with dose reduction and/or interruption. Recently, we reported a retrospective analysis of patients with transfusion-dependent anemias during a decade of deferasirox treatment indicating stable and a lack of any progressive worsening of renal function (Origa R *et al. Blood* 2016).
Aims: To assess serum creatinine (SCr) during long-term deferasirox treatment in subgroups of Italian patients with transfusional hemosiderosis who participated in the deferasirox registration studies and were then followed retrospectively.
Methods: Italian patients with β thalassemia, SCD, myelodysplastic syndromes or other anemias who received ≥1 deferasirox dose in the registration studies (studies 105, 106, 107, 108 or 109), had ≥1 post-baseline (BL) SCr measurement, and had medical records available were included. SCr values were collected retrospectively in 3-month periods from registration trial end until the latest patient assessment. Primary endpoint was SCr over time. SCr values during the retrospective period were evaluated by subgroups: here we report those who received only deferasirox and those who received no deferasirox but other ICT during the retrospective period.
Results: 282 patients were included in the retrospective study who received ≥1 deferasirox dose in registration studies; of these, during the retrospective period, 98 (35%) received only deferasirox (group A) and 62 (22%) received no deferasirox but other ICT (group B). In group A, mean (SD) age at first quarter was 25.9 (12.1) years and 36 (37%) were male; in group B, mean (SD) age at first quarter was 27.0 (10.9) years and 25 (40%) were male. The proportion of pediatric patients was 28% (n=27) in group A and 19% (n=12) in group B. Mean (SD) duration of deferasirox exposure in group A was 7.5 (1.7) years; mean daily deferasirox dose was 1440.0 (423.6) mg.
In both subgroups analyzed, mean SCr was within normal limits and remained stable over time during the retrospective period (Figure 1). Analysis in adults showed mean SCr values were stable over time. As expected in growing children who are gaining height and weight, pediatric mean SCr absolute values increased from baseline in proportion with an almost linear increase in muscle mass over time.

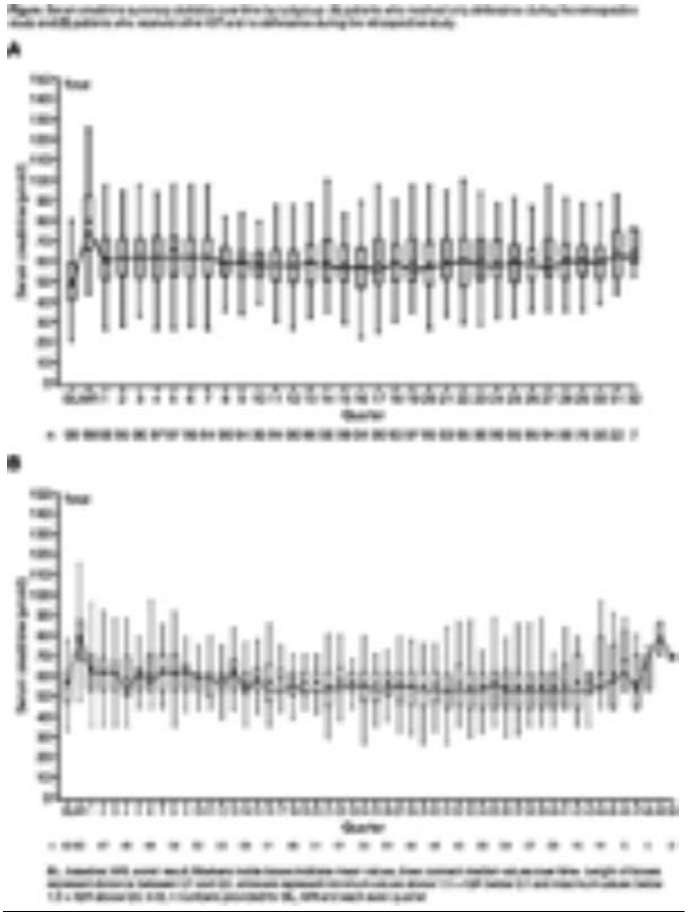

Figure 1.

Summary/Conclusions: This retrospective study of long-term renal safety in patients receiving iron chelation for transfusional iron overload suggests that long-term deferasirox treatment, or administration of ≥1 deferasirox dose followed by other chelators, did not have an overall detrimental long-term effect on renal function as monitored by SCr. This analysis provides no evidence of progressive renal function worsening over time, which is consistent with previous results demonstrating deferasirox has a mild, generally reversible renal hemodynamic effect.

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WHEN IRON LEADS TO RED CELLS (AND *VICE VERSA*): A COMPREHENSIVE PHENOTYPE -TOWARDS NGS/WES PATHWAY FOR THE DIAGNOSIS OF RED CELL AND IRON DISORDERS

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Background: Despite thorough clinical and biological phenotypic investigations, a number of red cell disorders remain uncharacterized. In addition, some of them are accompanied by overt iron overload and can be initially misdiagnosed as disorders of genes involved in iron metabolism. Next Generation Sequencing (NGS) has become an attractive technique to decipher constitutional disorders as it allows analyzing a large number of disease-causing genes. However an initial complete clinical, biological and morphological assessment is mandatory to correctly orientate towards the correct gene panel and to interpret the various molecular variations identified. We have set up in our center, a specialized outpatient consultation for both iron and red cell disorders. Specialized phenotypic investigations including biological and morphological tests followed by standard or second level genotyping can be prescribed.
Aims: The aims of this study was to characterize the molecular background of patients with iron or red cell disorders, or with a possible combination of both, and to propose key steps towards this genetic diagnosis.

Methods: A cohort of 25 well characterized patients was analyzed. Eighteen were initially referred to our center for unexplained hyperferritinemia (HF), two for proven iron overload (IO) by MRI, 2 for chronic hemolysis and 3 for aregenerative anemia. A set of phenotypic tests was systematically assessed, including CBC, reticulocyte count, serum haptoglobin and measure of the Liver Iron Content (LIC) by MRI. For all patients with HF, causes linked to hepatic disease, inflammation and cancers were ruled out and standard HFE genotyping was performed. Phenotypic investigations failed to clearly identify the cause of the disorder. Therefore, each patient was tested for a panel of 32 genes involved either in iron homeostasis or hereditary anemias, using NGS. Libraries were obtained using the Custom SureSelectQXT Target Enrichment system (Agilent, Santa Clara Ca USA) and sequenced on a MiSeq platform (Illumina, San Diego, Ca, USA). Each deleterious variation was independently checked using conventional Sanger sequencing. Written informed consent was obtained from all the patients for NGS genetic analyses.
Results: Initial phenotypic reassessment allowed classifying the patients into 5 different groups: 1/ isolated hyperferritinemia (n=1) 2/ HF and IO (MRI ≥90 µmole/g dry weight) (n=17); 3/ hemolytic anemia (HA) without IO (n=2) ; 4/ HA and IO (n=2); 5/ aregenerative anemia with IO (n=3). Among patients with an initial diagnosis of iron disorder, the reticulocyte count identified 2 undiagnosed chronic fully compensated hemolysis. Systematic screening using the gene panel identified a total of 14 sequence variations of clinical significance in 9 different genes and 9 patients. An isolated mutation was found in 7 and 2 patients with an initial diagnosis of iron or of red cell disorder respectively. A combined anomaly of red cell and iron genes was identified in 3 patients who displayed IO and compensated hemolysis or anemia. Digenism involving an HFE C282Y/wt or C282Y/H63D genotype and another "iron gene" was also shown in 3 patients with IO (without anemia or hemolysis). No sequence variation of clinical significance was found in the sequenced genes of eleven of the studied patients.
Summary/Conclusions: On the phenotypic point of view, the present study highlights the importance to check for hematological data (CBC and reticulocytes) in patients with HF, because this can allow discovering fully compensated hemolysis and bringing towards a red cell disorder. On the other hand, it also underlines the importance to systematically check for IO all patients with a red cell disorder, who may display high LIC. Our present genotypic data (and previous ones) suggest a relative frequency of combined inherited disorders of iron and red cells, making the combined search for both disorders quite relevant in clinical practice. This is now possible with the use of NGS analysis, which allows sequencing large numbers of genes. For those patients with no identified mutation, approaches using whole exome or genome can be proposed as the next step.

P290

CHANGES IN LIVER IRON CONCENTRATION R2 MRI MEASUREMENT ACROSS DIFFERENT CHELATION REGIMENS IN PATIENTS WITH HEMATOLOGICAL DISORDERS: REAL-LIFE EXPERIENCE FROM LICNET

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Background: The liver plays a central role in iron regulation and remains the primary site of iron storage, with liver iron concentration (LIC) being a strong surrogate of total body iron. Both R2 and T2* can accurately measure LIC. R2 MRI is a robust and validated technique (FerriScan® Resonance Health Limited, Claremont, WA, Australia) approved by the FDA. The LICNET (Liver Iron Cutino NETwork) was established by Foundation Franco e Piera Cutino of Palermo and is addressed to diagnostics of liver iron overload by R2 MRI in subjects with hemochromatosis in haematological disorders. The LICNET protocol was approved on December 4, 2012 by our Ethics Committee. Baseline data from LICNET were, recently, published (Vitrano *et al.*, 2016, EJH).
Aims: The aim of this study was to evaluate longitudinal changes in LIC measurements across different iron chelation regimens in a real-life cohort of patients with transfusional iron overload included in LICNET.
Methods: This was retrospective cohort study of patients with haematological disorders attending 9 Italian centres participating in the LICNET who had two R2 MRI scans recorded in the database and receiving the same iron chelation in between. Bivariate comparisons were made using the chi-squared and Fisher's exact tests for categorical variables and the Wilcoxon or Mann–Whitney test for continuous variables.
Results: A total of 130 patients were evaluated in this analysis, with a median (range) age years of 35 (range: 6–78) and including 60 (46.2%) men. The underlying diagnoses were regularly transfused thalassemia major (n=86, 66.2%), thalassemia Intermedia (n=33, 25.4%), sickle cell disease (n=6, 4.6%), myelodysplastic syndrome (n=3, 2.3%), and Diamond-Blackfan anemia (n=2, 1,5%). The median duration (range) between the first and second MRI was 483 days (184-1076) and was comparable between iron chelation regimens. Median pre-transfusion hemoglobin level and blood requirement were similar at both MRIs.The median change in LIC (range) in mg Fe/g dw was not significant in patients receiving DFP (n=29, median change -1.9, p=0.55), DFX (n=52, median change -0.5, p= 0.515), DFO+DFP (n=10, median change -2.2, p=0.074), or other combinations (n=7, median change -1.3, p=1.000), while it decreased significantly on DFO monotherapy (n=32, median change -1.4,p=0.002). Among oral chelators, DFX showed to be more effective, during the period of the study, in stabilizing iron body burden in 65.4% patients, even if they had baseline LIC values <7mg Fe/g dw (median 4.0 mg Fe/g dw) and with similar response as combined treatment DFO+DFP (Figure 1).

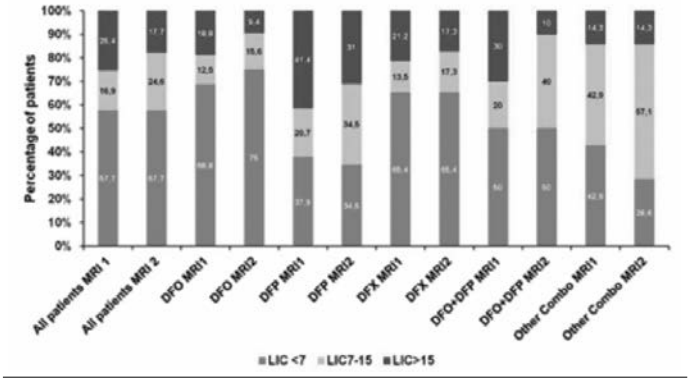

Figure 1.

Summary/Conclusions: This cohort study suggests that stabilization of LIC is achievable, during a median of 483 days, with different iron chelation regimens in real life experience, with considerable proportions of patients shifting to more favourable LIC categories. Therefore, the periodic determination of LIC by MRI has to be strongly recommended for management and prevention of iron overload and subsequent complications in haematological disorders.

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IN UTERO IRON STATUS AND AUDITORY NEURAL MATURATION IN FULL TERM INFANTS BORN TO MOTHERS WITH IRON DEFICIENCY ANEMIA

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Background: Iron deficiency is the most important cause of nutritional anemia and is the most common micronutrient deficiency worldwide, especially in developing countries. Iron is crucial for fetal brain development; hence, there is a paramount need for diagnostic neurofunctional screening that could identify low CNS iron stores. However, there are insufficient data regarding the effects of maternal iron deficiency anemia (IDA) on auditory neural maturation.
Aims: We aimed to evaluate the effect of maternal IDA on auditory neural myelination in full term neonates using the auditory brainstem evoked response (ABR) as noninvasive neurophysiological assessment tool.
Methods: This prospective case-control study was conducted at Ain Shams University Hospitals and included 100 neonates. Out of 223 pregnant women, 50 were diagnosed as having IDA anemia and 50 healthy mothers were enrolled as a control group. Neonates were studied focusing on anthropometric measures, hematologic profiles and iron status. Auditory brainstem response (ABR) test was done for the studied neonates within 48 hours after birth and at 3 months.
Results: Hemoglobin and iron profile were lower in neonates born to anemic mothers compared with controls. Of 100 neonates screened for ABR, 25 failed the test (all of them were born to anemic mothers). Neonatal birth weight, crown-heel length, BMI, occipitofrontal circumference (OFC) and mid arm circumference were significantly lower in neonates who failed the ABR test than those who passed it ($p<0.05$). Moreover, maternal and neonatal hemoglobin, red blood cells indices, serum iron, ferritin and transferrin saturation were significantly lower in neonates who failed the ABR test than those who passed it ($p<0.05$). Most neonates (88%) who failed the screening ABR test had latent iron deficiency (cord blood ferritin 11-75 µg/L). After 3 months, 85 underwent diagnostic ABR test which revealed significantly prolonged interpeak latencies I-III, III-V, and I-V among neonates of anemic mothers compared with the non-anemic group. All interpeak latencies were more prolonged in neonates with latent iron deficiency and those born to mothers with serum ferritin <15 µg/L. Logistic regression analysis showed that maternal hemoglobin and mean corpuscular volume were the significant independent variables that could predict neonatal ABR results.

Summary/Conclusions: IDA during late pregnancy adversely affects cord blood iron and hearing status. ABR results are closely related to the severity of maternal and neonatal iron status. Antenatal screening of pregnant mothers is needed to improve fetal iron status and prevent abnormal auditory maturation.

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THE RELATIONSHIP BETWEEN SERUM FERRITIN AND LIVER IRON CONCENTRATION IN PEDIATRIC CANCER SURVIVORS

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Background: There is increasing recognition that pediatric cancer survivors are at risk of transfusion-related iron overload related to intensive treatment regimes and improved survival rates. Current screening approaches rely on serum ferritin (SF). However, little is known about the SF to liver iron concentration (LIC) relationship in pediatric cancer survivors and whether SF thresholds derived from other iron overload disorders or age groups are appropriate.
Aims: The aim of this study was to investigate the relationship between SF and LIC in pediatric cancer survivors and to determine SF thresholds for predicting clinically significant LICs in this patient group.
Methods: In this retrospective study, patient data were extracted on survivors with elevated ferritin or iron overload from the University of Minnesota Childhood Cancer Survivor Program research database. All patients were enrolled into the database via an informed consent process according to the guidelines of the University of Minnesota Institutional Review Board. Survivors were re-consented once they reached 18 years of age. Seventeen individual survivors were identified where both SF and LIC data were available and the time between the SF and LIC measurement was less than 30 days. Eleven of the 17 survivors had multiple SF measurements producing a final dataset with 34 pairs of SF and LIC measurements. Blood for serum ferritin was collected during clinic visits and analyzed by the University of Minnesota Medical Center, Fairview CLIA-certified clinical laboratory. Liver iron concentration measurements were made using spin density projection-assisted R2-MRI (FerriScan®). Linear regression was used to determine the relationship between SF and LIC. Receiver operating characteristic (ROC) curve analysis was used to assess the sensitivity and specificity of SF concentrations for predicting LIC.
Results: The average age of the cohort (6 females and 11 males) at their first SF/LIC measurement was 18.3 years (range 9 to 30.3 years). Acute lymphoblastic leukemia (N=5) and acute myeloid leukemia (N=4) were the most common diseases and 15 of the 17 survivors had received a haematopoietic stem cell transplant (HSCT). The average length of time between the final treatment and the first SF/LIC measurement was 5.4 years (range 0 to 12.5 years). A linear fit to all 34 LIC-SF measurement pairs (Figure 1) produced a gradient of 63 ± 15 (mg ferritin). (g dry liver tissue)/(mg Fe)/(L serum) and an intercept of 509 ± 157 mg ferritin/L (r^2=0.36). The ROC curve analysis (Table 1) indicated that, in this cohort, a SF cut-off of 1270 mg /L potentially has good sensitivity and specificity for predicting a LIC above 15 mg Fe/g and a SF cut-off of 1076 mg /L has poor diagnostic performance for predicting a LIC above 7 mg Fe/g.

Table 1. ROC Curve Analysis.

LIC threshold (mg Fe/g dw)	SF (mg /L)	Sensitivity (95% CI)	Specificity (95% CI)	AUC (SE)
> 7	1076	0.68 (0.43 – 0.87)	0.93 (0.68 -1.00)	0.82 (0.07)
> 15	1270	1.00 (0.48 – 1.00)	0.90 (0.73 – 0.98)	0.92 (0.05)

AUC, area under the receiver operating characteristic curve.

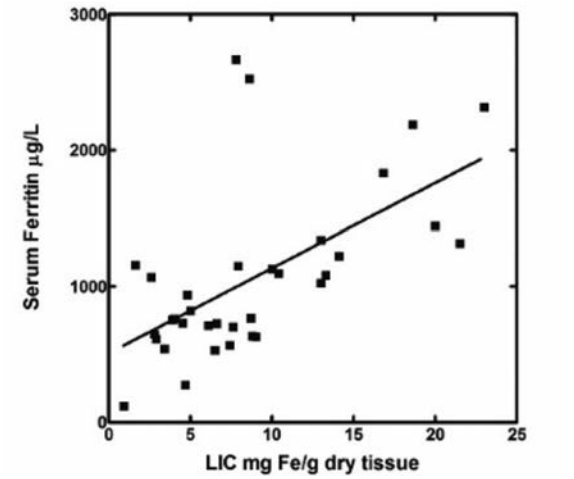

Figure 1.

Summary/Conclusions: In this study of pediatric cancer survivors, the gradient between SF and LIC and the SF cutoffs identified for predicting clinically important LIC values are considerably lower than observed for thalassemia or adult HSCT patients. This difference in the relationship between SF and LIC for different patient and age groups highlights the difficulty in relying on SF to screen for and define iron overload.

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DECREASED MCP-1 LEVELS IN PATIENTS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA: A CYTOKINE SIGNATURE OF IRON DEFICIENCY

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Background: Sustained iron deficiency is a major determinant of erythropoietin (Epo) resistance and consequent persistence of anemia in severely affected Hereditary Hemorrhagic Telangiectasia (HHT) patients with uncontrolled chronic bleeding. The mechanisms underlying erythroid suppression in these cases are still not fully understood.
Aims: We sought to investigate the potential impact of pro-inflammatory cytokines on the erythroid suppression associated with extreme iron deficient in HHT patients, focusing on MCP-1, recently described as a negative regulator of cellular iron uptake.
Methods: the study includes 18 HHT patients, 9 males and 9 females, aged 32-79 years , followed at the Hematology Service of CHP-HAS from 2013 to 2017. They all had history of persistent epistaxis (with variable frequency and severity) with or without gastrointestinal bleeding. The most severe cases (n=6) were resistant to iron treatment being transfusion dependent. Blood samples were collected in all cases for determination of erythroid parameters (including reticulocyte counts, Epo and soluble transferrin receptors (sTfR) levels) iron parameters (transferrin saturation, serum ferritin and hepcidin) and a cytokine profile (GM-CSF, IFN-γ, IL-10, Il-15, IL-1β, IL6, TNF-α, IP-10 and MCP1). The same parameters were determined in a group of 16 patients (5 males and 11 females aged 31-81 years) with iron deficiency (ID) due to chronic gastrointestinal bleeding under intravenous iron treatment and in a control group of 21 apparently healthy blood donors (9 males and 12 females aged 39-62 years). Magnetic Resonance Imaging (MRI) was used to assess tissue iron stores in liver, spleen and bone marrow.
Results: Severe anemia with absolute iron deficient (confirmed by appropriate hepcidin downregulation and absence of bone marrow iron stores by MRI) was evident in transfusion dependent HHT patients (TDHHT). Epo resistance in these cases was evidenced by an exponential increase of Epo levels correlated with parameters of severe anemia and ID with highly increased sTfR but inappropriate reticulocyte counts. Significantly decreased MCP-1 levels were observed in TDHHT patients but also in the other iron deficient groups. No significant alterations were observed in other cytokines except for IP-10 which was also decreased in TDHHT patients. In general, there is a linear decrease of MCP-1 with decreasing Hgb and increasing Epo levels. This effect, however, seems to be "blunted" in severely anemic TDHHT patients with Epo levels above 200 UI/L.
Summary/Conclusions: What is the sensing pathway downregulating MCP-1, and whether an insufficient MCP-1 downregulation contributes to Epo resistance and persistence of severe anemia in TDHHT patients, these are pending questions deserving further investigation.

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FERRIC CARBOXYMALTOSE VERSUS IRON SUCROSE COMPLEX IN WOMEN WITH IRON DEFICIENCY ANEMIA – A RANDOMISED CONTROLLED TRIAL

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Background: Anemia is a condition in which the number of red blood cells or their oxygen-carrying capacity is insufficient to meet physiologic needs, which vary by age, sex, altitude, smoking, and pregnancy status. The WHO Global Database on Anaemia for 1993–2005, covering almost half the world's population, estimated the prevalence of anaemia worldwide at 25 percent. India falls in 'severe' category of public health significance. Ferric carboxymaltose (FCM) comprises of a macromolecular iron hydroxide complex of polynuclear Fe^{3+} hydroxide tightly bound in a carbohydrate shell. The molecular structure of ferric carboxymaltose ensures controlled delivery of iron within cells of reticuloendothelial system and subsequent delivery to the iron binding proteins fer-

ritin and transferrin, with minimal risk of release of large amounts of ionic iron in the serum.
Aims: To compare safety and efficacy of Ferric Carboxymaltose (FCM) with Iron Sucrose complex (ISC) regarding improvement in haematological parameters and side effects in women with iron deficiency anemia (IDA).
Methods: Prospective randomized controlled study conducted in department of Obstetrics & Gynecology, in a tertiary care hospital in Delhi, India. 60 women having Iron deficiency Anaemia with Hb 6-8 g% were randomized 1:1 into two groups and were given 1000mg parenteral iron. One group received intravenous 500mg Ferric Carboxymaltose on day 0 and 8. 200mg Iron Sucrose complex was given in second group on alternate days for 5 doses. Haematological parameters - Hb. Reticulocyte count, RBC indices, S. ferritin; clinical parameters - fatigue, dyspnoea on exertion and adverse effects were studied on day 0, 7, 14 & 28.
Results: Two FCM infusions *vs* five ISC infusions were required. On day 28 Hb increment ≥3g%seen in 63,33% and MCV>80FL seen in 100% of FCM group *vs* 0%and 43.33% in ISC group. FCM group had 3.17 g/dl increment in Hb *vs* 1.9 g/dl in ISC group. S. Ferritin increased to 147ng/ml in FCM group *vs* 98 ng/ml in ISC group. Significant improvement in RBC indices & rectic count was seen in FCM group. Earlier and significant improvement in fatigability & dyspnoea on exertion was observed in FCM group. Both groups had similar safety profile except for thrombophlebitis was observed in 6.67% FCM group *vs* 50.00% ISC group.
Summary/Conclusions: Intravenous Ferric Carboxymaltose is more effective and safer than Iron Sucrose complex in treatment of Iron deficiency anaemia.

Lymphoma biology

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GENOME-WIDE ASSOCIATION STUDY OF HODGKIN LYMPHOMA IDENTIFIES HISTOLOGY-SPECIFIC ASSOCIATIONS AND TRANSCRIPTIONAL REGULATORS OF DISEASE SUSCEPTIBILITY

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Background: Several susceptibility loci for Hodgkin lymphoma (HL) have been reported, however much of the heritable risk and biological relevance remains unknown.
Aims: To identify novel risk loci for HL and histological subtypes and to further our understanding of how genetic risk loci influence disease susceptibility.
Methods: To our knoweldge, we have performed the largest genome-wide association study of HL totalling 5,156 cases and 16,763 controls across 10 million single nucleotide polymorphisms. We have integrated gene expression, chromatin state, transcription factor (TF) binding and capture Hi-C in model B-cells to functionally annotate new and existing risk loci.
Results: We identified risk loci for all HL at 6q22 (rs9482849, *PTPRK*, P=1.52 × 10^{-8}) and for nodular sclerosis HL (NSHL) at 3q28 (rs4459895, *LPP*, P=9.43 × 10^{-17}), 6q23 (rs6928977, *AHI1*, P=4.62 × 10^{-11}), 10p14 (rs3781093, *GATA3*, P=9.49 × 10^{-13}), 13q34 (rs112998813, *UPF3A*, P=4.58 × 10^{-8}) and 16p13 (rs34972832, *CLEC16A*, P=1.29 × 10^{-8}). Additionally, independent loci within the HLA region were observed for NSHL (rs9269081, HLA-DPB1*03:01, Val86 in HLA-DRB1) and mixed cellularity HL (rs1633096, rs13196329, Val86 in HLA-DRB1). Expression quantitative trait loci were observed in lymphoblastoid cells from 825 individuals at 6q23 (*AHI*, P_{SMR}=8.63x10^{-6}) and10p14(*GATA3*, P_{SMR}=4.70x10^{-8}). Across new and established risk loci we confirmed a significant enrichment of DNase hypersensitivity in GM12878 cells (P=1.20 × 10^{-5}), as well as regulatory elements in primary B-cells (P=6.0 × 10^{-6}) and GM12878 cells (P=6.85 × 10^{-3}). Analysis of ChIP-seq data on 82 transcription factors (TFs) in GM12878 cells, showed an over-representation of the binding of TFs that play a central role in B-cell signalling-networks such as RELA (nuclear factor NF-kappa-B p65), EBF1 (early B-cell factor 1), RUNX3 (runt-related transcription factor 3) and BATF (basic leucine zipper transcription factor, ATF-like).
Summary/Conclusions: These observations support the assertion that risk loci for HL mediate their effects through B-cell developmental networks, and are involved in transcriptional initiation and enhancement. Furthermore, our findings emphasise the differences between the major subtypes, which are likely reflective of differences in disease aetiology.

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SOX11 PROMOTES TUMOR PROTECTIVE MICROENVIRONMENT INTERACTIONS IN MANTLE CELL LYMPHOMA

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Background: Mantle Cell lymphoma (MCL) is one of the most aggressive lym-

phoid neoplasms characterized by highly infiltrated tumor cells in lymphoid tissues and extra nodal sites. The patients have short responses to current therapies and frequent relapses. However, recent studies have identified a subset of MCL with indolent clinical behavior that tends to present with leukemic disease instead of extensive nodal infiltration, and that is characterized by the absence of the transcription factor SOX11 [SRY (*Sex determining region-Y)-box11*]. However, SOX11 oncogenic pathways driven MCL tumor progression are poorly known.
Aims: The goal of our study was to identify the spectrum of genes regulated by SOX11 in malignant lymphoid cells and provide insights on how the constitutive overexpression of SOX11 may contribute to the oncogenic development of MCL.
Methods: We first generated a stable transduced SOX11-silenced MCL cell line with reduced SOX11 protein levels by infecting MCL cell lines with lentiviral particles carrying shRNA plasmids specifically targeting SOX11. SOX11-positive MCL cell line was infected with the empty vector and used as a control. These two MCL cell lines were injected in two different mice models to analyze *in vivo* the tumorigenic potential of SOX11, subcutaneous (sc) and intravenous (iv) xenograft tumor models. To analyze the crosstalk between MCL and microenvironment, we did *in vitro* cocultures experiments using accessory cells at the tumor microenvironment, as endothelial and bone marrow mesenchymal cells.
Results: In the sc mice model, we observed that SOX11 silencing reduced tumor growth compared to SOX11-positive control tumors. We analyzed the gene expression profiling of these xenograft tumors and of SOX11-positive and negative primary cases and we observed than different microenvironment-related signatures were enriched in SOX11-positive compared with SOX11-negative cells, as angiogenesis, migration and stromal stimulation. By ChIP-chip data, we observed than SOX11 is regulating transcription of different genes involved in these signatures, between them PDGFA. This data indicated a role for SOX11 in the crosstalk of MCL with tumor microenvironment. We found that SOX11 promotes angiogenesis in MCL cells through PDGFA regulation, promoting tumor growth and vasculature. Inhibition of PDGFA on endothelial cells impaired SOX11-enforced angiogenesis in MCL, and the treatment of SCID mice with a PDGFA inhibitor reduced tumor growth and angiogenesis of SOX11-positive MCL xenograft tumors. We also observed that SOX11 promotes migration, pseudoemperipolesis (migration of tumor cells beneath stromal cells) and cell adhesion mediated-drug resistance (CAM-DR) in MCL cells, increasing their survival and proliferation, and that these mechanisms were reduced in SOX11-negative cells. In the iv mice model, we observed that SOX11-positive cells were able to migrated and infiltrated bone marrow and lymph nodes, whereas SOX11-negative cells were retained in peripheral blood.
Summary/Conclusions: In conclusion, our results show that SOX11 is regulating essential processes involved in aggressiveness of MCL tumor cells, as angiogenesis, invasion and drug resistance. Inhibition of SOX11-target genes may represent an efficient strategy for the treatment of aggressive MCL.

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AICDA DRIVES EPIGENETIC HETEROGENEITY IN GERMINAL CENTER-DERIVED LYMPHOMAS AND ACCELERATES LYMPHOMAGENESIS

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Background: Diffuse large B-cell lymphomas (DLBCLs) are aggressive tumors derived from germinal center (GC) or post-GC B cells. Previous work from our group established that inferior outcome in DLBCL is associated with higher degrees of intra-tumor and inter-tumor cytosine methylation heterogeneity, although the molecules driving this epigenetic perturbation remain unknown.
Aims: We investigated the contribution of activation-induced cytidine deaminase (AICDA) to cytosine methylation heterogeneity in DLBCLs. AICDA is highly expressed in GC B cells where it drives somatic hypermutation (SHM) and also mediates DNA hypomethylation and epigenetic heterogeneity. AICDA is also expressed in a subset of DLBCLs and high level of AICDA in CHOP-treated DLBCL patients is associated with unfavorable prognosis. Thus, we hypothesized that AICDA could contribute to a more aggressive behavior of DLBCLs by facilitating epigenetic plasticity through the redistribution of cytosine methylation. We propose: AIM1. To characterize the effects of AICDA on the methylome and transcriptome of neoplastic B cells. AIM2. To determine the contribution of AICDA-mediated epigenetic modifications to DLBCL pathogenesis.
Methods: We overexpressed AICDA in bone marrow cells from VavP-*Bcl2* transgenic mice, which develop B cell lymphomas of GC origin. We transplanted AICDA-overexpressing (VavP-*Bcl2*+*Aicda*) or control (VavP-*Bcl2*) cells into lethally irradiated recipients. We studied survival, characterized disease biology and analyzed epigenome, genome and transcriptome of lymphomas. In addition, we studied GC B-cells from WT and *Aicda*$^{-/-}$ mice and analyzed the epigenome of primary DLBCLs samples, classified as AICDA^{high} and AICDA^{low} cases according to their AICDA expression.
Results: We observed more aggressive lymphoma phenotype in VavP-*Bcl2*+*Aicda* mice (n=7) compared to VavP-*Bcl2* mice (n=6), based on greater disruption of the splenic architecture and higher degree of B cell infiltration in organs such as lung, liver and kidney. Notably, the overexpression of AICDA reduced significantly the lifespan of the mice (Log-rank test p=0.0289). Neoplastic B cells from VavP-*Bcl2*+*Aicda* (n=4) and VavP-*Bcl2* (n=4) mice displayed similar mutation and indel burdens, suggesting that the more aggressive phenotype of AICDA-overexpressing mice was not likely due to increased mutagenesis. We profiled then the DNA methylation landscape of neoplastic B cells from VavP-*Bcl2*+*Aicda* and VavP-*Bcl2* mice. A principal component analysis of all CpGs, represented by its mean DNA methylation and heterogeneity (interquartile range) differences across replicates, revealed methylation loss and increased intertumor heterogeneity within VavP-*Bcl2*+*Aicda* methylomes compared to VavP-*Bcl2* (49,750 AICDA-perturbed CpGs). These altered CpGs were depleted in promoters and enriched in introns and intergenic regions. We observed a remarkably similar pattern of focal heterogeneity and demethylation in primary DLBCLs with high AICDA compared to low AICDA expression (37,557 AICDA-perturbed CpGs); and a reciprocal pattern of gain of methylation and reduced methylation heterogeneity in *Aicda*$^{-/-}$ compared to *Aicda*$^{+/+}$ GC B cells (64,323 AICDA-perturbed CpGs), suggesting a conserved epigenetic function of AICDA in GC B cells and human and mouse GC-derived lymphomas. Finally, we found significant overlap between genes affected by AICDA-perturbed CpGs in human AICDA high DLBCLs and murine VavP-*Bcl2*+AICDA lymphomas (P=2.21e-23) and with the genes affected by AICDA in GC B-cells (P=8.48e-33).
Summary/Conclusions: Our results demonstrate that AICDA acts as a methylome modifier in GC-derived lymphomas, introducing epigenetic heterogeneity, which may provide tumor cells with higher capacity to adapt to an evolving environment. These findings are relevant not only for B-cell lymphomas, but also for other types of cancer expressing cytosine deaminases.

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XPO1 INHIBITION SYNERGIZES WITH BCR INHIBITION, BLOCKS TUMOR GROWTH AND PROLONGS SURVIVAL IN A BIOLUMINESCENT ANIMAL MODEL OF PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

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Background: Primary central nervous system lymphoma (PCNSL) is an non-Hodgkin lymphoma localized in the CNS. Approximately 95% of PCNSL are classified as diffuse large B-cell lymphoma (DLBCL), being most of them related to activated B-cell type (ABC-DLBCL). PCNSL is associated with poor prognosis, particularly because of the difficulty for drugs to cross the blood brain barrier. High dose methotrexate is the most effective treatment, but relapse is very common and salvage treatment options are scarce. Also, in patients with systemic lymphoma, the secondary infiltration of the CNS is a fatal event, with a global overall survival of less than six months. Therefore, the development of new drugs with ability to penetrate the CNS is highly needed. Selinexor (KPT-330) is a Selective Inhibitor of Nuclear Export (SINE) that inactivates XPO-1 protein and induces anti-tumor effects mainly due to forcing nuclear retention and activation of tumor suppressors. Selinexor has shown excellent brain penetrance and promising results in pre-clinical models of glioblastoma and can inhibit both BCR and NF-kB signaling in malignant B-cells.
Aims: In order to provide a pre-clinical rationale for the design of new therapies for patients with CNS lymphoma our main aim is to assess the role of XPO-1 inhibition in intracerebral xenograft murine models.
Methods: We *in vitro* tested the sensitivity of DLBCL cell lines to selinexor and ibrutinib by MTS and AnnexinV/PI assay. We established an orthotopic xenograft model of PCNSL by stereotactic injection of OCI-Ly10 (ABC, MYD8 and CD79b mut) cells expressing luciferase into the cerebral parenchyma of nude athymic mice. We longitudinally quantified intracerebral tumoral growth by bioluminescence detection.
Results: To compare the sensitivity of DLBCL cell lines to selinexor we determined the IC50 in terms of survival and proliferation in 4 ABC and 5 GCB DLBCL cell lines. DLBCL cell lines had equivalent sensitivity to selinexor, regardless cell of origin (COO). In detail, survival by AnnexinV-PI exclusion showed that mean ID50 for ABC cell lines was 4.98 μM +/- 3.6 and 6.3 μM +/- 3.8 for GCB (p=0.9). Proliferation by MTS was also blocked by selinexor (mean ID50 for ABC-DLBCL was 1.35 μM +/- 0.7 *vs* 16.16 μM +/- 11.17 for GCB-DLBCL (p=0.41)). Since SINE compounds have been shown to inhibit the BCR, we next tested the potential synergy between ibrutinib and selinexor. In 3 out of 4 ABC-DLBCL cell lines there was a strong synergy. In contrast, none of the 3 GCB-DLBCL cell lines analyzed were sensitive to up to 100 μM ibrutinib; interestingly, however, treatment with selinexor sensitized SUDHL4 cells to ibrutinib and showed strong synergism between the two drugs. Finally, we established an orthotopic xenograft model of PCNSL by stereotactic injection of OCI-Ly10 cells expressing luciferase into the cerebral parenchyma of nude athymic mice. Eleven days after the injection of cells all animals had developed detectable tumors confined to the CNS. Tumor size

was measured and animals were randomly distributed into drug or vehicle group. At this time point mice were treated with 5mg/kg of selinexor or vehicle via oral gavage three times a week; subsequently, bioluminiscence was assessed twice a week. Treatment with selinexor significantly increased mice survival, with a median survival of 48 days in the treatment group compared to 34 days in the vehicle group ($p<0.0001$; Figure 1A). Mice in the treatment group showed a significantly slower increase in tumor size (two-way ANOVA: $p<0.0001$; Figure 1B). Specific time-point analysis showed that differences were significant as soon as 8 days after treatment. At final point, histopathological analysis showed diffuse infiltration in meninges and cerebral parenquima of highly proliferative CD20-positive B-cells. Currently, we are evaluating the synergy between ibrutinib and selinexor *in vivo*. For that we have used the same experimental setting and assigned 12 mice to each of the following groups: selinexor only (5mg/kg three times a week via oral gavage), ibrutinib only (25mg/kg daily in drinking water), combination or vehicle. Results will be available at the time of the meeting.

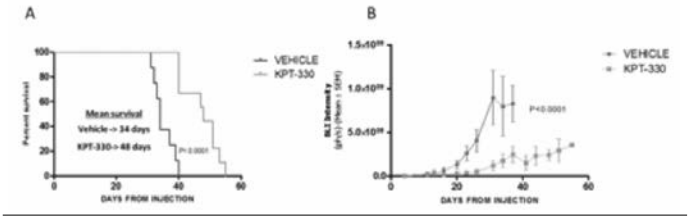

Figure 1.

Summary/Conclusions: Selinexor inhibits proliferation and survival of DLBCL cell lines regardless of COO and it can synergize with ibrutinib. Treatment of mice with CNS confined ABC-DLBCL with selinexor significantly reduces tumor growth and increases survival. Our results provide pre-clinical evidence for the development of selinexor as new therapeutic option for PCNSL or DLBCL with CNS involvement.

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MOLECULAR HETEROGENEITY IN PERIPHERAL T-CELL LYMPHOMA NOT OTHERWISE SPECIFIED REVEALED BY COMPREHENSIVE MUTATIONAL PROFILING

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Background: Peripheral T-cell lymphomas (PTCLs) are a highly heterogeneous group of mature T-cell neoplasms. In particular, accounting for the majority of PTCL, PTCL-not other specified (PTCL-NOS) is a diagnosis of exclusion and as such, is expected to include many heterogeneous tumors. In fact, recent genetic studies have suggested that a subset of PTLC-NOS is closely related to angioimmunoblastic T-cell lymphoma (AITL); both lymphoma types show follicular helper T-cell (TFH) phenotypes and share mutational targets in common, such as *RHOA*, *TET2*, *DNMT3A*, and *IDH2*. However, with the lack of comprehensive genetic analyses, the molecular pathogenesis is poorly understood in the majority of PTCL-NOS cases.
Aims: The aim of this study is to clarify a landscape of somatic mutations in PTCL-NOS.
Methods: We performed whole-genome/exome and transcriptome sequencing of PTCL-NOS and other related PTCLs, followed by targeted-capture sequencing of candidate drivers in T-cell lymphomas in 100 PTCL-NOS samples.
Results: Consistent with previous reports, *TET2* (38%) was the most frequently mutated gene in PTCL-NOS, followed by *RHOA* (28%), *TP53* (18%), *KMT2C* (13%), *IDH2* (11%), and *PLCG1* (11%). Frequently altered genes included signal transduction molecules (such as *RHOA*, *PLCG1*, *STAT3* and *SOCS1*), chemokine receptors (*CCR4* and *CCR7*), epigenetic modifiers (*TET2*, *KMT2C*, *IDH2*, *DNMT3A*, *CREBBP*, and *KDM6A*), and molecules associated with immune evasion (*HLA-A*, *HLA-B*, *B2M*, and *CD58*). Novel targets of recurrent mutation were also identified, including *PDCD1*, *YTHDF2*, and *LRP1B*, which were frequently targeted by nonsense and frameshift mutations distributed throughout the entire genes. Among these, *PDCD1* encodes PD-1, which transmits an inhibitory signal from PD-L1 and PD-L2 ligands, and therefore loss of function of this gene is predicted to enable malignant T-cells to escape from the negative signaling. By contrast, recurrent mutations in *YTHDF2* and *LRP1B* mutations in T-cell lymphomagenesis is unexpected. These genes encode a reader protein of N6-methyladenosine (*YTHDF2*), and a member of the low density lipoprotein receptor family (*LRP1B*). Although the function of these genes in T-cells are unknown, our findings suggest their unresolved roles, whose dysfunction may lead to malignant T-cell proliferation.Finally, we investigated the co-occurrence between frequently mutated genes in PTCL-NOS. In accordance with previous observation, mutations characteristic of TFH lymphomas (*TET2*, *RHOA*, *IDH2*, and *DNMT3A*) tended to co-occur in a subset of PTCL-NOS cases, but were almost mutually exclusive with mutations in *TP53* and chemokine receptor genes. These observations further support the molecular distinction between TFH and non-TFH lymphomas in PTCL-NOS: the former is more-related to AITL and discriminated from the latter in terms of their mutational profiles.
Summary/Conclusions: In summary, our findings illustrate the landscape of somatic alterations in PTCL-NOS and provide a novel insight into their genetic and molecular heterogeneity, which should help to devise a novel molecular classification of PTCLs and to exploit a new therapeutic strategy to combat these intractable T-cell malignancies.

P300

A COMPREHENSIVE PORTRAIT OF THE DNA METHYLOME OF 866 SAMPLES FROM DIFFERENT B CELL NEOPLASMS: BIOLOGICAL INSIGHTS AND CLINICAL APPLICATIONS

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Background: In the last years, a large body of evidence has been accumulated demonstrating that DNA methylation is not only widely altered in B-cell lymphoid tumors (and cancer in general) but it is also defining cell lineage and maturation stage. However, an integrative study of the whole DNA methylome of neoplastic B cells from different maturation stages has not been performed yet.
Aims: The aim of this study was to extensively dissect the dynamics of DNA methylation in B-cell neoplasias in the light of normal B cell maturation program. The ultimate goal of this study was to generate new clinically relevant knowledge with diagnostic and prognostic value.
Methods: Our dataset included whole-genome bisulfite sequencing data (n=57) and high-density methylation arrays (n=1161) from acute lymphoblastic leukemia (ALL), mantle cell lymphoma (MCL), Burkitt lymphoma (BL), follicular lymphoma (FL), diffuse large B cell lymphoma (DLBCL), chronic lymphocytic leukemia (CLL) and multiple myeloma (MM) patients as well as from ten different normal B cell subpopulations. As DNA methylation estimates in neoplastic samples are influenced both by tumor cell content and composition of the micro environment, we developed a new method to deconvolute and *in silico* purify the methylation signal of tumors arising in different niches (bone marrow, peripheral blood an lymph node). The data were analyzed by a series of bioinformatic and biostatistical approaches and correlated with clinical variables.
Results: The initial bioinformatic approach to purify of DNA methylation signals in B cell tumors revealed that samples with less than 55% tumor cell content could not be accurately purified. This strategy reduced the initial 1,044 tumor samples to 866. An unsupervised principal component analysis of in silico purified data revealed that each type of B-cell neoplasm clusters separately. ALLs clustered closer to precursor B cells, CLL and MCL closer to mature B cells and both DLBCL and MM showed the largest deviation from normal B cells. We then performed a differential methylation analysis comparing each sample *vs* normal B cell maturation stages, and thoroughly annotated the results to biological and clinical features. From the clinical perspective, we identified that for tumor samples with similar cellular origin, the higher the epigenetic deviation from healthy B cells (number of DNA methylation changes) the worse the clinical behavior of the patients. Furthermore, for each tumor entity, we could identify from 5 to 19 epigenetic biomarkers that could classify each entity with high sensitivity and specificity.
Summary/Conclusions: In this study, we show that *in silico* purification of DNA methylation data is a powerful strategy to accurately measure DNA methylation alterations in tumor cells. Using a large dataset, we have developed a set of epigenetic biomarkers with high differential diagnostic power and identified that the epigenetic drift is a universal prognostic factor that can be applied to different B cell tumors.

P301

ACTIVATION OF RHOA-VAV1 SIGNALING AXIS IN ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA

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Background: Angioimmunoblastic T-cell lymphoma (AITL) is a distinct subset of peripheral T-cell lymphoma with follicular helper T-cell (TFH) features. We and others previously found mutations of *RHOA*, encoding p.Gly17Val (G17V RHOA mutation) together with those in an epigenetic regulator, tet methylcytosine dioxygenase 2 in up to 70% of AITL and other TFH lymphoma (a subgroup of peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS)) samples. RHOA, a small GTPase, is converted from the GDP-bound inactive form to the active GTP-bound form by guanine nucleotide exchange factors (GEFs). The G17V RHOA mutant has been shown to be defective in RHOA signaling, because it does not bind GTP. Therefore, it has remained unknown how G17V RHOA is involved in lymphomagnesis. VAV1 serves as an important mediator of T-cell receptor (TCR) signaling pathway through its GEF-dependent and –independent function. VAV1 activation is tightly regulated by autoinhibitory mechanisms in the unstimulated state. Phosphorylation of VAV1 occurs within seconds in response to antigen stimulation of the TCRs by Syk and Src-family tyrosine kinases and initiates downstream TCR signaling.
Aims: We aim at clarifying the downstream signaling of the G17V RHOA mutant in AITL/TFH lymphoma.
Methods: Proteomic screening was performed to identify G17V RHOA-specific binding partners. Binding was validated by co-immunoprecipitation of G17V RHOA and the candidate partners. Simultaneously, RNA sequencing was performed for 9 PTCL samples, including 6 AITL and 3 PTCL-NOS. Targeted deep sequencing of *VAV1* was performed for 126 PTCL samples, including 69 AITL and 57 PTCL-NOS, 37 of which had *RHOA* mutations. The specific binding partner proteins of the G17V RHOA mutant were examined by high throughput screening in Jurkat cells. Nuclear factor of activated T cell (NFAT) activity in response to TCR stimulation was examined in Jurkat cells expressing wild-type (WT) and G17V RHOA mutant, and WT and various VAV1 mutants. Whole transcriptome was compared in Jurkat cells inducibly expressing each cDNA, in conditions with or without TCR stimulation. Expression of phospho-Vav1 was examined by immunostaining for AITL/TFH lymphoma samples.
Results: Proteomic screening identified the VAV1 protein as a G17V RHOA-binding partner. RNA sequencing identified a fusion gene involving *VAV1* and *STAP2* in an AITL sample without RHOA mutations. Moreover, targeted sequencing of *VAV1* identified 2 in-flame deletion mutations in an acidic region (c.C518_529del:p.173_177del and c.C494_520del:p.165_174del) in AITL samples and 2 missense mutations in a zinc finger and SH3-SH2-SH3 module (c.G1668C:p.Glu556Asp and c.C1844T:p.Pro615Leu) in PTCL-NOS and AITL samples, respectively. Phosphorylation of VAV1 at Tyr 174 was enhanced in Jurkat cells expressing the G17V RHOA or VAV1-STAP2 cDNA than those expressing each WT cDNA or mock. Phosphorylation was blocked by the dasatinib at 1-10 nM concentrations. The G17V RHOA, VAV1-STAP2 and various VAV1 mutants enhanced NFAT reporter activities and interleukin-2 (IL-2) mRNA levels compared to their WT or mock in Jurkat cells. Dasatinib completely blocked both of these TCR indicators at the concentration range similar to block VAV1 phosphorylation. Moreover, the aberrant reporter activity was also blocked by the dasatinib treatment. The levels of IL-2 mRNA were higher in Jurkat cells expressing either G17V RHOA or VAV1-STAP2 than those with their WT or mock. Gene set enrichment analysis showed that cytokine and chemokine-related pathways were enriched in Jurkat cells expressing the G17V RHOA compared to those with WT or mock. Finally, phospho-VAV1 was co-stained with PD-1, a TFH marker, in 7 out of 10 PTCL samples with *RHOA* or *VAV1* mutations.
Summary/Conclusions: The G17V RHOA and VAV1 mutants both intensify the TCR pathway through hyper-phosphorylation of Vav1. Our data suggest that the RHOA-VAV1 axis in AITL/TFH lymphoma may contribute to their clinical features and stand as a possible new therapeutic target.

P302

STAT3 IS CONSTITUTIVELY ACTIVATED AND CAN BE A THERAPEUTIC TARGET OF JAK INHIBITORS IN CHRONIC ACTIVE EPSTEIN-BARR VIRUS INFECTION

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Background: Chronic active Epstein-Barr virus infection (CAEBV) is a rare disorder characterized by clonal proliferation of EBV-infected T or NK cells and associated with severe systemic inflammation. Chemotherapy-resistant lymphoma or hemophagocytic lymphohistiocytosis can develop during the course of CAEBV, and the only curative treatment strategy is hematopoietic stem cell transplantation. In addition, why EBV persistently infects T or NK cells and how the disorder develops in patients have not been elucidated yet. The outcome of CAEBV remains poor, and the establishment of an effective chemotherapy based on the molecular mechanisms of CAEBV development is an urgent issue.
Aims: We designed this study to investigate STAT3 activation and its contribution to CAEBV development, because it was recently indicated that STAT3 was constitutively activated in some T- or NK-cell malignancies. We also examined the effects of JAK inhibitors on CAEBV.
Methods: The EBV-positive T- and NK-cell lines SNT8, SNT15, SNT16 and the NK-cell lines SNK1, SNK6, SNK10 were examined. EBV-positive T or NK cells were obtained from peripheral blood mononuclear cells (PBMCs) of CAEBV patients who were diagnosed according to the previously described diagnostic criteria (Blood 2012; 119:673-86). To detect and isolate EBV-infected cells, T and NK cells were separated from PBMCs using magnetic beads. Gene expression was examined using one-color microarray-based analysis (Agilent Technologies, Santa Clara, CA, USA). The direct sequencing analysis of exons 19 to 24 of STAT3, which encode the SH2 domain, was performed using primers from the previous report (N Engl J Med 2012; 366: 1905-13). EBV-negative T- and NK-cell lines and PBMCs from healthy donors were used as negative controls. Cell survival and apoptosis were examined by an XTT assay and Annexin V assay, respectively. The mRNA expression of cytokines was examined by TaqMan® Gene Expression Assays.
Results: STAT3 was constitutively phosphorylated on Y705 and S727 and was localized in the nucleus in EBV-positive T- or NK-cell lines and PBMCs from the CAEBV patients, as indicated by western blotting. The microarray analysis of EBV-positive T or NK cells derived from CAEBV patients demonstrated that the expression of STAT3-responsive genes, including interferon-γ, were upregulated in these cells compared with EBV-negative cells. No mutation was detected in the SH2 domain of STAT3 in patient-derived cells by direct sequencing. The JAK inhibitors ruxolitinib and tofacitinib suppressed STAT3 activation and cell survival by inducing apoptosis of the cell lines and PBMCs from CAEBV patients. Ruxolitinib also inhibited the mRNA expression of TNF-α and interferon-γ in CAEBV patient-derived cells.
Summary/Conclusions: STAT3 is constitutively activated in EBV-positive T or NK cells from CAEBV patients. The inhibition of STAT3 by ruxolitinib could be an attractive and effective treatment for CAEBV by suppressing not only EBV-infected cell survival but also the accompanying inflammation.

P303

RECURRENT MUTATIONS IN MICRO-RNA BINDING SITES MAY BE POTENTIALLY RELEVANT IN FOLLICULAR LYMPHOMA

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Background: Follicular lymphoma (FL) is the most common low grade B cell malignancy accounting for ~20% of all non-Hodgkin lymphomas. Approximately 30% of the FL cases suffer a histological transformation to a much more aggressive subtype of lymphoma drastically reducing the overall survival from 10 years to just 14 months. Despite being a critical event during disease progression it is molecularly poorly understood and no biomarkers exist to predict this phenomenon. Previous studies have suggested the possibility that deregulation of microRNA expression (miRNAs, small endogenously produced non-coding RNAs) could be implicated in the development of FL disease as well as in the transformation event. We hypothesise that mutations in miRNA binding sites may also have a role in this process.
Aims: We set ourselves to find predictive biomarkers of transformation for FL, with a special focus on sequence variants affecting miRNA binding sites.
Methods: We interrogated whole genome sequencing (WGS HiSeq, Illumina) data from sequentially obtained samples of 6 FL patients that underwent trans-

formation using a bespoke bioinformatic pipeline based on TargetScan prediction algorithm in order to identify mutations in putative miRNA binding sites. Once identified, in order to validate them and test their recurrence in an extended cohort (60 samples from 31 FL patients who underwent transformation plus 21 samples of non-transformed FL patients) we designed an Ampliseq (Ion Torrent, Life Technologies) NGS custom panel. Finally, we selected a number of variants for assessing the variant effect on the miRNA:mRNA interaction, by means of a combination of an *in silico* predictive algorithm and *in vitro* luciferase assays.

Results: 36% of somatic variants from WGS data arose in 3´UTR, and 68% of these were putative miRNA-binding sites (525 mutations in 497 genes). Interestingly, the ontogeny analysis showed that these mutations were not randomly distributed but rather there was enrichment in genes associated with haematological malignances ($P=2.18x10^{-4}$).We then validated 85% of these mutations using targeted resequencing and found a total of 103 recurrent variants located in putative miRNA binding sites. QC criteria filtering led us to prioritise 38 variants in 25 genes to be functionally tested. Crucially, ontogeny analysis showed that these genes were highly enriched for GC-like B-cell lymphoma genes ($P=4.39x10^{-5}$), strongly suggesting that these variants may have a biological significance in the disease. We then performed an *in silico* approach based on TargetScan miRNA target prediction algorithm to evaluate the effect of the mutations on the binding of the miRNAs to their target sites. Based on these results we prioritized some of these genes to perform luciferase assays. We experimentally demonstrated not only that the majority of these *loci* are *bona fide* miRNA targets sites, but also that the presence of a number of these variants cause a dysregulation of the normal miRNA regulatory activity (Figure 1).

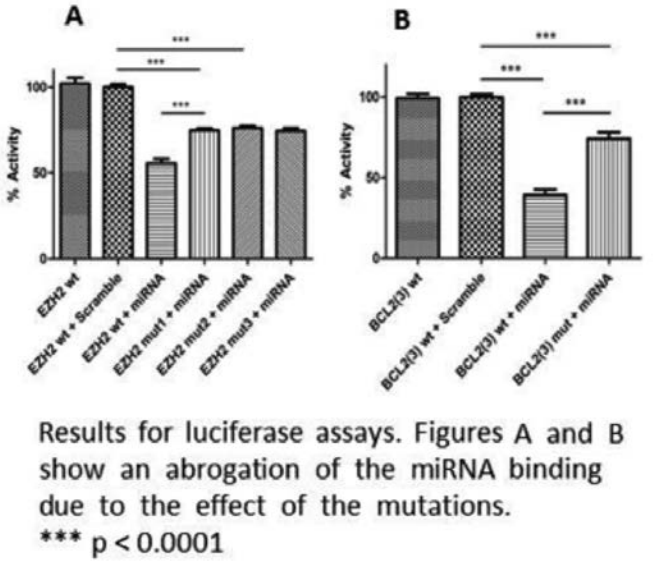

Figure 1.

Summary/Conclusions: Our data show that the identified mutations do not occur randomly, but preferentially in putative microRNA binding sites of genes related to lymphomagenesis, supporting their role in FL pathogenesis. Furthermore, the presence of some of the identified variants in miRNA binding sites indeed promotes a dysregulation of the normal miRNA regulatory activity, suggesting that they might have a biological significance in FL.

P304

CLINICAL IMPACT OF TP53 AND KMT2D MUTATIONS IN MCL RECEIVING HIGH-DOSE THERAPY AND AUTOLOGOUS TRANSPLANTATION: UPDATED RESULTS FROM THE FONDAZIONE ITALIANA LINFOMI MCL0208 PHASE III TRIAL

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Background: Within the landscape of mutated genes in mantle cell lymphoma (MCL), only *TP53* disruption has been so far associated with outcome.

Aims: Here we present the clinical update of the deep sequencing MCL gene panel analysis in the prospective FIL-MCL0208 phase III trial (NCT02354313, high-dose immunochemotherapy followed by autologous transplantation for untreated, advanced stage <65 years MCL) based on the data from the second interim analysis.

Methods: A targeted resequencing gene panel, including coding exons and splice sites of the *ATM*, *BIRC3*, *CCND1*, *KMT2D*, *TP53*, *TRAF2*, *WHSC1*, and *NOTCH1* genes was analyzed in tumor DNA from baseline bone marrow CD19+ purified MCL cells and, to filter out polymorphisms, in the paired normal genomic DNA (55% of cases) using a TruSeq Custom Amplicon target enrichment system followed by deep next generation sequencing (Illumina, median depth of coverage 2356x). Variants represented in >10% of the alleles were called with VarScan2 with the somatic function when the paired germline DNA was available. For patients lacking germline DNA, a bioinformatics pipeline including a number of stringent filters was applied to protect against the misclassification of polymorphisms as somatic variants. Clinical data were updated at the time of the second interim analysis (January, 2017).

Results: Out of the 300 enrolled patients, 174 were evaluable for mutations. Median follow-up of the cohort was 36 months, and 3-years PFS and OS were 67% and 86%, respectively. Patients not included in the study, due to unavailable tumor DNA (n=126) showed superimposable clinical features and outcome. Mutations of *TP53* (8% of cases) and *KMT2D* (11% of cases) associated with an increase in the hazard of progression both in univariate analysis as well as after adjusting for MIPI, Ki67 and blastoid variant: HR 3.87 (95% CI 1.64 to 9.13), p<0.002 and HR 3.66 (95% CI 1.77 to 7.56), p<0.001, respectively. These results translated into an increase of the hazard of death in both *TP53* and *KMT2D* mutated patients both in univariate analysis as well as adjusting for MIPI, Ki67 and blastoid variant HR 4.26 (95% CI 1.34 to 13.57), p=0.014 and HR 3.09 (95% CI 1.07 to 8.86), p=0.036, respectively. On these bases, a survival model was proposed based on the *TP53* and *KMT2D* mutation status: 3-years PFS and OS were 26% and 64% for patients carrying either *TP53* or *KMT2D* mutations or both *vs* 75% and 92% for patients without any of these mutations (Figure 1).

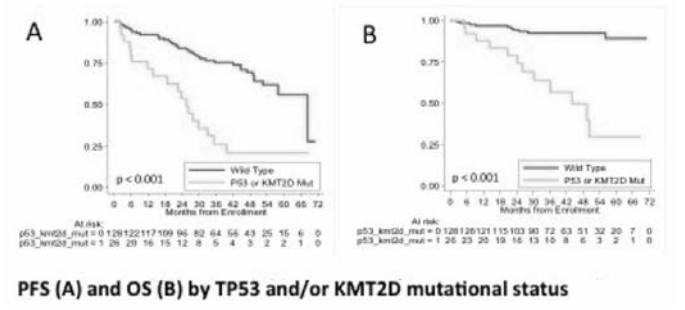

Figure 1.

Summary/Conclusions: The updated clinical results of the FIL-MCL0208 trial show that: i) both *TP53* and *KMT2D* mutations independently associate with shorter PFS and OS in younger MCL patients receiving high-dose therapy; ii) *KMT2D* mutations seem to be as detrimental as *TP53* mutations, at least in terms of PFS; iii) given the negative prognostic impact of these mutations, they might be used to select high-risk patients for novel therapeutic approaches.

Multifaced aspects of bleeding disorders

P305

A LOOKBACK AT VWD TYPE 2A AND 2M CLASSIFICATION IN A LARGE COMPREHENSIVE HAEMOPHILIA CENTRE.

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Background: Von Willebrand Disorder (VWD) has a prevalence of approximately 1% in the general population and is due to quantitative deficiencies or qualitative defects of the Von Willebrand Factor (VWF) protein. VWF is a large meltimeric protein with multiple functions. It carries and protects factor VIII and helps in the binding of FVIII, platelets and the vascular endothelium at sites of injury. VWF binding to platelets is through several receptors most notably the glycoprotein 1b (GP1b) and collagen exposed at site of injury is important for VWF adhesion to the subendothelial matrix forming an adhesive anchor. Classification of VWD is based on the quantitative deficiencies (Type 1 and 3) and VWD type 2 are qualitative defects of the VWF protein with or without quantitative deficiency as well. Type 2 VWD is further subdivided into type 2A,2B,2M and 2N. These subtypes depend on a number of laboratory assays that measure the FVIII activity, VWF protein level (VWFAg assay) and the function of the protein i.e its ability to bind to 1) FVIII(FVIII - VWF binding assays),2) platelets (VWF ricof assay) and 3) collagen (VWFCB assay). Other tests include ristocetin induced platelet aggregation (RIPA), multimer analysis, assay ratios and VWF genetic analysis. No single commercially available laboratory method can achieve to test all the parameters required to clinch the accurate diagnosis of the subtypes of VWD. Use of those multiple assays with VWF ricof/VWFAg ratio, VWF CB (VWF-CB)/ (VWF Ag) ratio have helped in the better identification of VWD and the subtypes.
Aims: To assess recent various VWF investigation panels and assay ratios, VWF genetic analysis, multimeric patterns of the VWF protein in accurate diagnosis of the VWD subtypes. VWD 2A and 2M shows similarities in certain aspects and it is important to differentiate these 2 subtypes as new therapies become available and personalised treatment approaches of VWD become a reality.
Methods: Clinicians who have made a diagnosis of VWD for individuals referred for a bleeding state work up would classify the subtypes of the VWD according to the results of the investigations available at the time of seeing the patients. All patients with an inherited bleeding disorder would then be registered in the centre and details would be put into a database. We have looked back into the database from the period of 2000 to end of 2016 and focussed on the VWD types 2A and 2M. Current VWD diagnostic panel in our centre includes the following tests: FVIII one stage assay, VWFAg Elisa,VWF ricof; Platelet agglutination method, VWF CB Elisa methods, VWF multimeric analysis by gel chromatography and VWF exon 27/28 genetic mutations are routinely done. New information and new set of results for the registered patients have been taken into account the classification of VWD type 2A and 2M and the database are updated.
Results: In the VWD database 38 patients classified as 2M and 19 patients as type2A have been recorded from 2000 to end of 2016. With the updated results and genetic analysis and the response to DDAVP, around 30% of the patients have had their subtypes changed. This exercise confirms that no singular test can be used to accurately diagnose the VWD and its subtypes and illustrates the importance of DDAVP testing and the difficulty of interpreting assay ratios for subtyping when VWFricof levels are <15u/dl.
Summary/Conclusions: VWD may be misdiagnosed, underdiagnosed or overdiagnosed. Appropriate and complete investigative panel is necessary for complete classification of VWD and its subtypes.

P306

RETROSPECTIVE EVALUATION OF PHENOTYPE AND MANAGEMENT OF DYSFIBRINOGENEMIA AND HYPODYSFIBRINOGENEMIA IN A COHORT OF ITALIAN PATIENTS

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Background: Dysfibrinogenemia (DF) and hypodysfibrinogenemia (HDF) patients (pts) experience hemorrhages or thromboses, and the clinical management can be difficult.
Aims: Aim of this study is to obtain information on DF/HDF clinical phenotype and management.
Methods: This is a spontaneous, retrospective, multicenter national study. Data are collected from clinical records.
Results: Forty-one pts have been enrolled in 3 centers: 35 DF (85%), 6 HDF (15%); 18M, 23F. Median follow-up: 7.4 months (1-203). Median age at diagnosis: 36 years (range 3-81). Median fibrinogen activity/antigen level: 53 mg/dL (0-156) and 250 mg/dL (66-380), respectively. Fourteen pts experienced hemorrhages: epistaxis, hematuria (presence of kidney stones), hematomas, ecchimoses, menometrorrhagia, and gastro-intestinal (presence of esophageal varices). No specific therapy was administered. A portal venous thrombosis occurred in 1 DF splenectomized patient in absence of replacement therapy; he was treated with warfarin without anti-hemorrhagic prophylaxis. Forty-one minor/major surgeries were performed in 23 pts. In 10/41 (24%) cases, prophylaxis was administered [fresh frozen plasma in 3, fibrinogen concentrate (FC) in 1, tranexamic acid in 6]; in 5/41 (12%) cases, low molecular weight heparin (LMWH) was administered; no hemorrhage occurred. Thirteen pregnancies were initiated in 9 women. In 1 case, LMWH prophylaxis was administered during pregnancy, and in 1 other during puerperium. In 2 cases, FC was administered at the time of spontaneous delivery (SD). Nine SD and 4 cesarian sections were performed without complications.
Summary/Conclusions: Pts from this case series experienced few hemorrhagic/thrombotic events. The majority was asymptomatic and the most severe events were related to concomitant pathologies. Nonetheless, this study has the potential to collect data from a numerous population of pts who live in the same country, and therefore to provide useful information to better characterize and manage these rare diseases. .

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OSTEOPOROSIS IN PATIENTS WITH HEMOPHILIA

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Background: Osteoporosis is often a co-morbidity of hemophilia, which exacerbates the hemophilic arthropathy and affects the long-term stability of the components after the arthroplasty. We present our results for the presence of osteoporosis in 148 patients with haemophilia and hemophilic arthropathy.
Aims: To prevent progression of hemophilic arthropathy and increase the long-term stability of the components after the arthroplasty.
Methods: . In the period from 2015 to 2016. the presence of osteoporosis surveyed 148 patients with haemophilia who are hospitalized in the department of reconstructive orthopedics for patients with hemophilia (Moscow, Russia): 121 (81.8%) - hemophilia A, 21 (14.2%) - and hemophilia B 6 (4%) – haemophilia with inhibitor. The average age of the patients was 39.3 years (range 10 to 69 years). 121 patients with hemophilic arthropathy performed primary total arthroplasty (98 knee, 20 hip, 3 shoulder joints); 18 patients underwent revision arthroplasty (5 - purulent infection, 7 - instability of the implants, 4 - fractures, 2 - loss of motion in the operated joint). 40 patients underwent ultrasound densitometry.
Results: As a result of ultrasound densitometry in 17.5% (7 patients) of cases revealed osteopenia and 20% (8) T-highest index. 105 patients underwent histological study in which 93 (88.6%) bone resorption, 58 (55.2%) intraosseous hemorrhage which 53 (50,5%) cases were accompanied by bone resorption. In total (histologically and of ultrasound densitometry) 99(66.9%) patients with haemophilia had osteopenia.
Summary/Conclusions: The data indicate that osteoporosis at patients with haemophilia considerably more common than in the general population. Intraosseous hemorrhage identified in more than half of the cases, exacerbate the decline in bone mineral density.

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PREVALENCE OF GENETIC MARKERS OF OXIDATIVE STRESS IN PATIENTS WITH SEVERE HEMOPHILIA FROM NORTH-WESTERN RUSSIA

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Background: Severe haemophilia (SH) is often complicated by chronic arthropathy due to recurrent haemorrhagic events and activation of such biological mechanisms as oxidative stress (OS) and inflammation. We have previously shown that the biochemical markers of OS and/or deficiency of antioxidant system (AOS) are frequently seen in SH patients affected with joint(s) destruction. Until now, there is a little data on the frequency of genetic variants predisposing to OS or decreased AOS activity in patients with SH.
Aims: To assess the prevalence of several genetic variants predisposing to OS or decreased AOS activity in patients with SH from North-Western Russia (NWR).
Methods: We studied 71 men with severe haemophilia A or B (62 and 9 patients, respectively). Osteoarthritis of large joint(s) was detected in each

patient, with the rate of recurrent haemorrhagic events in joint(s) from 6 to 13 per year. The control group consisted of 255 age-matched healthy men. Gene polymorphism of apolipoprotein E (ApoE e2/e3/e4), paraoxonase (PON1 Gln192Arg), methylenetetrahydrofolate reductase (MTHFR C677T), catalase (CAT C-262T) and plasmatic glutathione peroxidase (GPX3 T-65C) was studied by PCR-RFLP technique. Statistical differences between the patient and control groups were assessed by Fisher's exact test. Odds ratios (OR) with their 95% confidence intervals (CI) and p-value were calculated by using GraphPad Prism 5.0 software.
Results: We found abnormal distribution of ApoE genotypes in the patient group. Absence of ApoE e3 allele was observed in 7 (9.9%) men with SH and 8 (3.1%) controls (OR=3.4, 95% CI: 1.2-9.7, p=0.025). In particular, the frequency of ApoE e2/e2 genotype was 10-fold increased in patients when compared to healthy men (4.2% *vs* 0.4%, OR=11.2, 95% CI: 1.1-109.5, p=0.034). ApoE e2/e4 and e4/e4 genotypes were also more prevalent in SH than in the control group (2.8% *vs* 0.8% and 2.8% *vs* 2.0%, respectively). In the patient group, we observed the positive association between the PON1 192Gln/Gln variant and heterozygous GPX3 -65TC genotype (OR=5.8, 95% CI: 1.3-25.7, p=0.021). Simultaneous presence of these genetic variants was more than 5-fold frequently found in SH than in controls (8.5% *vs* 1.6%, OR=5.5, 95% CI: 1.3-22.8, p=0.016).
Summary/Conclusions: Our results indicate that OS-provoking variants of ApoE, PON1 and GPX3 genes are frequently seen in SH patients with chronic arthropathy and joint(s) destruction.

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THE ROLE OF DNA METHYLATION AND EXPRESSION OF MMP-2 AND MMP-9 IN PATHOGENESIS OF INTRACEREBRAL HEMORRHAGE IN CONGENITAL FACTOR XIII DEFICIENCY

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Background: Congenital factor XIII deficiency (CFXIIID) is a rare bleeding disorder. Intracerebral hemorrhage (ICH) is a leading cause of mortality and morbidity in this disorder. Matrix metalloproteinase-2 (MMP-2) and MMP-9 are reported to be associated with ICH.
Aims: The purpose of this study was to investigate the association of MMP-2 and MMP-9 methylation and their expression with ICH.
Methods: Patients with abnormal clot solubility test as well as a positive family history of FXIII deficiency were participated in the study. Methylation status was analyzed by Bisulfite Sequencing PCR. Gene expression in mRNA and protein levels was assayed by Quantitative real-time RT-PCR and ELISA, respectively.
Results: We found an unmethylated profile for both MMP-2 and MMP-9 in patients with ICH. Both of these genes were partially methylated in controls. Percent of methylated CGs are also higher for MMP-9 than MMP-2. Higher expression of MMP-9 in both of mRNA and protein levels was found in ICH compared to non-ICH group. However, there were no significant differences in MMP-2 expression levels (neither mRNA nor protein) between two groups.
Summary/Conclusions: Our findings showed that gene methylation contributes effectively in regulation of MMP-9 expression. Furthermore, our data suggest that MMP-2 expression in CFXIIID may not be controlled by gene methylation alone because methylation status of this gene did not correlate with expression levels (neither mRNA nor protein). Further investigations are needed for better understanding the exact role of these MMPs in the pathogenesis of ICH in CFXIIID and also identifying the regulatory mechanisms.

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GENETIC CONFIRMATION AND FINDING NOVEL MUTATIONS IN GLANZMANN THROMBASTHENIA AND VON WILLEBRAND DISEASE FAMILIES BY DIAGNOSTIC EXOME SEQUENCING

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Background: Congenital platelet function disorders and von Willebrad disease (vWD) are very heterogeneous group resulting in primary hemostatic defects. Physicians generally have difficulty to confirm them due to complicated diagnostic technique.
Aims: We intended to apply diagnostic exome sequencing (DES) for genetic confirmation and finding causative variants in children with primary hemostatic problems.
Methods: Library preparation was performed with TruSight One sequencing panel (Illumina, USA), which enriches about 4,800 genes with clinical relevance. Massively parallel sequencing was conducted with NextSeq (Illumina). Variants were annotated with population databases (1000 Genomes Project, Exome Variant Server, Exome Aggregation Consortium) and disease databases (OMIM). For missense variant, in-silico analysis was done with SIFT, PolyPhen-2, and MutationTaster. Candidate variants were confirmed by Sanger sequencing and family study. For *VWF* gene, multiplex ligation dependent probe amplification assay was also done using SALSA MLPA probemix P011-B3/P012-B3. Among variants from genes of primary interest, common variant with minor allele frequency ≥1% using population databases were filtered out. In addition, variants detected in more than 2% in in-house database were further filtered out to remove population specific polymorphism or platform specific errors. For VWF exons of either incomplete coverage or low mapping quality due to highly homologous region (exon 26, 24), additional Sanger sequencing was performed. Genes of primary interest were those associated with platelet dysfunction; vWD (*VWF*), Bernard–Soulier syndrome (*GP1BA, GP1BB, GP9*), Glanzmann thrombasthenia (GT) (*ITGA2B, ITGB3*), Thromboxane A2 receptor defect (*TBXA2R*), ADP receptor defect (*P2RY12*), Gray platelet syndrome (*NBEAL2*), Quebec platelet disorder (*PLAU*), ARC syndrome (*VPS33B*), Hermansky-Pudlak syndrome (*HPS1, AP3B1, HPS3, HPS4, HPS5, HPS6, DTNBP1, BLOC1S3*), Chediak-Higashi syndrome (*LYST*), Griscelli syndrome (*MYO5A, RAB27A, MLPH*), Scott syndrome (*ABCA1*).
Results: Twelve children with easy bruising, frequent epistaxis, or menorrhagia and their family members were enrolled. Two unrelated children were confirmed as GT. One proband had compound heterozygous variants of c.1913+5G>T and c.1451G>T (p. Gly484Val) in *ITGB3*. The former was pathogenic which results in aberrant splicing and the latter is novel. The other proband had homozygous variant of c.1913+5G>T in *ITGB3*. Three unrelated children were confirmed as vWD. One proband had compound heterozygous variants of c.2574C>G (p.Cys858Trp) and c.3390C>T (p.Pro1127_Gly1180delinsArg) in *VWF*, especially the latter synonymous variant previously confirmed to be resulted in exon 26 skipping. Another proband had a novel variant, c.2008C>T (p.Arg670Cys). The last proband had a known *VWF* pathogenic variant of c.1728G>T (p.Met576Ile).
Summary/Conclusions: DES is a valuable method to confirm GT or vWD. Further study is needed to find out unidentifiable mutations by this strategy.

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HPA-3A/3A GENOTYPE IS A POSSIBLE RISK FACTOR OF SEVERE HEMORRHAGIC SYNDROME IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA

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Background: The main clinical manifestation of primary immune thrombocytopenia (ITP) is hemorrhagic syndrome (HS) of different severity-from a lack or minimal cutaneous hemorrhages to severe life-threatening bleeding. It is well known, that there is no stable correlation between the platelets count or other parameter(s) and the hemorrhage grade in ITP patients. Possibly, the genetically-based individual mechanisms of immune response impairment could affect the clinical course of ITP, in particular, the severity of HS.
Aims: To reveal genetic risk factor(s) for severe HS in patients with chronic ITP.
Methods: A total of 67 patients (58 women and 9 men) with chronic ITP were involved in the study. The median age of the group was 57 years (range: 24-77). The mean duration of ITP was 7 years (2-48). Hemorrhage was graded according to WHO scale. Taking into account the severity of HS, all the patients were divided into two groups. The first group included 40 patients with HS of 0-1 grade and the second consisted of 27 patients with HS of 2-3 grade. All patients of the second group needed the use of different methods of emergency haemostatic therapy and we consider it as a "severe ITP". We analyzed DNA polymorphism of 8 genes responsible for the formation of specific human platelet alloantigen systems (HPA-1, -2, -3 and -5) or associated with impaired immune response (IL-1B, IL-6, IL-10 and TNF-A). The differences in genotype frequencies between the groups 1 and 2 were assessed by Fisher's exact method. Odds ratios (OR), their 95% confidence intervals (CI) and p-values were calculated with GraphPad Prism 5.0 software.
Results: The frequency of HPA-3a/3a (GpIIb 2622TT, 843 Ile/Ile) genotype was more than 2-fold increased in ITP patients with severe HS (55.6% *vs* 25.0% in the group with HS of 0-1 grade; OR=3.8, 95% CI: 1.3-10.7, p=0.02). HPA-1a/1a and HPA-2a/2a genotypes were also more frequently seen in patients with HS of grade 2-3 when compared to the group HS 0-1 (77.8% *vs* 72.5% and 92.6% *vs* 80.0%, respectively), but these differences were not statistically different (p=0.78 and p=0.19, respectively). Moreover, in the group with "severe ITP" we found almost 2-fold increase of the IL-6 -174CC genotype frequency (26.9% vs.15.0% in HS 0-1; OR=2.1, 95% CI: 0.6-7.1, p=0.34). Patients positive for IL-10 -592A allele were also more frequently seen in the group with HS of 2-3 grade (48.1% vs.26.3% in HS 0-1; OR=2.6, 95% CI: 0.9-7.4, p=0.11).
Summary/Conclusions: Our data indicate that HPA-3a/3a variant could be a possible risk factor for severe HS in ITP patients.

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AN ALGORITHM TO IDENTITY CASES OF SEVERE HEMORRHAGE IN ROUTINELY COLLECTED HEALTHCARE DATA

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Background: Many patients with a hematological malignancy have an increased risk of hemorrhages. Research addressing the causes of these hemorrhages, especially those on major hemorrhages, are hampered by the difficulty to find sufficient and representative cases of major hemorrhage. Unfortunately, electronic health records generally do not codify hemorrhages.
Aims: The aim of this study was to develop an algorithm that can be used to find patients who suffered from major hemorrhages (WHO grade 3 or 4) within electronic health records.
Methods: An algorithm was developed using electronic health record data of a cohort of patients with acute leukemia, who received platelet transfusions between June 2011 and December 2015 at the Leiden University Medical Center in the Netherlands. Chart review was performed for a stratified, random sample of observation days. Discriminative performance of three indicators was assessed: CT-brain, drop in hemoglobin level and transfusion need within 24 hours. The cut off values for hemoglobin drop and transfusion need with the best discriminating capacity and CT-brain were entered in the final algorithm. The C-statistic was calculated and calibration plots were made. The algorithm will be externally validated in two other academic hospitals.
Results: The derivation cohort consisted of 255 patients comprising 10,638 observation days and chart review was performed for 353 days. The incidence of major hemorrhage was 0.22 per 100 observation days. The final algorithm consisted of information on CT-brain (yes/no), a hemoglobin drop of ≥2.8 g/dl and the need of six or more transfusions (yes/no). The C-statistic of the algorithm was 0.93 (95% confidence interval (CI) 0.86 to 0.99). The incidence of bleedings with all grades of severity was 8.4 per 100 days. The algorithm for bleedings of all grades had a c-statistic of 0.54 (CI 0.53 to 0.55). The results of the external validation are not available yet.
Summary/Conclusions: An algorithm using information on CT-brain, hemoglobin drop and transfusion can accurately identify cases of major hemorrhage within electronic health care data. External validation will be performed.

Myelodysplastic syndromes – Clinical 1

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MOLECULAR MECHANISMS AND CLINICAL SIGNIFICANCE OF REDUCED PTPN1 EXPRESSION IN MYELODYSPLASTIC SYNDROMES

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Background: Previously we determined common deleted region (CDR) of del(20q) observed in MDS by CGH-array. Our data showed that the *PTPN1* gene is located within CDR of del(20q). The *PTPN1* gene encodes PTP-1B, a non-receptor type protein tyrosine phosphatase, which is involved in multiple physiological and pathological cellular processes via dephosphorylation of several tyrosine kinases, and other molecules. Although roles of PTP-1B in normal and pathological hematopoiesis has not been elucidated, it may function negative regulator for cellular processes mediated by tyrosine kinases, including JAK2, and SRC. We hypothesized that the *PTPN1* gene is a target gene disrupt by del(20q), resulting in haplo-insufficiency, and involved in MDS molecular pathogenesis.
Aims: We attempted to examine PTPN1 expression level in bone marrow cells of MDS patients with or without del(20q), and to investigate its clinical and biological significance.
Methods: Total RNA was extracted for cDNA synthesis from bone marrow samples taken at the time of diagnosis with written informed consent from patients and control subjects were used for the present study. Real-time RT-PCR was carried out to quantify PTPN1 expression by the TaqMan probe method using an ABI 7500 real-time PCR system (Applied Biosystems). Data including patients' demographic, disease status, medical history, clinical and laboratory findings, and outcome, were collected from medical records and laboratory data base. A non-parametric Mann-Whiteny-Wilcoxon test was used to examine whether expression levels among groups are statistically different. The Kaplan-Meier model was used to analyze the impact of PTPN1 expression on overall survival, and log-rank test was used for statistical analysis. We also examined the effect of 5-azacytidine treatment on PTPN1 expression in primary bone marrow cells from MDS patients. Bone marrow cells were cultured with or without 5mM of 5-azacytidine for 48 hours. Expression level of PTPN1 was examined by quantitative RT-PCR described as above.
Results: A total of 118 MDS patients, 71 males and 47 females with median age of 68 years (range: 20-91 years) and 19 control subjects were included in the present study. The patients were classified as RCUD (n=18), RCMD (n=58), RARS (n=8), RAEB-1 (n=20), and RAEB-2 (n=14) according to WHO classification. Relative PTPN1 expression level was significantly decreased in MDS patients with del(20q) (P<0.001) compared with control subjects. Moreover, relative PTPN1 expression level in MDS patients without del(20q) also significantly decreased (P<0.001). Expression patterns of PTPN1 among five WHO-subtypes, were statistical different (*P*=0.0201). Median values of relative PTPN1 expression level in RCUD, RCMD, RARS, RAEB-1, and RAEB-2 were 1.52, 1.95, 1.94, 1.46, and 1,26 respectively. Relative PTPN1 expression level in WHO-subtypes with high blast counts (RAEB-1 and RAEB-2) was significantly lower than that in WHO-subtypes with less blast counts (RCUD, RCMD, RARS) (median value: 1.41 *vs* 1.89, *P*=0.0074). To investigate prognostic implication of PTPN1 expression in MDS, we analyzed impact of PTPN1 expression on overall survival (OS). Based on PTPN1 expression level, 118 patients were divided into four groups, high (Q1), intermediate (Q2, Q3), and low (Q4) quartiles. Kaplan-Meier analysis demonstrated that the lowest quartile (Q4) showed significantly worse survival compared with remaining quartiles (Q1, Q2, Q3) (*P*=0.048). The estimated 5-year OS rates in Q1-3 group and Q4 group were 69% and 49.8%, respectively. We examined whether PTPN1 expression is induced by 5-azacytidine in primary bone marrow cells of 17 MDS patients. Real-time PCR analyses indicated that 5-azacytidine treatment significantly induced PTPN1 expression.
Summary/Conclusions: The present study demonstrated that PTPN1 expression is reduced in MDS patients by haplo-insufficiency due to del(20q) and methylation of promotor region of the *PTPN1* gene. Low PTPN1 expression is associated with advanced disease and poorer clinical outcome, indicating that PTPN1 expression level could be a useful prognostic marker in MDS. .

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MOLECULAR MARKERS PREDICTING RESPONSE TO AZACITIDINE TREATMENT FOR MYELODYSPLASTIC SYNDROMES

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Background: DNA hypomethylating agents (HMAs) comprise standard therapy for non-transplant-candidate high-risk myelodysplastic syndromes (MDS). However, little is known about the exact mechanism of their effects to MDS or no reliable makers predicting the response to HMAs have been developed, although a recent study reported a very high response rate of *TP53*-mutated AML and MDS to decitabine.
Aims: The purpose of this study is to elucidate the clonal dynamics and molecular signatures that correlate with response to azacitidine therapy for MDS, focusing on the role of *TP53*-mutations.
Methods: We conducted a prospective multicenter trial of azacitidine treatment for high-risk MDS patients, in which the efficacy was compared between the 5-day and 7-day regimens. A total of 107 patients were enrolled between 2013 and 2016. For all cases, a bone marrow specimens collected before treatment was analyzed for mutations using targeted-capture sequencing. Mutations were also interrogated after 4 cycles of azacitidine therapy in 48 (45%) cases. An additional 24 cases was also analyzed for mutations who received azacitidine therapy for MDS and whose bone marrow specimens were available both before and after therapy. RNA baits were designed for detection of both oncogenic variants in 67 known driver genes in myeloid neoplasms and copy number alterations on the same platform. Response was evaluated according to the IWG 2006 criteria. We also evaluated the difference in the size of clones showing the maximum allelic burden between pre- and post-treatment specimens (ΔTCF: tumor cell fraction).
Results: On average, 2.7 mutations (range 0-9) were detected per sample before azacitidine treatment. *TP53* represented the most common mutational target affecting 29 (27%) and 10 (42%) cases in the on- and off-protocol cohort, respectively, followed by *ASXL1, RUNX1, TET2*, and *SRSF2*. *TP53*-mutated cases had significantly lower number of driver mutations (1.7 *vs* 3.1/sample, p<0.001) and higher number of copy number changes (9.6 *vs* 2.1, p<0.001), compared with unmutated cases. Clinical response was observed in 25 cases in the on-protocol cohort, including 6 complete remission (CR) (5.6%) and 19 marrow CR (mCR) (17.8%) and 7 (29%) cases (all CR) in the off-protocol cohort. Notably, CR was obtained almost exclusively in *TP53*-mutated cases (5/6 and 5/7 CR cases in the on- and off-protocol cohort. No other mutations were significantly associated with clinical response. Median time to CR was 119 days (range: 81–721), which lasted for a median duration of 217 days (range 10 –783). ΔTCF was evaluable for 62 cases who had one or more follow-up specimens and carried at least one mutation in either pre- or post-treatment with an average of –0.075 (range: –0.75–0.72). ΔTCF was significantly lower in responders than non-responders (–0.18 *vs* –0.0002, p=0.0068) and in *TP53*-mutated than unmutated cases (–0.25 *vs* 0.0058, p=0.001).
Summary/Conclusions: Our study revealed a significant positive association of *TP53* mutations with favorable responses to azacitidine for MDS, although the response was transient and the expected response rate seems to be much lower compared to that reported for decitabine. Given that decitabine is not approved for MDS in many areas (*e.g.* EU and Japan), our results suggests a potential role of azacitidine as a key agent to improve the notoriously dismal clinical outcomes of *TP53*-mutated tumors. Further study should be warranted to confirm its efficacy and to develop an optimal post-remission therapy to overcome the short remission period.

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UPDATED RESULTS FROM PHASE 2 STUDY OF GUADECITABINE FOR PATIENTS WITH UNTREATED INT-2/HIGH RISK MYELODYSPLASTIC SYNDROMES OR CHRONIC MYELOMONOCYTIC LEUKEMIA

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Background: Improving the current response and survival outcomes of patients with higher risk MDS and CMML is fundamental. Guadecitabine is a next generation hypomethylating agent with increased length of exposure compared to decitabine and clinical activity in patients with MDS.
Aims: To evaluate the activity of guadecitabine in previously untreated patients with higher-risk MDS or CMML.
Methods: We conducted a single arm phase II clinical trial of guadecitabine at a dose of 60mg/m^2 sc daily for 5 days (days 1-5) every 28 days for patients with newly diagnosed MDS or CMML classified as Intermediate-2 or High risk by IPSS. Primary endpoint was complete response (CR). Responses were evaluated following the revised 2006 International Working Group criteria. Sequencing data was obtained at the time of pre-treatment evaluation by the use of a 28-gene next generation sequencing platform. Study included stopping rules for response and toxicity. Overall survival (OS) was censored at the time of transplant.
Results: A total of 53 patients have been enrolled: 50 (94%) are evaluable for toxicity and 44 (83%) for response. Median age is 67 years (49-87). A total of 43 (86%) patients have MDS and 7 (14%) have CMML. A total of 21 (42%) have complex karyotype. Sequencing data was available in 48 (96%) patients with *TP53* mutations being the most frequently detected in 36% patients. After a median of 6 treatment cycles (1-20), the ORR is 71% including 32% CR. Median best response occurred by 3 cycles (1-6). Seven (21%) out of 33 evaluable patients achieved a complete cytogenetic response. Ten (20%) subjects proceed to allogeneic stem cell transplantation. Median follow up was 6.3 months (0-23). Median OS is 14.1 months (CI 13.3-14.9 months) and median EFS is 8.4 months (CI 5.6-11.2 months). Forty-five (90%) patients experienced at least one AE during therapy. Most common grade 1-2 AEs included fatigue (66%), nausea (38%) and dyspnea (26%). Dose reductions due to cytopenias were required in 17 (34%) patients. Early 8-week mortality occurred in 3 (6%) patients.
Summary/Conclusions: Guadecitabine is well-tolerated and active in patients with higher-risk MDS and CMML even in the presence of adverse biological features such as high frequency of complex karyotype, therapy related disease and *TP53* mutations.

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AZACITIDINE IMPROVES OUTCOME IN HIGH RISK MDS PATIENTS WITH CHROMOSOME 7 ABNORMALITIES: RETROSPECTIVE COMPARISON OF GESMD AND GFM REGISTRIES

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Background: A benefit of treatment with azacitidine (AZA) in higher-risk (intermediate-2 and high risk by IPSS) Myelodysplastic syndromes (HR-MDS) patients with abnormalities of chromosome 7 (Abn 7) has been suggested in relatively small studies.
Aims: Our purpose was to confirm this benefit in a larger patient series.
Methods: Retrospective study of 235 HR-MDS patients with Abn 7 treated with AZA (n=115) *vs* best supportive care (BSC; n=120), assessing AZA treatment as time-varying variable in multivariable analysis.
Results: Seventy-four (64%) of AZA patients had de novo MDS and 41 (36%) had therapy related (secondary MDS), compared to 70 (90%) and 8 patients (10%) in the BSC group (*P*=0.0001). According to WHO 2008 classification, 65% in the AZA group and 48% in the BSC groups had refractory anemia with excess of blasts type 2 (RAEB-2) or secondary acute myeloid leukemia (AML with <30% of blasts) (p=0.015). The AZA and BSC groups were well balanced in terms of age, gender, cytogenetic risk category, and IPSS risk. In the AZA group, 55% patients were IPSS high-risk and 45% intermediate-2-risk and 61% had Complex-K, 23% non-complex -7, 14% non-complex del(7q), and only 2 patients (1.8%) had non-complex 7p-. Nevertheless, regarding MDS classification and MDS subtype (de novo *vs* secondary) was unbalanced with more patients with RAEB-2+AML (65% *vs* 48%, p<0.015) and secondary MDS (36% *vs* 10% <0.001), in the AZA group as compared to BSC patients. Median follow-up time from diagnosis was 47.5 months (95% CI: 24.2 – 122.9) in the AZA group and 59.8 months (95% CI: 15.5 - not reached) in the BSC group (*P*=ns). Median time from diagnosis to AZA treatment was 2 months (range 0 – 66.2). Ninety-two patients (80%) received AZA according to the conventional 7 days every 28 days schedule whereas 20% received 5-day cycles. The median number of AZA cycles received was 5 (range, 1-32). Response to AZA: Twelve patients were not evaluable for response according to IWG 2006 criteria because no complete data was recovered. In the 103 patients evaluable for response in the AZA group, the overall RR (ORR) was 37.9% (39/103), including 14.6% CR and 23.3% SD with HI. Among AZA non-responders (62.1%), 27.1% had SD without HI, 23.3% PD, and 11.7% (n=12) had early death (8, infection; 1, bleeding, 3, unknown cause). According to cytogenetic, the ORR was 38.1% in patients with CK, 32% in patients with non complex -7 and 46.2% in patients

with non complex del(7q) (*P*=ns for complex *vs* non complex, chi-square test). The ORR was 37.5% in "*de novo*" and 38.4% in secondary MDS, respectively (*P*=ns). Impact of AZA treatment compared to BSC on overall survival: Results of this multivariable analysis of OS at different time points are presented in Table 2. Chromosome 7 cytogenetic categories and IPSS retained a poor prognosis over time with a constant value of poor prognosis. AZA treatment had a favorable impact on OS during the first 3 years of treatment, compared to BSC, confirming results obtained in univariable analysis. Nevertheless, the benefit of AZA treatment as compared to BSC approach decreased as time spends and the HR value increased over time: HR of 0.3 at 6 months, 0.5 at 1 year and 0.7 at 2 and 3 years after treatment. (Figure 1). This benefit was present in all chromosome 7 categories with a a trend towards better impact among patients with complex karyotype but no significant differences between the 3 categories (-7, del(7q) and CK).

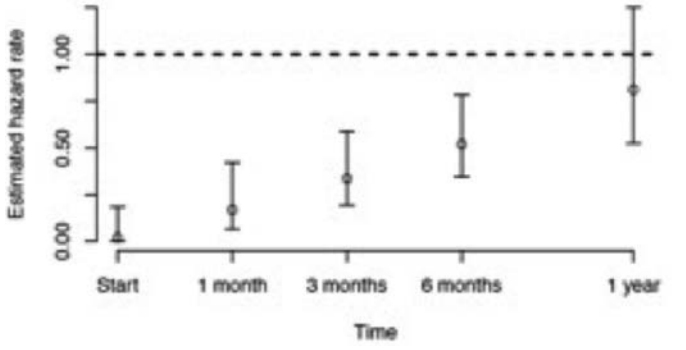

Figure 1.

Summary/Conclusions: This study confirms the benefit of AZA treatment on outcome in patients with HR-MDS and cytogenetic abnormalities involving chromosome 7.

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UN UPDATE OF A PHASE II EXPLORATORY STUDY OF OPN-305, A TOLL-LIKE RECEPTOR 2 ANTIBODY, IN PATIENTS WITH LOWER RISK MYELODYSPLASTIC SYNDROMES WITH PRIOR HYPOMETHYLATING AGENT THERAPY

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Background: Alterations of innate immune signaling, including overexpression of TLR2, are common in MDS. Significant TLR2 overexpression in MDS bone marrow CD34+ cells, especially after HMA therapy, has been reported. OPN-305 is a fully humanized antagonistic IgG4 kappa monoclonal antibody to TLR2 which significantly increases the formation of erythroid colonies (CFU-E) in BM CD34+ cells isolated from pts with lower-risk MDS *in vitro*.
Aims: To evaluate the potential therapeutic value of OPN-305 in patients (pts) with MDS
Methods: We designed a phase I/II trial of OPN-305 for pts with Low or Int-1 risk MDS by IPSS after failure to prior therapy with a HMA (≥4 cycles). Pts where required to be transfusion dependent (≥2 units in 8 weeks). Pts with isolated del(5q) should have received therapy with lenalidomide. Because, OPN-305 had not been previously used in pts with hematological malignancies, the study had an initial phase of N=10 pts using OPN-305 at a dose of 5 mg/kg every 4 weeks for a maximum of 9 cycles. Therapy could be repeated as long as there was no excess toxicity or progression. If after 16 weeks of therapy, there was no response, azacitidine on a 3 day schedule, could be added to OPN-305. Responses were evaluated following the revised 2006 IWG criteria. This initial cohort allowed evaluation of toxicity, pharmacokinetic analysis, receptor occupancy, and sequential analysis of cytokine profile. An extension dose escalation phase to 10mg/kg was planned for N=30 pts.
Results: At the time of this report, 31 pts have been enrolled, 11 at the initial 5 mg dose and 21 at 10 mg/kg. A total of 21 pts are evaluable for toxicity and response. Median age was 72 years (range 42-87). Nine (43%) pts were classified as Low risk and 12 (57%) as Intermediate-1 risk by IPSS. Thirteen pts had normal karyotype, 2 del(5q), 2 trisomy 8, 1 del(20q), 1 monosomy Y and 2 other single or double abnormalities. Median number of prior therapies was 2 (range 1-4) with a median duration of prior therapies of 23 months (range 6-56). A total of 5 (29%) pts developed AEs related to OPN-305. All AEs were grade 1 with gastrointestinal disorders being the most frequent (23%). At this point, no significant drug related toxicity has been documented with no excess infectious complications. Overall response rate in the form of hematological improvement was 53% (8/21) with 3 (20%) pts achieving transfusion independence and 5 (33%) minor hematological improvement. Half-lives of OPN-305 in serum were >200 h at 5 mg/kg and >300 h at 10 mg/kg. There was a greater-than-dose proportional increase in mean OPN-305 exposure (AUC) between 5 and 10 mg/kg. PK profiles after repeated dosing at 5 mg/kg in N=2 subjects and pre-dose (trough) levels in other subjects indicated some variability in the potential for accumulation. TLR-2 receptor occupancy in blood PBMCs and bone marrow aspirates was complete in virtually all samples taken after OPN-305 administration. There is no evidence of treatment related anti-drug antibodies. Compared with baseline, no significant changes of IL-23, IL-18, IFN-r, IL-10, IL-1β, IL-6, IL-12 (p40), IL-12 (p70) and IL-8 levels where observed among responders or non-responders or based on OPN-305 dosing. A trend to increased response was observed in patients with higher TLR2 expression, with no differences in response based on cytogenetic or mutational profile.
Summary/Conclusions: Treatment with OPN-305 in pts with previously treated lower-risk MDS was well tolerated with no significant toxicities and 53% ORR including 20% transfusion independence, and potential association between TLR2 levels and response.

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IN PATIENTS UNDEGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR MDS DEVELOPMENT OF CHRONIC GVHD COULD AMELIORATE THE ADVERSE IMPACT OF SPECIFIC SOMATIC MUTATIONS

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Background: Approximately 90% of patients with Myelodysplastic Syndromes (MDS) have somatic mutations in driver genes detected by Next Generation Sequencing (NGS). In the last years, several studies have related these mutations with prognosis, disease characteristics and response to therapy, including allogeneic Hematopoietic Stem Cell Transplantation (HSCT). Development of Chronic Graft Versus Host Disease (cGVHD) has been reported as one of the most powerful antineoplastic mechanisms after HSCT.
Aims: To evaluate the impact of specific somatic mutations in patients with MDS undergoing HSCT and if the development of cGVHD can modify their prognosis.
Methods: The results of HSCT in 115 MDS patients from five centres in Spain were retrospectively analyzed. Bone marrow samples were collected a median of 27 days prior to transplant and DNA was screened for somatic mutations by NGS, using a NextSeq platform (Illumina). Two myeloid genes panels that included the most frequently mutated genes in myeloid malignancies were used.
Results: Median age was 53 years (range from 19 to 70). Fifty-eight percent were male and 79.13% were classified as *de novo* MDS. According to WHO 2008 classification 4 (3.5%) were RCUD, 2 (1.8%) RARS, 22 (19.50%) RCMD, 28 (24.8%) RAEB-1, 32 (28.3%) RAEB-2, 12 (10.6%) Unclassifiable MDS, 9 (8%) CMML and 4 (3.5%) were AML (FAB RAEB-T). Among patients with calculated Revised IPSS (R-IPSS) (85 of 115 patients) 2 (2.4%) had very low risk, 15 (17.6%) low risk, 21 (24.7%) intermediate risk, 22 (25.9%) high risk and 16 (18.8%) had very high risk; 9 patients with CMML (10.6%) were categorized separately. Among patients with known karyotype (101 of 115), 7 of them (6.9%) had a complex kariotype (CK). Regarding mutational study, 44 patients (38.3%) didn't shown any mutation before transplant; 27 patients (23.5%) had 1 mutated gene, 15 (13%) had 2, 19 (16.5%) had 3, 6 (5.2%) had 4, 3 (32.6%) had 5 and only 1 patient (0.9%) had 6 different mutated genes. The most frequently mutated genes were: *TP53* in 15 patients (13%), *SRSF2* in 14 (12.2%), *TET2* in 13 (11.3%), *DNMT3A* in 9 (7.8%), *RUNX1* in 9 (7.8%), *SF3B1* in 9 (7.8%) and *ASXL1* in 8 (7%) patients. After a median of follow up for survivors of 2.02 years, Overall Survival (OS) was 48.1% (63.4% at 1 year; median 5.96). Patients were divided into 2 groups: group 1, with 2 or less mutated genes (74.5%) and group 2, with more than 2 mutated genes (25.2%). Group 2 had a lower OS (46.9% *vs* 69.6% at 1 year; p=0.035) and a higher Cumulative Incidence of Relapse (CIR) (25.3% *vs* 10.1% at 1 year; p=0.007). Development of cGVHD significantly improved outcome in both groups (Figure 1). Univariate analysis determined that developing of cGVHD, CK, number of mutated genes (more than 2 mutated genes) and mutations in *TET2* significantly impacted on outcome. Nevertheless, only the development of cGVHD as a time-dependent variable (HR 0.046, 95%CI 0.016-0.138, p<0.001) and *TET2* mutations (HR 2.562, 95%CI 1.018-6.447, p=0.046) significantly influenced on OS in multi-

variate analysis. We also observed the unfavourable impact of *TP53* mutations on relapse risk: CIR was 41.7% (95% CI 22.5-77.1) at 1 year for *TP53* mutated *vs* 9.8% (95% CI 5.3-18.1) at 1 year for non *TP53* mutated patients (p=0.006).

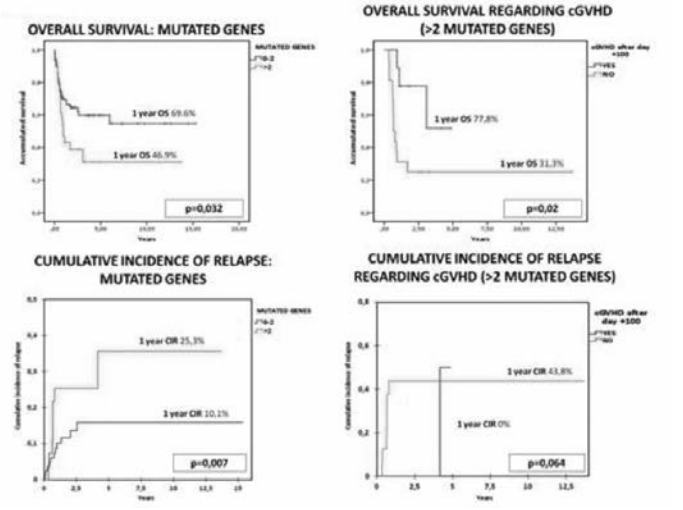

Figure 1.

Summary/Conclusions: We conclude that the number of mutated genes prior to transplant could be a prognostic factor of OS and CIR. Mutations in some genes, like *TET2* and *TP53,* could also have an adverse impact on outcome. However, cGVHD could ameliorate the poor prognosis of somatic mutations in transplanted patients with MDS.

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VOSAROXIN PLUS AZACITIDINE TREATMENT FOR PATIENTS WITH MYELODYSPLASTIC SYNDROME: A PHASE 1/COHORT EXPANSION STUDY

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Background: Although hypomethylating agents are the mainstay of treatment for myelodysplastic syndromes (MDS), these agents result in remissions in a minority of patients and are not curative. Vosaroxin is a first-in-class quinolone derivative that intercalates DNA and inhibits topoisomerase II. Vosaroxin is active with a tolerable safety profile in acute myeloid leukemia (AML) and the novel combination of vosaroxin and azacitidine was found to be synergistic in primary myeloblasts.
Aims: This phase 1/cohort expansion study was designed to determine the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of vosaroxin when given in combination with azacitidine, and to evaluate the efficacy and safety of the combination treatment.
Methods: Patients with MDS ≥18 years old with cytopenias requiring transfusions, an IPSS score of intermediate (INT)-1 or greater, or chronic myelomonocytic leukemia were eligible. Vosaroxin (initial dose: 50 mg/m^2/d) was administered on Days 1 and 4, and azacitidine (75 mg/m^2/d) on Days 1-7 of a 28-day cycle, in an outpatient setting, for up to 6 cycles in a 3+3 design (additional cycles were permitted if a clear benefit for the patient was demonstrated). Once the MTD was determined, an expansion cohort of 20 evaluable patients (≥1 cycle) was enrolled.
Results: A total of 35 patients enrolled in the dose escalation (n=13) and expansion (n=22) phases. The median age of the entire cohort was 66 years (range 38-77) with IPSS scores of low (n=1); INT-1 (n=13); INT-2 (n=15); and high risk (n=6). The median ECOG score for the entire cohort was 1 (range 0-2). In the dose escalation phase, at the initial dose of vosaroxin 50 mg/m^2/d (n=6), the median number of total cycles was 2 (range: 1-4); 2 of 6 patients experienced a DLT at this dose (grade 4 hyperbilirubinemia and grade 4 neutropenia >42 days). At the de-escalated dose of 34 mg/m^2/d (n=7), the median number of cycles was 2 (range: 1-18); 1 patient experienced a DLT at this dose (grade 4 mucositis). The MTD of vosaroxin was determined to be 34 mg/m^2/d and this dose was used in the expansion phase. Incidences of ≥grade 3 non-hematologic adverse events considered possibly, probably, or definitely drug-related for the total cohort (n=35) included infections (n=38), non-neutropenic fever (n=1); neutropenic fever (n=23); bleeding (n=9); and GI (mucositis/colitis/dysphagia; n=4). Two deaths were considered possibly treatment-related (sepsis and diffuse alveolar hemorrhage). No cardiac toxicity attributable to study treatment was observed, even with prolonged therapy. Of the 35 enrolled patients, 32 have completed ≥1 cycle and are evaluable for response. Among these patients, median number of cycles completed is 3 (range, 1-18), with 5 still continuing with therapy. Best response rates are shown in the Table 1. The median number of cycles to best response was 1 (range: 1-6). Sixteen patients have received transplant to date.

Table 1.

Best Response, n (%)	Total Cohort Evaluable patients (N=32)	34 mg/m^2/d Cohorts Evaluable patients (n=27)
Overall Response Rates		
CR + PR + HI	18 (56)	15 (56)
CR + PR + mCR + HI	25 (78)	21 (78)
CR	**6 (19)**	**6 (22)**
PR	**1 (3)**	**1 (4)**
mCR/mCR-HI	**14 (44)**	**11 (41)**
mCR	7 (22)	6 (22)
mCR-HI-erythroid	1 (3)	1 (4)
mCR-HI-neutrophil	2 (6)	1 (4)
mCR-HI-platelets	3 (9)	2 (7)
mCR-HI-neutrophil/platelets	1 (3)	1 (4)
SD/SD-HI	**10 (31)**	**8 (30)**
SD	6 (19)	5 (19)
SD HI-neutrophil	1 (3)	0
SD HI platelets	2 (6)	2 (7)
SD HI-neutrophil/platelets	1 (3)	1 (4)
PD	**1 (3)**	**1 (4)**

CR, complete response; HI, hematologic improvement; mCR, marrow complete response; PD, progressive disease; PR, partial response; SD, stable disease

Summary/Conclusions: The MTD of vosaroxin in MDS patients was 34 mg/m^2/d when given on Days 1 and 4 with a fixed dose of 75 mg/m^2 of azacitidine on Days 1-7. The major non-hematologic toxicities were infections, febrile neutropenia, and bleeding. The combination of vosaroxin and azacitidine showed promising activity with responses rates comparable or better than those generally observed with azacitidine alone. Additionally, the transplant rate observed was encouraging in this patient population.

Myeloma and other monoclonal gammopathies - Biology

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ADVANCED STAGE MYELOMA IS CHARACTERIZED BY A SIGNIFICANT INCREASE OF MUTATIONS IN GENES ASSOCIATED WITH DRUG RESPONSE

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Background: The amount of genomic data available in Multiple Myeloma (MM) is exponentially increasing, however, hardly any of that information is translated into the clinic. A number of genes has been associated with resistance to commonly used anti MM compounds. This, most importantly, includes immunomodulators (IMiDs) and proteasome inhibitors (PIs). However, no mutation screening has yet been amended to our MM routine diagnostic workflows. We investigated 458 MM patients by targeted sequencing, including the largest cohort of previously treated MM patients so far. We identified an increased mutation incidence in treated patients, yet unreported mutations and functionally validated a subset.
Aims: To describe the mutational spectrum in genes of pathways targeted by standard of care (SOC) therapies in a cohort of pretreated and previously untreated patients.
Methods: Tumor-germline paired samples of five contributing sites were pooled (Würzburg, Heidelberg, Madrid, Rotterdam and Mayo Clinic). Analysis included 310 untreated and 148 IMiD and/or PI treated patients. Targeted sequencing was performed using the M^{3}P (v2.0 or v3.0) gene selection, that includes most commonly mutated MM genes, actionable drug targets and genes being associated with drug resistance. Average sequencing depth increased 700X. Functional analyses of *PSMB5* mutations were conducted using Sleeping beauty vectors transposed into AMO1 cell line.

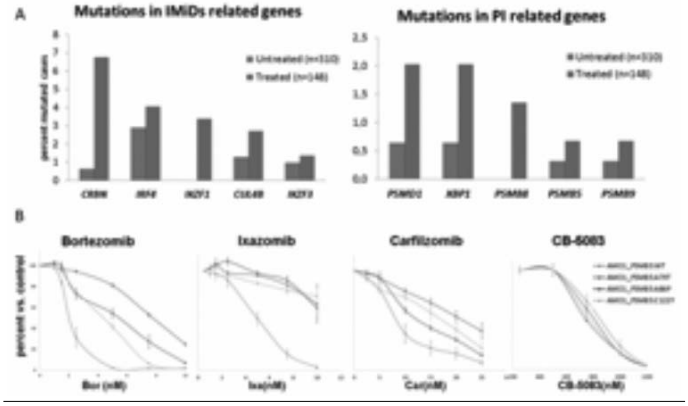

Figure 1. A: Mutation incidence in IMID related and PI related genes. B: Functional analysis of *PSMB5mut* expressing AMO-1 cells with different PI inhibitors and the P97 inhibitor CB-5083.

Results: Our analysis included five genes each with known association to drug response to IMiDs (*CRBN*, *CUL4B*, *IKZF1*, *IKZF3* and *IRF4*) and PIs (*PSMB5*, *PSMB8*, *PSMB9*, *PSMD1* and *XBP1*). Based on the increased sequencing depth, the mutation incidence in untreated patients is higher than in the CoMMPass dataset (IMiDs: 5.8% *vs* 3.9%; PIs: 1.9% *vs* 1.4%). Furthermore, pretreated patients showed a significant mutational increase compared with untreated pts (IMiDs: 19.7%, Z-score: -4.2, p<0.001; PIs: 7.3%, Z-score: -2.6, p=0.009). We observed a Gly159Arg mutation within the Lenalidomide (Len) degron sequence of *IKZF3* in a patient progressing on Len and Pomalidomide (Pom), as well as two XBP1 truncating mutations in PI refractory patients. Of note, among three treated cases with mutations in the β5 (*PSMB5*) or β5i (*PSMB8*) PI binding subunit of the proteasome, one patient harbored not less than 4 subclonal mutations. This is the first description of *PSMB5* mutations in human MM, identified in a patient with long term history of PI treatment. All mutations were located in or close to the Bor binding site of *PSMB5*. The functional analysis demonstrated induction of resistance not only to Bor ($IC_{50}^{PSMB5wt}$= 2 nM *vs* $IC_{50}^{PSMB5mut}$= 4.5-8 nM), but also to the second generation PI Ixazomib ($IC_{50}^{PSMB5wt}$= 5.2 nM *vs* $IC_{50}^{PSMB5mut}$= N/A) and Carfilzomib ($IC_{50}^{PSMB5wt}$= 8 nM *vs* $IC_{50}^{PSMB5mut}$= 13-22 nM). Of interest, the P97 blockade of the protein homeostasis by the investigational compound CB5083 remains still possible in the mutated cell lines and the resistance can be overcome. Finally, Pom treatment eradicated two of the *PSMB5* containing subclones (Figure 1).
Summary/Conclusions: Under the selective pressure of anti-MM therapy the incidence of mutations in genes associated with drug resistance increases over time. Resistance mechanisms evolve in parallel in competing (sub)clones of the disease, mimicking phenotype and behavior. Remarkably, despite our restrictive gene selection, a quarter of our treated cohort is affected by at least one mutation. Aim of future therapy may be the eradication of selected clones or subclones, which, according to our data, appears possible.

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ILF2-YB1 INTERACTION MODULATES RNA SPLICING TO INDUCE RESISTANCE TO DNA-DAMAGING AGENTS IN 1Q21-AMPLIFIED MULTIPLE MYELOMA

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Background: The 1q21 amplification, which occurs in approximately 40% of de novo and 70% of relapsed Multiple Myeloma (MM), is among the most frequent chromosomal aberrations in MM patients and is considered a very high-risk genetic feature that is especially correlated with disease progression and drug resistance. The 1q21 amplicon contains many genes, and while it is unlikely that all contribute to the pathobiology of high-risk MM, the critical genes that do drive this high-risk phenotype have not yet been fully clarified. Identifying such genes and their contributions to this phenotype would enable the development of new and effective targeted therapy strategies for high-risk MM and thus improve their survival outcomes.
Aims: In our study we wanted to investigate the biological and molecular mechanisms behind the 1q21 amplification's contribution to high-risk MM with the ultimate goal of obtaining a list of validated therapeutic targets to inform the design of novel translational clinical trials for this subgroup of patients.
Methods: We conducted a high-resolution analysis of recurrent copy number alterations and expression profiles in a collection of 254 MM samples included in MMRC database. To define the discrete minimal common 1q21 region that is recurrently amplified in MM, we used Genomic Identification of Significant Targets in Cancer, a systematic method that identifies regions of genome that are recurrently amplified or deleted across a set of samples. These genes were enlisted into an *in vitro* screening strategy that employed a single-shRNA-per-96-well approach and GFP-competitive cell growth assay to identify 1q21 genes whose loss of function resulted in the selective death and/or growth inhibition of MM cells carrying the 1q21 amplification but not MM cells without the 1q21 amplification.
Results: We identified MCL1, UBAP2L, INTS3, LASS2, KRTCAP2 and ILF2 as potential 1q21-specific vulnerability targets whose expression is driven by copy number. We functionally validated, both *in vitro* and *in vivo*, Interleukin-2-enhancer binding factor 2 (ILF2) as a key 1q21 amplification-specific gene. Our results show that ILF2 impacts homologous recombination (HR) and induces resistance to DNA damaging agents routinely used in the treatment of MM, which is consistent with the observation that ILF2 expression correlates with poor survival in MM patient treated with high-dose melphalan followed by tandem autologous transplantation. On the mechanistic level, ILF2 interacts with numerous RNA binding proteins directly involved in the regulation of DNA Damage Response (DDR) by modulating alternative splicing of specific pre-mRNAs. RNA sequencing experiment confirmed that ILF2 knockdown results in aberrant splicing of genes involved in the DDR pathways and, strikingly, ILF2 RIP-seq analysis showed that ILF2 directly binds to transcripts involved in the regulation of the HR pathway, including components of BRCA1 protein complex. Furthermore, we found that ILF2 mediates drug resistance in dose-dependent manner by modulating YB-1 nuclear localization and interaction with the splicing factor U2AF65 to promote mRNA processing and stabilization of DDR genes in response to DNA damage (Figure 1).
Summary/Conclusions: In conclusion, our study reveals an intimate relationship among 1q21 amplification, mRNA splicing and DNA repair in the control of DDR in MM. On the basis of our findings, we propose that 1q21-driven ILF2 overexpression deregulates HR by stabilizing the mRNA splicing of critical HR

effectors, which enables genomic instability, promotes adaptive mechanisms to genotoxic stress, and enhances cell survival, thereby promoting drug resistance and disease progression. Given that 1q21 amplification is one of the most frequent copy number alterations in cancer, synthetic lethality approaches based on targeting gain-of-function associated to ILF2 may have a broad spectrum of application to potentiate the sensitivity of cancer cells to chemotherapeutic agents.

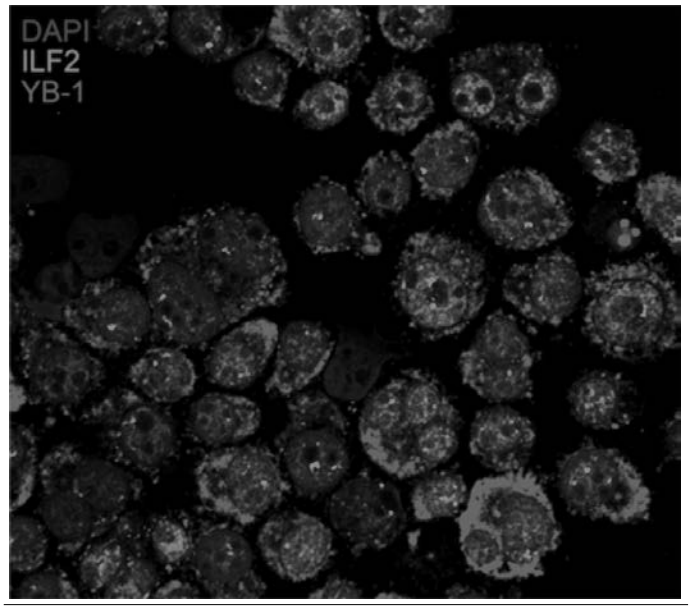

Figure 1.

P322

PROGNOSTIC IMPLICATION OF SOMATIC MUTATIONS BY NEXT GENERATION SEQUENCING: AN ANALYSIS FROM THE MMRF COMMPASS STUDY IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS

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Background: High throughput techniques, such as next generation sequencing, are becoming an appealing approach to characterize multiple myeloma (MM) genomic profiles and better define risk assessment. However, the clinical relevance of such approaches is still largely unknown. The Multiple Myeloma Research Foundation (MMRF) CoMMpass trial (NCT01454297) has collected data from 1154 newly-diagnosed MM patients enrolled worldwide. Comprehensive analysis of somatic mutations in MM cells at diagnosis could unravel prognostically relevant disease characteristics not detectable with traditional approaches.
Aims: We analyzed data from the interim analysis 8 cohort (August 2015) to create a prognostic model.
Methods: CD138+ purified MM specimens from bone marrow aspirates and peripheral blood cells were collected at diagnosis. Whole exome libraries from both tumor and constitutional DNA samples were created. Somatic single nucleotide variants (SNV) were identified, only nonsynonymous SNV were included in the analysis. We evaluated the impact on progression free survival (PFS) of recurrently mutated genes (with at least a nonsynonymous SNV with an allele frequency of more than 5% in more than 10 patients) in a multivariable Cox model adjusted for international staging system (ISS) and cytogenetic profile (high risk, standard risk and missing). A backward selection based on the Akaike Information Criterion (AIC) was used to identify the final Cox model used to create a scoring system.
Results: 517 patients with baseline somatic mutation data were included in the analysis. Median age at diagnosis was 64 years (range 27-93), all patients received novel agents as first line treatment, 236 (45.6%) received autologous stem cell transplantation (ASCT). The most recurrent mutated genes were KRAS (25%) and NRAS (19.5%). Consistently with other works, DNA allele frequency data revealed that, in the great majority of cases, only a subclonal portion of MM cell DNA harbors a selected somatic SNV (data not shown). Based on the impact on PFS of recurrently mutated genes, a scoring system was developed. Four groups were identified according to the mutational status of 9 genes selected in a nonbiased manner (Table 1): group I (score 0-2, 17%); group II (score 3, 51%), group III (score 4-5, 26%) and group IV (score >5; 6%). After a median follow-up of 371 days, the 18-month PFS was 93% for group I, 85% for group II, 73% for group III and 40% for group IV (Figure 1). The hazard ratio was 2.31 (p=0.118) for group II *versus* group I, 4.45 (p=0.006) for group III *versus* I and 17.38 (p<0.001) for group IV *vs* I. The prognostic trend of the score was confirmed in different patient subgroups including ASCT/no ASCT, standard/high risk cytogenetic profile, ISS I, II, or III. Of note, 23% of patients in group I had ISS III and 34% of patients in group IV had ISS I.

Table 1.

Mutational score obtained by adding score for each parameter (total points).

Gene	Mutated Yes/No	Score assigned
PRRC2C	Yes	3
USH2A	Yes	2
RBP3	Yes	2
PKHD1	Yes	2
HRNR	Yes	2
FAT4	No	2
KRAS	Yes	1
FAT3	Yes	1
NRAS	No	1

Additive total score	
Group I	0-2
Group II	3
Group III	4-5
Group IV	>5

PFS stratified according to mutational score subgroups. Group I (score 0-2, red), group II (score=3, pink), group III (score 4-5, blue), group IV (score >5, purple).

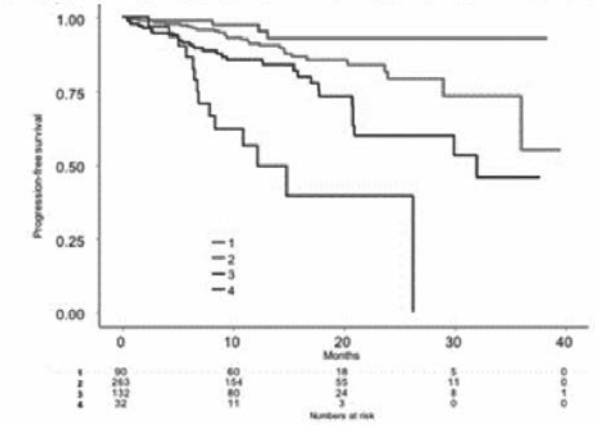

Figure 2.

Summary/Conclusions: The use of a prognostic model based on the mutational status of 9 recurrently mutated genes could improve risk assessment of newly-diagnosed MM patients. Longer follow-up and validation in independent cohorts of patients are needed to confirm our findings. Updated results with a longer follow-up will be presented at the meeting.

P323

TARGETING GENE DEPENDENCY OF 1Q AMPLIFICATION IN MULTIPLE MYELOMA

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Background: Gain of 1q is one of the most frequent copy number variations across cancer types and in Multiple Myeloma (MM). Gain of 1q is associated with a poor outcome, indicating it is a potential driver in MM progression and resistance to treatment. While the whole 1q arm can be amplified in some cases, a specific minimal amplified region has been identified by CGH array, including approximately 500 genes in the 1q21.1-23.3 region. However, the driver genes in the 1q region are unknown.
Aims: We hypothesize that specific genes present in the 1q minimal amplified region are critical regulators of clonal evolution and tumor progression in MM.
Methods: To explore gene dependency in 1q21.1-23.3 in MM, lung, breast and

ovarian cancers, we performed an shRNA targeted screen, using the C911 technology as a control. We used 14 cell lines including MM, lung, and breast cancer cell lines. We designed a pooled library targeted shRNA/C911 screen containing 6 shRNAs along with their matched control for each of the 500 genes in the 1q21.1-23.3 region, including lncRNA and miRNA in addition to protein coding genes. The pooled library contained 6500 shRNAs, including C911 controls. The differential expression from each shRNA and its own C911 control was calculated and we used the RIGER software to call hits, using the Kolmogorov-Smirnov algorithm. To complement the 1q-targeted shRNA screening, we studied both the Achilles dataset and patients' gene expression profiling from the Multiple Myeloma Genomic portal (MMGP) and the Cancer Genome Atlas (TCGA). Using a list of candidate genes, we then interrogated a large expression-profiling resource developed by the Library of Integrated Network-based Cellular Signatures (LINCS) program to identify potentially active drug targeting our candidate genes. Finally, a targeted drug screening was performed using 179 compounds identified through the LINCS program and using the FDA-approved drug library. Compounds with higher toxicity were further validated in 1q+ (OPM2, H929 and KMS11) and non 1q cell lines (KMS18).
Results: We were able to identify 10 candidate genes, for which knockdown significantly impaired proliferation of gain of 1q cell lines. Several candidate genes were identified as being the top genes preferentially affecting the proliferation of gain of 1q cell lines. To further confirm that our candidate genes are overexpressed in gain of 1q patients, we studied publicly available gene expression profiling from the MMGP and TCGA. Five of the 10 genes were significantly overexpressed in patients with gain of 1q. We then generated a gain of 1q signature by analyzing publicly available gene expression profiling from patients with MM, lung and breast cancers. Each dataset was divided equally into training and validation sets. We identified differentially expressed genes in tumors with gain of 1q *vs* no gain of 1q in these three different cancer types in the training sets. We then combined the three signatures to generate a 'pan-cancer' gain of 1q core signature, which was significantly enriched by using Gene Set Enrichment Analysis (GSEA) in patients with gain of 1q in the validation sets (shown here for MM). We next queried the core signature against the MSigDB 'c2' canonical pathways and 'c3' transcription factor pathways in GSEA and consistently identified a significant enrichment of cell cycle and E2F pathways. A targeted drug screen was then performed using FDA approved drugs and based on specific targets identified in the LINCS database. Compounds that showed high toxicity were further validated in 1q *vs* non 1q+ MM cell lines, and indeed showed significant differential activity of these compounds on 1q *vs* non 1q+ MM cell lines.
Summary/Conclusions: Gain of 1q is one of the defining features of high-risk MM and is associated with adverse outcomes. We developed a systematic approach to determine gene dependencies in gain of 1q MM combining a loss-of-function pooled screen, a computational approach and a drug screen to identify novel therapeutic targets in MM .

P324

DUAL INHIBITION OF DNMT1 AND EZH2 CAN EFFECTIVELY OVERCOME BOTH INTRINSIC AND ACQUIRED RESISTANCE OF MYELOMA CELLS TO IMIDS

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Background: The introduction of novel agents for the treatment of multiple myeloma (MM), mainly proteasome inhibitors and immunomodulatory agents (IMiDs), has significantly improved the survival rates of the patients, and both classes of drugs stand as the main treatment options for MM. Several studies have identified Cereblon (CRBN) as the direct target of not only thalidomide, but also lenalidomide and pomalidomide, and suggested that its expression is essential for the anti-myeloma effect of these drugs. However, even though the expression levels of CRBN have been associated with response to IMiDs, not all studies have confirmed this finding, as for example patients or cell lines with high levels of CRBN might not exhibit sensitivity to IMiDs. Thus, the expression of CRBN does not consistently explain the lack of IMiD sensitivity and the precise mechanisms behind IMiD resistance still remain elusive.
Aims: The aims of this study were to examine the importance of epigenetic modifications for the expression of CRBN and subsequently the development of IMiD resistance, as well as investigate whether restoration of sensitivity to IMiDs is feasible through epigenetic reprogramming by epigenetic modulators.
Methods: For the development of IMiD-resistant cell lines (OPM2-LR and-PR, H929-LR and -PR), we treated OPM2 and NCI-H929 continuously with increasing doses of either lenalidomide or pomalidomide for 4-6 months, until cell viability and proliferation were not affected. Nucleosome positioning (chromatin accessibility) was assessed using Acce*Sssl*ble, with all the analyses performed in the statistical software R, using the package *minfi*. RNA-seq is currently being performed for OPM2, NCI-H929 their IMiD-resistant as well as resensitized counterparts (N=10) in collaboration with BGI, using BGISEQ500 platform.
Results: Using AcceSsslble, we we found that acquired resistance for both lenalidomide and pomalidomide was primarily associated with a global decrease in chromatin accessibility and to a much lesser extent with DNA methylation changes. Interestingly, neither CRBN nor any of the other molecules involved in the CRBN pathway (IKZF1, IKZF3, IRF4) exhibited changes in either promoter DNA methylation or chromatin accessibility. We then treated the IMiD-resistant cells with the combination of 5-Azacytidine (a DNMT1 inhibitor) and EPZ-6438 (an EZH2 inhibitor) for 48 hours, before exposing the cells again to IMiDs, and found that resistant cells treated with this combination showed increased sensitivity to both lenalidomide and pomalidomide, with significantly increased apoptotic response, similar to the sensitive cell lines. Even more interestingly, we found that the treatment with 5-Aza and EPZ-6438 almost completely restored the global chromatin accessibility changes associated with acquired IMiD resistance back to the initial state, even though the same was not observed for DNA methylation changes. Finally, the combination of 5-Aza and EPZ-6438 was also effective in sensitizing the majority of cell lines with intrinsic resistance to IMiDs. We also observed that treatment with the combination of 5-Aza and EPZ-6438 failed to induce a significant upregulation of CRBN in the IMiD-resistant cell lines, thus suggesting that the process of resensitization to IMiDs is CRBN-independent. Moreover, we show that the degradation of IKZF1 is abrogated on the resistant cell lines, and remains as such despite their resensitization, meaning that the CRBN-IKZF1/IKZF3 pathway might be bypassed and other important regulatory networks might be as important for sensitivity to IMiDs. Therefore, we are currently performing RNA-seq, which might, in combination with accessibility data, give information about the regulatory mechanisms behind acquired IMiD resistance.
Summary/Conclusions: In conclusion, our study is the first one to ever show that acquired IMiD-resistance is mainly an epigenetic event that is potentially reversible through a combination of two epigenetic compounds, 5-Azacitidine and EPZ-6438. These drugs have been shown to have low levels of toxicity, thus making them very good candidates for a prospective phase I study to examine their potential as "IMiD-resensitizers", which may improve the outcome treatment of MM patients with drug-resistant myeloma clones and a potentially high-risk disease.

P325

MULTILAYER EPIGENOMIC ANALYSES REVEAL OF NEW CANDIDATE ONCOGENES INVOLVED IN THE PATHOGENESIS OF MULTIPLE MYELOMA

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Background: Most of the published omics studies in multiple myeloma (MM) have focused on the analysis of the genome, transcriptome and DNA methylome. Over the last years, the chromatin structure and histone modifications are emerging as essential epigenetic layers to understand gene deregulation in cancer, although this field remains widely unexplored in MM.
Aims: We herein aim to elaborate a comprehensive description of the MM epigenome including multiple layers of information.
Methods: We performed ChIP-seq of six histone modifications with non-overlapping functions (H3K4me3, H3K4me1, H3K27ac, H3K36me3, H3K27me3, and H3K9me3), ATAC-seq for chromatin accessibility, Whole Genome Bisulfite Sequencing (WGBS) for DNA methylation, and RNA-seq for gene transcription in purified bone marrow plasma cells from four MM patients and, as healthy controls, naive B cells, germinal center B cells, memory B cells and plasma cells. Data were extensively mined using a battery of different bioinformatic tools.
Results: An initial unsupervised approximation showed that each epigenetic layer cluster MM patient cells separately from normal B cells, which in turn also show differences according to their maturation stage. Moreover, an integrative analysis of ChIP-seq data from six histone marks allowed us to segment the genome into functional chromatin states, such as promoters, enhancers, transcribed regions and repressed/heterochromatic regions. In order to detect regions with significant differences in chromatin activity between MM and normal plasma cells, we elaborated a new algorithm that allowed us to transform the qualitative chromatin state data into a quantitative chromatin activation score (ChromAS). When we compared the ChromAS between MM and normal plasma cells, we detected over 13000 regions with differential activity in MM, from which near 90% were gaining activity in MM, suggesting a widespread activation of their chromatin landscape. To further characterize this phenomenon, we calculated the mean ChromAS per gene and performed a K-means clustering of MM and control cells. Interestingly, we identified the presence of a cluster comprising over 2000 genes whose chromatin was increasing activation in MM as compared to all normal cells. These findings were further validated by ChIP-seq in an additional series of 10 MM patients. We next focused on the genes that gained *de novo* activity in MM and were completely inactive (*i.e.* heterochromatic) in normal

cells. Out of this list, we observed that two adjacent genes, *PRDM5* and *NDNF,* were co-activated in MM. The analysis of their expression in additional patient cohorts indicated that their co-upregulation is a consistent event in MM pathogenesis and that their levels were negligible in bone marrow and tonsillar plasma cells. When analyzing chromatin topology by 4C-Seq, we identified 3D interactions between both gene loci only in MM cells, suggesting that DNA looping between the two genes may be related to their co-activation in MM. Finally, knockdown of each of these genes using inducible shRNAs, decreased cell proliferation and induced apoptosis in MM cells.

Summary/Conclusions: Collectively, our initial exploration of histone modification profiles in MM has revealed an extensive activation of the MM chromatin landscape, which targets new candidate oncogenes. Reversing this global activation by epigenetic drugs, such as BET inhibitors, may represent an attractive therapeutic option for MM.

P326

CLINICAL IMPLICATIONS OF CLONAL CD34+ CELLS IN STEM CELL HARVEST FROM PATIENTS WITH PLASMA CELL DYSCRASIAS

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Background: Introduction of novel treatments; Lenalidomide, high-dose alkylating agents (Melphalan) conditioning prior to autologous stem cell transplant (ASCT) over the last few decades has improved overall survival in patients with Multiple Myeloma (MM). In spite of enhanced survival rates, some therapies (especially Lenalidomide maintenance post ASCT) have come under scrutiny for causing therapy related myeloid neoplasms and second primary malignancies (SPM) like Myelodysplastic syndrome (MDS) and Acute myeloid Leukaemia (AML). Clonal haematopoiesis resulting in sequential accumulation of a combination of driver-passenger genetic mutations (in upto 80% of MDS & >95% AML patients) steer MDS/AML disease pathogenesis and clinical outcome. Therefore, we hypothesised that detection of Clonal Hematopoiesis of Indeterminate Potential (CHIP) in haematopoetic stem cells (HSCs) prior to ASCT in patients with MM treated with a range of therapies could be utilised for predicting patients at risk of developing SPMs *i.e.* MDS/AML.

Aims: To ascertain baseline mutational spectrum [especially low-level clones with variant allele frequency (VAF) ≥5%] of MDS/AML associated gene mutations in HSCs prior to ASCT in order to predict patients at risk of clonal evolution, transformation to MDS/AML.

Methods: DNA was isolated from mononuclear cells (MNCs) collected by leucopheresis prior to ASCT from 128 MM patients. A customised amplicon-based Illumina MiSeq panel was used for the sensitive interrogation of 24 most common genes harbouring mutations in MDS/AML (splicing factor genes; *SF3B1*, *SRSF2*, *U2AF1* and *ZRSR2*, genes implicated in epigenetic regulation; *TET2*, *IDH1/2*, *ASXL1*, *EZH2* & *DNMT3A*, known oncogenes/genes involved in cell signalling/transcription regulation and cohesion complex; *TP53*, *FLT3*, *NRAS*, *KRAS*, *ETV6, RUNX1*, *CCBL*, *C-KIT*, *JAK2*, *MPL*, *CEBPA*, *STAG2, GATA2*, KDM6A and *NPM1*). Variant analysis was performed using Illumina Variant Studio (≥5% VAF & read depth ≥150X threshold). To accommodate for the lack of germ line material to confirm the somatic nature of the variants, SNPs occurring at a frequency of ≥0.001% in the healthy population [*e.g.* dbSNP132, UCSC genome browser, Exome sequencing project (esp6500), Exome Aggregation Consortium (ExAc)] were excluded.

Results: Seven patients (6.25%) contained heterozygous somatic mutations (VAF range 7-50%) in *DNMT3A*, *IDH1*, *IDH2*, *TET2*, *ETV6* and *CBL* genes (Table 1). Four missense mutations identified in *DNMT3A* were aggregated in the Mtase domain responsible for its methyltransferase activity indicating a strong intent to abrogate this function. Previous studies confirm R882 variant (accounts for ~ 60% *DNMT3A* mutations) as a founder lesion in MDS/AML strongly associated with clonal haematopoiesis and HSC differentiation. Missense mutation in *CBL* (I429F) has been previously reported in CMML cases *(* while translocations and deletions of *ETV6* are more common in AML M0 (5%) compared to mutations suggesting its role as a tumor-supressor . Genes identified in our cohort are frequently associated with MDS & AML; *IDH1*/*IDH2* (5 & 20%), *TET2* (12 & 20%), *DNMT3A* (8 & 20%) and associated with poor prognosis (*DNMT3A, IDH1/IDH2*). SNP array karyotyping on 4/7 cases (patients 1-4) displayed no chromosomal abnormalities. Median age at diagnosis in these four cases was 65 (range 60-70), long-term follow up (3-5 yrs) revealed relapse of MM in patient 1 and 3, acute kidney injury with myeloma in patient 2 and transformation to AML in patient 4.

Summary/Conclusions: Our data identifies for the first time a subgroup of MM patients (6.25%) with no morphological evidence of MDS/AML prior to ASCT but harbouring CHIP in CD34^{+} harvest stem cells and later developing MDS/AML. These findings are pivotal for identification of such patients at risk of clonal evolution and transformation prior to ASCT since it can be a significant parameter in determining appropriate treatment modality *i.e.* whether or not to employ CHIP harbouring CD34^{+} harvest stem cells as therapy for these patients.

Table 1.

	Gene	VAF (%)	Amino Acid
Patient 1	IDH2	50.8	G145fsX
Patient 2	TET2	9.74	T1149fsX
Patient 3	DNMT3A	14.35	R882H
Patient 4	DNMT3A	7.58	A662V
Patient 5	DNMT3A	13.26	A788G
	DNMT3A	5.24	R676Q
	TET2	7.61	A1882D
	ETV6	16.95	E250K
Patient 6	CBL	21.15	I429F
Patient 7	IDH1	48.53	T106M

Seven patients harbouring mutations in MDS/AML associated genes. Variant allele frequency (VAF) is indicated as a percentage of the total read depth.

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PATHOPHYSIOLOGICAL FUNCTIONS AND CLINICAL IMPACT OF THE NEW IMMUNORECEPTOR SLAMF3 IN MULTIPLE MYELOMA

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Background: The signaling lymphocytic activation molecule family 3 (SLAMF3) is a member of the immunoglobulin superfamily expressed on T, B, and natural killer cells and modulates the activation and cytotoxicity of these cells via self-ligand binding. SLAMF3 is also expressed on plasma cells from patients with multiple myeloma (MM), although its role in MM pathogenesis remains unclear.

Aims: To clarify this, we investigated the expression and functions of SLAMF3 in MM.

Methods: 1) Two hundred thirty patients comprising 153 newly diagnosed (19 asymptomatic and 134 symptomatic) MM patients, 30 refractory/relapsed MM patients, and 47 patients with monoclonal gammopathy of undetermined significance were enrolled. SLAMF3 and CD138 expression levels on clonal plasma cells were analyzed using flow cytometry (FCM). Soluble SLAMF3 (sSLAMF3) serum levels were measured using ELISA. 2) Drug sensitivity to antimyeloma agents (melphalan and bortezomib) and the proliferation potential in MM cell lines KMS18 and U266 were analyzed using FCM and the MTT assay. SLAMF3 knockdown MM cell lines were obtained using the lentiviral shRNA system and siRNA. Stable transfected KMS34 cell lines overexpressing full-length SLAMF3 and cytoplasmic domain-truncated SLAMF3 (ΔSLAMF3) were established through corresponding vectors. Single-nucleotide polymorphism (SNP) genotyping was analyzed by real-time PCR. The adaptor protein of SLAMF3 was identified by Western blotting and immunoprecipitation.

Results: 1) SLAMF3 was highly expressed on plasma cells in almost all MM patients, even in relapsed/refractory disease, although CD138 expression levels were decreased in some with advanced disease. 2) The proliferative potential and percentage of antimyeloma agent-induced apoptosis in SLAMF3^{high} cells were significantly higher and lower than in SLAMF3^{low} cells, respectively. The cell proliferation and drug resistance in SLAMF3-expressing KMS34 cells were promoted in comparison with ΔSLAMF3 and control cells. That malignant potential in MM cells was cancelled by SLAMF3 knockdown. Furthermore, the proliferation of MM cells and resistance to antimyeloma agents were inhibited by anti-SLAMF3 antibody. Adaptor proteins, SHP2 and GRB2, were expressed in MM cell lines, but neither SAP nor EAT-2 were. SLAMF3 interacted directly with SHP2 and GRB2, and SHP2 also interacted with GRB2. SHP2 inhibitor-treated or SHP2/GRB-knockdown cells had characteristics similar to SLAMF3-knockdown cells. 3) The frequency of GG genotypes of SLAMF3 SNP rs509749 in MM patients was 63.6% (n=28), of AG 29.5% (n=13), and of AA 6.8% (n=3). Patients with GG genotypes tended to have shorter overall survival times than patients with AG genotypes. 4) sSLAMF3 levels were significantly higher in symptomatic MM than in asymptomatic MM and markedly increased in advanced MM. MM patients with high levels (≥3.3 ng/ml, n=62) of sSLAMF3 progressed to the

advanced stage significantly more often and had shorter progression-free survival times than those with low levels (<3.3 ng/ml, n=32) (P=0.032).
Summary/Conclusions: This study revealed that SLAMF3 molecules consistently expressed on MM cells may transmit positive signals mediated via the complex of SHP2 and GRB2 by self-ligand interaction between MM cells and induce a high malignant potential in MM. Furthermore, high levels of serum sSLAMF3 may reflect MM disease progression and be a useful prognostic factor in MM. Thus, SLAMF3 molecules may be a new potential target for future immunotherapy and chemotherapy.

P328

TARGETING CD74 IN MULTIPLE MYELOMA WITH A NOVEL ANTIBODY DRUG CONJUGATE, STRO-001

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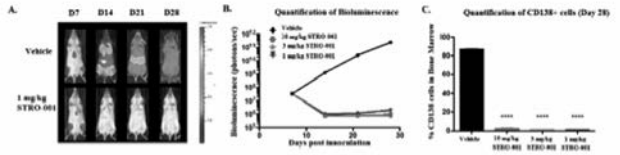
Background: CD74 is a transmembrane glycoprotein involved in MHC protein formation and transport. CD74 expression has been observed in up to 90% of B-cell malignancies, including multiple myeloma (MM), with minimal expression in normal tissues. CD74 is rapidly internalized, making it an attractive target for ADCs. STRO-001 is a novel ADC comprised of an aglycosylated anti-CD74 IgG1 human antibody (SP7219) conjugated covalently to the non-natural amino acid para-azido-methyl-L-phenylalanine (pAMF) with a non-cleavable dibenzocyclooctyne (DBCO)-maytansinoid linker-warhead. Highly efficient site-specific conjugation enabled by novel cell-free antibody production and click chemistry results in a well-defined homogeneous ADC drug product with a drug-antibody ratio (DAR) of 2.
Aims: The *in vitro* cytotoxicity and *in vivo* efficacy of STRO-001 was investigated in MM cell lines and xenografts. An exploratory toxicology study was conducted in a non-human primate model.
Methods: DBCO-Alexa647-conjugated SP7219 staining and flow cytometry were used for detection and quantitation of CD74 expression on MM cell lines. STRO-001 was used to determine the EC_{50} and percent span of killing in MM cell lines. The anti-tumor activity of STRO-001 was evaluated in the disseminated ARP-1 and MM.1S MM models. *In vivo* bioluminescence imaging (BLI) for animals bearing MM.1s-luc cells was performed using an IVIS Spectrum. BLI images were collected 7, 14, 21, and 28 days post-tumor cell inoculation. STRO-001 was administered to cynomolgus monkeys in an exploratory dose-escalating study of repeat IV doses of 1, 3, 10 and 30 mg/kg on days 1 and 15.
Results: *In vitro* cytotoxicity assays show nanomolar potency of STRO-001 in five MM cell lines: MC/CAR (EC_{50} 0.8 nM), ARD (EC_{50} 6.5 nM), MM.1S (EC_{50} 10-11 nM), U266B1 (EC_{50} 8.5-9.3 nM), and ARP-1 (EC_{50} 4.3-22 nM). CD74 cell surface expression is required for STRO-001 cytotoxic activity but expression level, as measured by antibody-binding capacity, does not correlate strongly with *in vitro* potency (R^2=0.5837 for MM cell lines). STRO-001 inhibits the growth of CD138+ plasma cells in bone marrow (BM) and formation of visceral tumors (p=0.002 for kidney; p<0.0001 for ovary) after 4 weekly doses of 3 mg/kg in the ARP-1 disseminated MM xenograft model. STRO-001 dosed at 3 mg/kg and 10 mg/kg weekly x 3 also eradicates malignant BM plasma cells by day 32 post-inoculation (p<0.0001) and prolongs survival in the MM.1S disseminated model. At termination of the study, 129 days post-inoculation, 100% of the STRO-001 treated animals survived and showed no evidence of disease with no CD138+ cells in their bone marrow, while mean survival of vehicle-treated control animals was 35 days with almost 50% of their bone marrow containing myeloma cells. BLI of luciferase-expressing MM.1S (MM.1S-luc) tumor cell lines enabled non-invasive quantitation of tumor burden. Single doses of 1, 3, and 10 mg/kg STRO-001 (administered on day 7 post-inoculation) resulted in eradication of myeloma by day 28 based on bioluminescence signal and quantification of CD138+ cells in bone marrow. In addition, STRO-001 produced a dose-dependent reduction in normal B-cells in cynomolgus monkeys, providing pharmacodynamic evidence of B-cell targeting (Figure 1).



NSG mice inoculated with MM.1S-luc cells received a single dose of vehicle, 1, 3, or 10 mg/kg STRO-001 at 7 days post-inoculation. (A) Representative *in vivo* images of luminescence showing MM.1S-luc cells in the bone marrow of live mice treated with vehicle or 1 mg/kg STRO-001 at different time points. (B) Quantification of bioluminescence in live mice treated with vehicle, 1 mg/kg STRO-001, 3 mg/kg STRO-001 and 10 mg/kg STRO-001. (C) Quantification of CD138+ cells in the bone marrow of mice treated with vehicle, 1 mg/kg STRO-001, 3 mg/kg STRO-001 and 10 mg/kg STRO-001 on day 28 post-inoculation. Asterisks indicate significant difference compared to control (****, p<.0001).

Figure 1.

Summary/Conclusions: STRO-001 demonstrates potent *in vitro* cytotoxicity in MM cell lines and reduces tumor burden in MM xenograft models, including significant prolongation of survival in the MM.1S model. Based on these encouraging observations, STRO-001 is advancing to the clinic for the treatment of CD74-expressing B-cell malignancies.

P329

GENOTYPE CHARACTERIZATION OF LIGHT CHAIN AMYLOIDOSIS BY WHOLE EXOME SEQUENCING

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Background: Immunoglobulin light-chain amyloidosis (AL) is a heterogeneous and multifactorial disease with high genetic complexity. Until now, no common factor o unique mutation associated with this disease has been described. Whole exome sequencing in Multiple Myeloma (MM) patient's tests allowed to known important genes and pathways that are involved in the disease. However, few evidences through next generation sequencing (NGS) analysis were described in AL. Consequently, the application of NGS technologies permits unraveling the genomic landscape of AL to better disentangle the biology of the disease, allowing the identification of new therapeutic targets as in MM.
Aims: Genotype characterization of novel molecular alterations in AL plasma cell by whole-exome sequencing technology.
Methods: We studied 40 paired samples (sorted pathological plasma cells and peripheral blood) from 20 patients with AL. Whole exome and regulatory regions were captured using Agilent's SureSelect Human All Exon V6+UTR kit and sequenced on the Illumina NextSeq 500 platform with pair-end sequencing technique with a global mean depth coverage of 70x, on target coverage of 96.5% and a Phred quality score of 91.3% up to Q30. Data were analyzed with Strelka software to discard germinal mutations, wANNOVAR for functional annotation, and a data reduction strategy to identify candidate variants.
Results: After analysis of patient samples we got an average of 76 (range 18-177) mutations per patient. 28.4% of the mutations was located on regulatory regions (5' UTR, 3' UTR). So far, we did not identify recurrent mutations between the patients, although some patients presented different mutations on the same gene.
The mutation pattern was very heterogeneous between patients. We identified alterations in genes involved in extracellular matrix (MMP2), cell proliferation, differentiation and development (TFGA), transcription factors (ZFHX3, HNRPNPL), adherent junction function (RASSF8), GTPases (RAPGEF2, RAB40A), and genes of the collagenase family (COL9A1, COL1A2) among others.
Summary/Conclusions: Taken together, these results suggest that the mutation pattern in AL is heterogeneous with no common mutated gene among all patients. However, we described novel mutations in the context of AL in regulatory genes or over-representing cancer-related pathways that can help to elucidate the molecular biology of the disease.

Myeloma and other monoclonal gammopathies - Clinical 1

P330

IMPROVED SURVIVAL IN 21,465 MULTIPLE MYELOMA PATIENTS: RESULTS FROM A POPULATION-BASED STUDY

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Background: Multiple myeloma (MM) is generally considered an incurable disease, however advances in the treatment options for MM have been great in recent years. Recent studies on these new agents indicate an improvement in survival, nevertheless population-based studies have had contradicting findings, especially in the elderly patients.
Aims: The aim of the study was to evaluate the survival of all patients diagnosed with MM in Sweden in the years 1973 to 2013 and to relate the survival pattern to trends in treatment strategies.
Methods: Patients diagnosed with MM in the period from January 1, 1973 to December 31, 2013 were identified from the Swedish Cancer Registry. Information on sex, date of birth, date of diagnosis, and date of death was collected. Relative survival ratios (RSRs) were used to provide a measure of excess mortality of MM patients compared to a comparable group from the general population. RSRs with 95% confidence intervals (Cis) were found for 1-, 5-, and 10-years for 4 calendar periods; 1973-1982, 1983-1992, 1993-2002, and 2003-2013 and furthermore for 6 age categories at diagnosis (0-40, 41-50, 51-60, 61-70, 71-80 and >80). Short-term survival, as defined by RSR of less than 3 months, was also defined for all calendar periods.
Results: A total of 21,465 patients (54% males, median age at diagnosis 72 years) with MM were recorded in the time period. Overall, the 1- and 5- and 10-year RSRs improved in the whole period, with the greatest improvement in the two most recent calendar periods. The 1-year RSR increased significantly between all calendar periods (0.69, 0.74, 0.77 and 0.82, respectively). The 5-year RSR increased significantly between the two last calendar periods (0.28, 0.31, 0.33 and 0.41, respectively; Figure 1) as well as the 10-year RSR (0.10, 0.12, 0.14 and 0.20, respectively). Short-term survival increased significantly between the first two and last two calendar periods (the RSR were 0.83, 0.88, 0.89 and 0.93 respectively). Females had a lower excess mortality compared to males (excess mortality ratio 0.91).

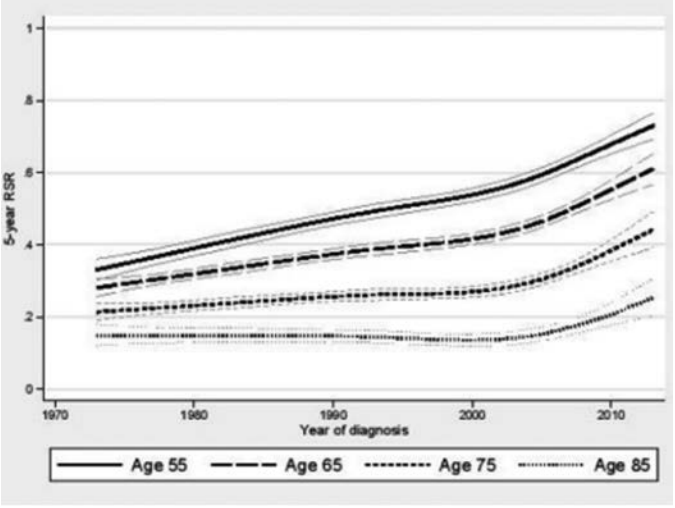

Figure 1.

Summary/Conclusions: In this population-based study, based on more than 21,000 MM patients diagnosed during more than a 40-year period, we showed that with an increased use of novel agents in MM patients, survival has improved significantly. This is especially prominent during the last 10 years. Our findings are important, since new agents are approved based on clinical trials, where certain groups, such as older patients and patients with significant comorbidities are often excluded.

P331

PROGNOSTIC IMPLICATIONS OF MULTIPLE CYTOGENETIC HIGH-RISK ABNORMALITIES IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA

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Background: Cytogenetic evaluation using fluorescence *in situ* hybridization (FISH) at the time of diagnosis is essential for initial risk stratification in multiple myeloma. The presence of specific cytogenetic high-risk abnormalities (HRA) is known to confer a poor prognosis, less is known about the cumulative effect of multiple such abnormalities.
Aims: To evaluate the prognostic implications of the presence of multiple HRA at the time of diagnosis.
Methods: We studied 1181 patients who were diagnosed with multiple myeloma between July 2005 and July 2015 at Mayo Clinic Rochester, underwent FISH evaluation within 6 months of diagnosis, and received first-line therapy with at least 1 novel agent (immunomodulator or proteasome inhibitor). HRA were defined as t(4;14), t(14;16), t(14;20), del(17p), and gain(1q). Bone marrow aspirates were evaluated for deletions, monosomies, trisomies, and tetrasomies using chromosome- or centromere-specific FISH probes. IGH rearrangements were evaluated using an IGH break-apart probe and evaluating up to 5 potential partners (FGFR3, CCND1, CCND3, MAF, and MAFB). Kaplan-Meier overall survival estimates were calculated and the log-rank test was used to compare overall survival in patients with and without HRA (stratified by the number of HRA). A multivariable-adjusted Cox regression model was used to assess the effect of HRA on overall survival adjusting for age, sex, International Staging System (ISS) stage, and first-line therapy (immunomodulator, proteasome inhibitor, upfront autologous hematopoietic stem cell transplantation). Patients diagnosed after 2014 (approximately 15% of the cohort) routinely underwent evaluation for gain(1q), therefore the hazard ratios represent conservative effect estimates. P-values below 0.05 were considered statistically significant.
Results: The median age at diagnosis was 65 years (28 - 95), 708 (60%) of the patients were male. There were 375 HRA in 327 patients (28% of the cohort): 170 (45%) del(17p), 110 (29%) t(4;14), 45 (12%) t(14;16), 8 (2%) t(14;20), and 42 (12%) gain(1q). Of the 280 patients with 1 HRA 130 (46%) had del(17p), 120 (43%) had a high-risk translocation, and 30 (11%) had gain(1q). Of the 46 patients with 2 HRA 34 (76%) had del(17p) and a high-risk translocation, 6 (13%) had a high-risk translocation and gain(1q), 5 (11%) had del(17p) and gain(1q), and 1 had 2 high-risk translocations. There was 1 patient with 3 HRA: del(17p) and t(4;14) and gain(1q). The median overall survival was 6.6 years (6.0 - 8.0) for the entire cohort (n=1181), 8.3 years (6.7 - 8.9) for those without HRA (n=854, 72%), 4.9 years (3.9 - 5.6) for those with one HRA (n=280, 24%), and 2.7 years (2.1 - 3.8) for those with 2 or more (2+) HRA (n=47, 4%). Figure 1 shows the Kaplan-Meier overall survival estimates stratified by the number of HRA (n=1181). The presence of 1 HRA (*versus* 0, HR 1.57, 95% CI 1.26 - 1.96, p <0.001, n=1181) and the presence of 2+ HRA (*versus* 1, HR 3.37, 95% CI 2.21 - 5.14, p <0.001, n=1181) were of prognostic significance after adjusting for age, sex, ISS stage, and first-line therapy. When adjusting for the revised ISS instead of the ISS the hazard was attenuated for 1 HRA (*versus* 0, HR 1.42, 95% CI 1.12 - 1.80, p=0.004, n=1087) and 2+ HRA (*versus* 1, HR 2.82, 95% CI 1.81 - 4.40, p <0.001, n=1087).

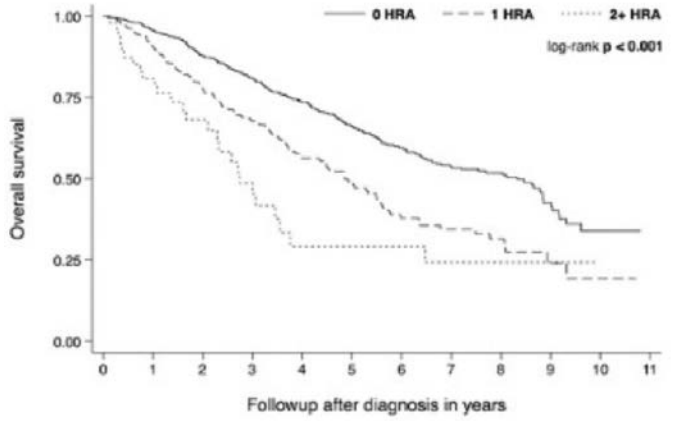

Figure 1.

Summary/Conclusions: Approximately 1 in 4 patients with newly diagnosed multiple myeloma presented with 1 HRA at the time of diagnosis, approximately 1 in 25 with 2 or more HRA. These patients experienced inferior overall survival suggesting a cumulative effect of multiple HRA.

P332

LENALIDOMIDE MAINTENANCE VS PLACEBO AFTER STEM CELL TRANSPLANT FOR PATIENTS WITH MULTIPLE MYELOMA: OVERALL SURVIVAL AND PROGRESSION-FREE SURVIVAL AFTER ADJUSTING FOR TREATMENT CROSSOVER IN CALGB

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Background: At a prespecified interim analysis (December 2009), the phase 3 CALGB/ECOG 100104 (Alliance) study results surpassed the prespecified superiority boundary (significantly improved progression-free survival [PFS] for lenalidomide [LEN] maintenance *vs* placebo [PBO] after SCT) and the majority of PBO arm patients without progressive disease (PD) crossed over to LEN maintenance. An updated analysis (cutoff March 2015), showed significantly longer overall survival [OS] with LEN maintenance (HR, 0.56; 95% CI, 0.42-0.76). However, the crossover from PBO to LEN makes it difficult to assess the true treatment effect of LEN.
Aims: To examine the effect of LEN *vs* PBO on OS and PFS from randomization, adjusting for effects of crossover.
Methods: The rank-preserving structural failure time model (RPSFTM; Robins, *Commun Stat Theory Methods*, 1991) was used for crossover adjustment; the iterative parameter estimation (IPE; Branson, *Stat Med*, 2002) algorithm was used as validation. Survival was partitioned assuming a residual LEN effect after discontinuation. A landmark analysis was also performed at the Dec 2009 interim for patients who remained on treatment. Patients in the trial provided informed consent.
Results: Patients were randomized to LEN maintenance (n=231) and PBO (n=229) (intent-to-treat [ITT] population); 76 patients without PD crossed over from PBO to LEN. The median time from randomization to crossover was 11.5 months. The relative treatment effect for OS and PFS increased for LEN *vs* PBO when adjusting for crossover using RPSFTM and IPE (Table 1). The landmark analysis at the Dec 2009 interim (PBO crossover, n=76; No crossover, n=34) showed the treatment effect is not dissimilar to the ITT analysis (HR 0.53; 95% CI, 0.25-1.13). Sensitivity analyses showed consistent results. Updated data will be presented at the meeting.

Table 1.

	OS[a]	PFS[a]
	LEN (n = 231) vs PBO (n = 229)	
ITT, Unadjusted	NR vs 79.0 0.56 (0.42-0.76)	58.4 vs 28.9 0.58 (0.46-0.73)
RPSFTM	NR vs 70.9 0.48 (0.34-0.69)[b]	58.4 vs 25.8 0.50 (0.39-0.63)[b]
IPE	NR vs 70.9 0.48 (0.33-0.68)[b]	58.4 vs 25.8 0.48 (0.36-0.64)[b]

[a]Data are median, months; HR (95% CI). [b]Bootstrapped 95% CI.

Support: U10CA180821, U10CA180882, CA180820. **ClinicalTrials.gov Identifier:** NCT00114101

Summary/Conclusions: Adjusting for the potential diluting effects of crossover reduced median OS and PFS with PBO, and improved the treatment effect in the ITT analyses for OS and PFS for LEN *vs* PBO maintenance after SCT. The statistical significance of the ITT analyses was maintained throughout.

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UPDATED RESULTS FROM ASPIRE AND ENDEAVOR, RANDOMISED, OPEN-LABEL, MULTICENTRE PHASE 3 STUDIES OF CARFILZOMIB IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background: In RRMM, carfilzomib, lenalidomide, and dexamethasone (KRd) was superior to Rd in ASPIRE (Stewart, N Engl J Med. 2015), and carfilzomib and dexamethasone (Kd) was superior to bortezomib and dexamethasone (Vd) in ENDEAVOR (Dimopoulos, Lancet Oncol. 2016) for the primary endpoint of progression-free survival (PFS) by independent review.
Aims: To report safety and efficacy data after 6-7 months of additional follow-up.
Methods: Adults with RRMM who received 1–3 prior regimens were randomised 1:1. In ASPIRE, patients received lenalidomide (25 mg) on days 1–21 and dexamethasone (40 mg) on days 1, 8, 15, and 22 (28-day cycle). KRd patients received carfilzomib on days 1, 2, 8, 9, 15, and 16 during cycles 1–12 (20 mg/m^2 [days 1 and 2 of cycle 1]; 27 mg/m^2 thereafter); carfilzomib was omitted on days 8 and 9 in cycles 13–18. In ENDEAVOR, Kd patients received carfilzomib (20 mg/m^2 on days 1 and 2 of cycle 1; 56 mg/m^2 thereafter) on days 1, 2, 8, 9, 15, and 16 and dexamethasone (20 mg) on days 1, 2, 8, 9, 15, 16, 22, and 23 (28-day cycle). In the Vd group, bortezomib was given (1.3 mg/m^2; intravenously or subcutaneously) on days 1, 4, 8, and 11, and dexamethasone (20 mg) on days 1, 2, 4, 5, 8, 9, 11, and 12 (21-day cycle). Comparisons were per stratified log-rank test; data presented here are per investigator assessment.
Results: In ASPIRE, 792 patients were randomised. Baseline characteristics were balanced between arms. At a median follow-up of 37.8 months (KRd) and 37.0 months (Rd), median PFS was 26.1 months (KRd) and 16.6 months (Rd) (hazard ratio [HR]: 0.67; 95% confidence interval [CI]: 0.56–0.80; P <0.0001); 18-month PFS rates were 64.5% (KRd) and 46.6% (Rd). Median time to progression (TTP) was 30.5 months (KRd) and 18.9 months (Rd) (HR: 0.62; 95% CI: 0.51–0.76; P <0.0001). Median time to next treatment (TTNT) was not estimable (KRd) and 24.3 months (Rd) (HR: 0.62; 95% CI: 0.50–0.77; P <0.0001). 16.8% (KRd) and 19.0% (Rd) of patients discontinued because of adverse events (AEs). Grade ≥3 AE rates were 5.9% and 2.2% for hypertension, 3.9% and 1.8% for cardiac failure, and 4.6% and 5.4% for peripheral neuropathy for KRd and Rd, respectively. In ENDEAVOR, 929 patients were randomised. Baseline characteristics were balanced between arms. At a median follow-up of 19.4 months (Kd) and 17.7 months (Vd), median PFS was 17.6 months (Kd) and 9.4 months (Vd) (HR: 0.53; 95% CI: 0.44–0.63; P <0.0001); 18-month PFS rates were 48.7% (Kd) and 23.9% (Vd). Median TTP was 19.4 months (Kd) and 10.2 months (Vd) (HR: 0.50; 95% CI: 0.42–0.60; P <0.0001). Median TTNT was 26.1 months (Kd) and 14.5 months (Vd) (HR: 0.49; 95% CI: 0.40–0.60; P <0.0001). 15.8% (Kd) and 14.9% (Vd) of patients discontinued because of AEs. Grade ≥3 AE rates were 13.8% and 3.3% for hypertension, 5.2% and 2.0% for cardiac failure, and 2.4% and 8.6% for peripheral neuropathy for Kd and Vd, respectively.
Summary/Conclusions: Consistent with the primary analyses, these results show that incorporation of carfilzomib into treatment regimens in patients with RRMM results in clinically meaningful improvements in PFS and a favourable benefit-risk profile.

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EFFICACY AND SAFETY OF DARATUMUMAB, LENALIDOMIDE, AND DEXAMETHASONE VERSUS RD ALONE IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA: UPDATED ANALYSIS OF POLLUX

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Background: Daratumumab, a human monoclonal antibody targeting CD38, significantly prolongs progression-free survival (PFS) and achieves deep and durable responses when combined with other established standard-of-care regimens in patients with RRMM.
Aims: To provide updated efficacy and safety data from POLLUX, a multicenter, phase 3, randomized study of DRd *versus* Rd in RRMM.
Methods: Eligible patients with ≥1 prior line of therapy were randomly assigned to Rd (25 mg PO lenalidomide on Days 1-21 of each every-4-week [Q4W] cycle; 40 mg dexamethasone weekly) with or without daratumumab (16 mg/kg IV once weekly for Cycles 1 and 2, every 2 weeks for Cycles 3-6, then Q4W until disease progression). Patients who were refractory to lenalidomide were excluded. Progression-free survival (PFS) was the primary endpoint. Bone

marrow samples were collected, and minimal residual disease (MRD) was assessed at the time of suspected complete response (CR) and at 3 and 6 months after suspected CR at 3 different sensitivity thresholds (10^{-4}, 10^{-5}, and 10^{-6}) using the ClonoSEQ™ next-generation sequencing-based assay (Adaptive Biotechnologies, Seattle, WA). Additional reflex testing using an anti-idiotype antibody was used to confirm CRs in cases in which daratumumab interference with serum M-protein quantitation was suspected in patients with possible CR.

Results: Patients received a median (range) of 1 (1-11) prior lines of therapy; 55% of patients had received immunomodulatory agents (IMIDs), and 18% had been exposed to lenalidomide. After median follow-up of 17.3 months, DRd significantly prolonged PFS compared with Rd alone (median: not reached *vs* 17.5 months; hazard ratio [HR], 0.37; 95% confidence interval [CI], 0.28-0.50; *P*<0.0001), with 18-month PFS rates of 76% and 49%, respectively. Responses continued to deepen in the DRd group with longer follow-up, with significantly higher overall response rate (ORR; 93% *vs* 76%) and rates of very good partial response (VGPR) or better (78% *vs* 45%) and CR or better (46% *vs* 20%) with DRd *versus* Rd alone (*P*<0.0001 for all). MRD-negative rates were >3 times higher with DRd compared with Rd alone at all 3 sensitivity thresholds (31.8% *vs* 8.8% at 10^{-4}; 24.8% *vs* 5.7% at 10^{-5}; and 11.9% *vs* 2.5%, at 10^{-6}; *P*<0.0001 for all), and MRD negativity was associated with prolonged PFS at 10^{-5} (Figure 1). Overall survival (OS) data are immature, with 40 (14%) deaths in the DRd group and 56 (20%) deaths in the Rd group (HR, 0.63; 95% CI, 0.42-0.95). Neutropenia was the most common grade 3 or 4 treatment-emergent adverse event (53% with DRd *vs* 38% with Rd), and no new safety signals were reported with longer follow up. We will present updated efficacy and safety data based on approximately 25 months follow up at the meeting.

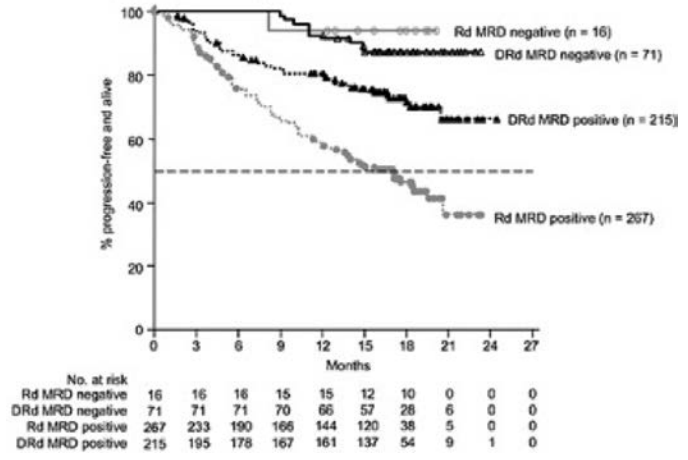

Figure 1.

Summary/Conclusions: DRd significantly improved outcomes compared with Rd alone, including PFS, ORR, depth of response, and MRD-negative rates, with a favorable safety profile that was maintained after longer follow up. These updated data continue to support the use of DRd in patients with RRMM who received ≥1 prior therapy.

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DARATUMUMAB-BASED COMBINATION REGIMENS IN ELDERLY (≥75 YEARS) PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA: SUBGROUP ANALYSIS OF THE PHASE 3 CASTOR AND POLLUX STUDIES

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Background: Daratumumab (D) used in combination with bortezomib and dexamethasone (Vd; CASTOR) or lenalidomide and dexamethasone (Rd; POLLUX) significantly prolongs progression-free survival (PFS) with a manageable safety profile compared with either Vd or Rd alone in patients (pts) with RRMM.

Aims: Here in this subgroup analysis we investigated the safety and efficacy of DVd and DRd in elderly pts aged ≥75 years from the CASTOR and POLLUX phase 3 studies.

Methods: Overall, pts enrolled in the CASTOR and POLLUX studies had ≥1 prior line of therapy. Pts in CASTOR received up to 8 cycles of Vd with or without D; pts in the DVd group then continued to receive D monotherapy q4w until disease progression or unacceptable toxicity. Pts in POLLUX were treated until progression. Dosing schedules for D (16 mg/kg) were different between CASTOR (qw in Cycles 1-3, q3w for Cycles 4-8, and q4w thereafter) and POLLUX (qw for Cycles 1-2, q2w for Cycles 3-6, and q4w thereafter). All elderly pts received a reduced dose of dexamethasone (20 mg once weekly *vs* 40 mg once weekly) in both studies.

Results: In CASTOR, 23/251 pts in the DVd group and 35/247 pts in the Vd group were ≥75 years; the median (range) age for this group of pts was 78 (75-88) and 78 (75-85) years, respectively, with 100% and 94% with an ECOG status ≤1. At a median follow-up of 13.0 months, discontinuation rates due to treatment-emergent adverse events (TEAEs) were similar with DVd and Vd (15% *vs* 20%). Common (≥10%) grade 3/4 TEAEs for DVd were thrombocytopenia (45% *vs* 37% with Vd), fatigue (15% *vs* 11%), pneumonia (15% *vs* 17%), and anemia (10% *vs* 11%). Infusion-related reactions (IRR) occurred in 13 (65%) pts, with 10% having grade 3/4 IRR, but no pts discontinued due to IRR. Median PFS was significantly prolonged with DVd *versus* Vd (not reached [NR] *vs* 8.1 months; hazard ratio [HR], 0.27; 95% confidence intervals [CI], 0.12-0.61; P=0.0007), consistent with the overall PFS observed in CASTOR (Figure). Higher overall response rate (ORR; 95% *vs* 79%) and rates of complete response (CR) or better (25% *vs* 3%) and very good partial response (VGPR) or better (70% *vs* 18%) were achieved with DVd *versus* Vd, respectively, consistent with the overall population. In the POLLUX study, 29/286 pts in the DRd group and 35/283 pts in the Rd group were aged ≥75 years; the median (range) age for this group of pts was 77 (75-89) and 78 (75-87) years, respectively, with 86% and 91% with an ECOG status ≤1. At a median follow-up of 17.3 months, 10% of pts in the DRd group and 11% in the Rd group discontinued due to TEAEs. Common (≥10%) grade 3/4 TEAEs for DRd were neutropenia (45% *vs* 31% with Rd), hypokalemia (14% *vs* 3%), and pneumonia (10% *vs* 11%). D-associated IRR occurred in 12 (41%) pts in the DRd group, with 4 (14%) pts having grade 3/4 IRR. No patient discontinued DRd because of IRR. Median PFS was significantly prolonged with DRd compared with Rd in the elderly subgroup (NR *vs* 11.4 months; HR, 0.19; 95% CI, 0.06-0.55; P=0.0007), consistent with the overall PFS observed in POLLUX (Figure 1). ORR was higher with DRd *versus* Rd (93% *vs* 77%), and rates of CR or better (52% *vs* 9%) and VGPR or better (72% *vs* 41%) were also higher with DRd *versus* Rd.

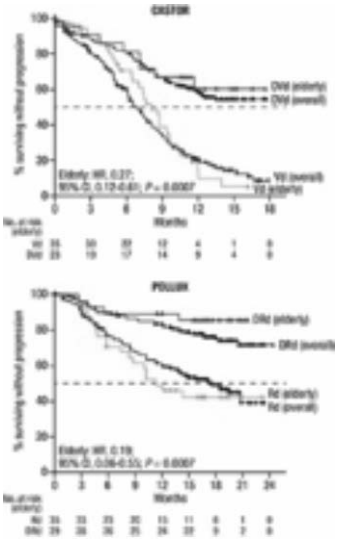

Figure 1.

Summary/Conclusions: The results in elderly pts were consistent with those observed in the overall study populations in terms of efficacy. Rates of most common grade 3/4 hematologic TEAEs in elderly pts were similar to that of the overall populations, and IRR were manageable. This subgroup analysis supports the addition of D to standard-of-care regimens in elderly pts with RRMM.

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ALL ORAL COMBINATION OF IXAZOMIB PLUS THALIDOMIDE AND DEXAMETHASONE FOR RELAPSED OR REFRACTORY MULTIPLE MYELOMA: INTERIM DATA OF AN ONGOING PHASE II TRIAL

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Background: Ixazomib is a novel, effective oral proteasome inhibitor with a favorable toxicity profile. Recent studies showed significant activity as single agent with dexamethasone and in combination with other agents. The Tourmaline trial showed superior PFS with ixazomib plus lenalidomide and dexamethasone in pts with relapsed or refractory myeloma (RRMM).
Aims: Here, we evaluate the activity and tolerability of ixazomib plus thalidomide and dexamethasone (IxaThalDex) in pts with RRMM.
Methods: Pts with RRMM and one or more prior lines of therapy (TX) with the following criteria were eligible: Measurable disease, ECOG PS ≤2, ANC ≥1000/μL, platelet count ≥50000μL, GFR ≥15mL/min. Treatment regimen: Ixazomib (4mg, d 1, 8 and 15), thalidomide (100mg/d), and dexamethasone (40mg d 1, 8, 15). Pts aged ≥75 years received lower doses of thalidomide (50mg/d) and of dexamethasone (20mg). Pts were scheduled for 8 cycles followed by ixazomib maintenance therapy (4mg, days 1, 8, 15 of a 28 cycle and 3mg in pts aged ≥75 years) for one year. Primary objective was PFS, and secondary objectives were ORR, OS, impact of cytogenetic risk and of renal impairment, safety and health related QoL
Results: Sixty-seven of 77 planned pts have been enrolled so far. The following patient characteristics were recorded in the intent-to treat group (ITT): median age: 67, range 41 to 84 years, ISS stage I: 28, II 22, III: 16, not known: 1, median number of prior TX lines: 1 (range: 1-8). 9 pts discontinued TX before completion of 2 cycles. Presently, 5 pts are too early for evaluation per protocol (PP). Full documentation of ≥2 cycles is available for 52 pts, with a median number of 4 cycles and a median F/U of 7.4 mos. A PR or better was achieved in 33 pts (63%), nCR: 2 pts (4%), VGPR: 10 (19%), PR: 21 (40%), MR: 2 (4%), yielding a clinical benefit rate (CBR) of 67%. FISH data are available in 43 of the 52 PP pts. ≥PR was seen in 11/18 (61%) pts with t (4; 14) and/or t (14; 16) and/or del17p and in 15/25 (60%) with standard risk cytogenetics. Median PFS at the time of reporting is 11.6 mos. in the ITT and 10.4 mos. in the PP group. Median OS has not been reached in neither group. Patients with high-risk cytogenetics did not show statistically significant differences in PFS (10.4 mos. *vs* 10.3 mos., p=0.882) and OS (11.6 mos *vs* median n.r., p=0.061). Global health status/QoL as assessed by EORTC QLQ C-30 instrument revealed clinical meaningful (>5 points) improvement in QoL, which was slightly more pronounced in pts with ≥PR (Figure1). Neutropenia was noted in 19 pts (28%), (15 grade 2 and 4 grade 3). Leucopenia was seen in 19 pts (28%), (14 grade 2 and 5 grade 3). 32 pts (47%) had anemia (23 grade 1/2 and 9 grade 3). Thrombocytopenia was recorded in 11 pts (14%, 4 grade 2 and 6 grade 3). The most frequent non-hematological toxicities were fatigue observed in 21 pts (32%) and infections noted in 27 pts (including 6 pts with pneumonia and 1 pt with sepsis). Polyneuropathy was seen in 19 pts (28%, 18 grade 1 or 2, one grade 3). During the study, the incidence of new PNP was relatively low (17 new and two worsening PNP).

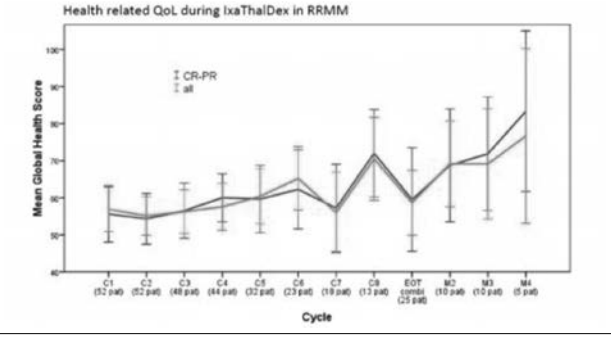

Figure 1.

Summary/Conclusions: The all oral IxaThalDex regimen showed an ORR of 63% with no difference in pts with/without high-risk cytogenetics, a CBR of 67%, and a PFS of 10.4 mos in pts with RRMM. The regimen was well tolerated and was associated with a low incidence of mainly grade ≤2 PNP, which required dose reduction in one patient only. Response rates improved with continuation of therapy and treatment was associated with an increase in health related QoL.

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EVALUATION OF GROWTH DIFFERENTIATION FACTOR-1 (GDF15) AS A NEW BIOMARKER FOR RENAL OUTCOMES IN DIFFERENT COHORTS OF PATIENTS WITH LIGHT CHAIN AMYLOIDOSIS

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Background: Growth differentiation factor-15 (GDF-15), is a member of the TGF-beta family, and is involved in several pathological conditions, including inflammation, cancer, cardiovascular, pulmonary and renal diseases. Serum GDF-15 levels add prognostic information to conventional prognostic factors, such as NT-proBNP and troponins, in cardiovascular disorders and has also shown to be associated with renal damage and risk of end stage renal disease in patients with diabetes. Increased serum GDF-15 levels have also shown to be correlated with early death and shorter survival independently of other cardiobiomarkers and Mayo stage. Because GDF-15 was also associated with renal outcomes we evaluated the prognostic value of GDF-15 levels in two independent cohorts of patients with AL amyloidosis and renal involvement who were treated in two different centers (Pavia Amyloidosis Center and Department of Clinical Therapeutics, Athens).
Aims: To evaluate the prognostic value of GDF-15 levels in independent cohorts of patients with AL amyloidosis and renal involvement .
Methods: Circulating levels of GDF-15 were measured by a novel pre-commercial immunoassay (Roche Diagnostics) in stored serum. The Pavia cohort included 135 and the Athens cohort included 76 patients with AL amyloidosis and renal involvement. Standard criteria were used for the diagnosis, evaluation of organ involvement and cardiobiomarker-based risk stratification. Renal staging was based on the system proposed by Palladini *et al.*, based on baseline proteinuria >5 gr/day and eGFR <50 ml/min.
Results: Median age and involved FLC levels were similar between the two cohorts. However, heart involvement was more common in Pavia cohort (72% *vs* 53% f, p=0.005). Mayo stage disposition was also different (17%, 46% & 37% for stage 1,2 & 3 in Pavia *vs* 30%, 43% & 27% in Athens cohort, p=0.08, but stage 3B was similar, 13% *vs* 12%). Also there were differences in peripheral nerve involvement (9% in Pavia *vs* 21% in Athens cohort, p=0.025). Median eGFR and renal stage distribution (26%, 54%, 20% *vs* 20%, 54%, 26% for renal stage-1, 2 & 3 respectively) were similar between the two cohorts (p=0.544). Median follow up for the Pavia cohort was 18 months and for the Athens cohort was 45 months (p<0.001). Survival at 2 years was 59% for Pavia and 56% for Athens cohort. Median GDF-15 levels was 3454 pg/ml in Pavia (range 624 to >100000) and 4152 pg/ml (range 626 – 71475) in Athens cohort (p=0.09), while 93% and 94% of patients in the two cohorts had GDF-15 levels >1200 pg/ml (the upper limit of normal for individuals without cardiovascular disease). We then evaluated the prognostic significance regarding renal outcomes (dialysis): GDF-15 >4000 pg/ml was associated with a HR of 6 (95% CI 2015.6, p=0.001) in Athens cohort (progression to dialysis within 2 years in 7% *vs* 47%); while, by applying the same cutoff in patients in Pavia cohort, 2-year dialysis rate was 10% *vs* 37% (HR: 3, 95% CI 1.6-15, p=0.004). Although renal stage discriminated 3 groups in univariate analysis in each cohort, in multivariate analysis, GDF-15 >4000 pg/ml outperformed renal stage by eGFR and proteinuria and was the only independent prognostic factor for progression to dialysis in each cohort (Figure 1).

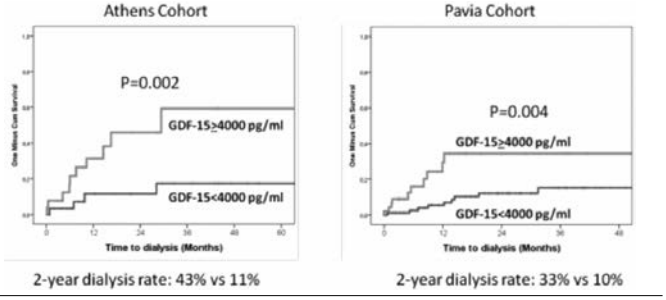

Figure 1.

Summary/Conclusions: Our study validated and confirmed in two independent cohorts, with differences in their characteristics, the prognostic value of GDF-15, which emerges as a novel biomarker with prognostic implications for different outcomes in patients with AL amyloidosis. Importantly, GDF-15 emerges as a strong biomarker for renal outcomes in patients with AL amyloidosis.

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AN OPEN-LABEL, PHASE 2 STUDY TO EVALUATE THE ORAL COMBINATION OF IXAZOMIB, CYCLOPHOSPHAMIDE AND DEXAMETHASONE IN TRANSPLANT-INELIGIBLE PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA

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Background: Proteasome inhibitor (PI)-based combinations are standards of care in all lines of MM therapy. As the treatment paradigm moves to focus more on extended therapy, new combinations are needed that will be efficacious and tolerable, while giving pts the flexibility of taking their treatment at home. Combinations of ixazomib, the first oral PI, with immunomodulatory drugs (IMiDs) are feasible and effective; however, there may be pts for whom the use of IMiDs is not desirable. Therefore, triplet combinations of ixazomib with alkylators have been studied.
Aims: This phase 2 study (NCT02046070) evaluated the safety and efficacy of the all-oral ICd regimen in transplant-ineligible pts with NDMM. Primary endpoint was rate of CR+VGPR during induction. Secondary endpoints included tolerability and toxicity, overall response rate (CR+VGPR+PR) throughout treatment, time to response, PFS, and quality of life (QoL).
Methods: Adult pts with NDMM who were transplant-ineligible were randomized (1:1) to receive oral ixazomib 4.0 mg plus oral cyclophosphamide 300 mg/m^2 (Arm A) or 400 mg/m^2 (Arm B) on days 1, 8, and 15, and dexamethasone 40 mg on days 1, 8, 15, and 22, for up to 13 x 28-day cycles as induction. Pts with ≥SD and an acceptable toxicity profile then received single-agent ixazomib maintenance therapy until PD, death, or unacceptable toxicity.
Results: 70 NDMM pts were enrolled (n=36 Arm A; n=34 Arm B): median age 73 years (range 61–87); 47% male; 31%/33%/29% ISS stage I/II/III MM; 50% had a cardiovascular/pulmonary comorbidity; 9% had high-risk cytogenetics (t(4;14), t(14;16), del 17p). At data cut-off (29 June 2016), pts had received a median of 19 cycles; 66% had completed 13 ICd induction cycles and proceeded to ixazomib maintenance therapy; 10% were ongoing on therapy, and 53% had discontinued due to AEs (24%), PD (16%), patient withdrawal (3%), or other reasons (10%). Confirmed responses by investigator assessment are shown in the Table 1. Median time to first/best response across arms was 2/4 months. After a median follow-up of 17.9/18.5 months in Arm A/B, median PFS was not reached. Combined PFS at 12/18/24 months was 81%/66%/59% (24-month PFS 64%/56% for Arm A/B). In Arm A/B, 94%/100% reported AEs; 72%/74% reported grade ≥3 AEs; and 47%/56% reported SAEs. The most common all-grade AEs were neutropenia (22 [31%]), anemia (19 [27%]), diarrhea, nausea, peripheral edema (each 18 [26%]), vomiting (15 [21%]), fatigue, and constipation (each 14 [20%]). The most common grade ≥3 AEs were neutropenia (22 [31%]), anemia (10 [14%]), lower respiratory tract and lung infections (9 [13%]), and supraventricular arrhythmias (5 [7%]). There were 5 on-study deaths, none considered related to treatment. QoL (by EORTC QLQ-C30; Global Health Status) was maintained from baseline during the study.

Table 1.

Confirmed responses by investigator assessment						
	Arm A (n=33)		Arm B (n=34)		Arm A + Arm B (N=67)	
Response, n (%)	Induction	Overall	Induction	Overall	Induction	Overall
CR	4 (12)	5 (15)	3 (9)	4 (12)	7 (10)	9 (13)
sCR	1 (3)	2 (6)	0	0	1 (1)	2 (3)
PR	22 (67)	22 (67)	21 (62)	20 (59)	43 (64)	42 (63)
VGPR	5 (15)	7 (21)	5 (15)	6 (18)	10 (15)	13 (19)
SD	4 (12)	6 (18)	6 (18)	6 (18)	10 (15)	12 (18)
PD	0	0	1 (3)	2 (6)	1 (1)	0
CR+VGPR	9 (27)	12 (36)	8 (24)	10 (29)	17 (25)	22 (33)
CR+VGPR+PR	26 (79)	27 (82)	24 (71)	24 (71)	50 (75)	51 (76)
CR, complete response; sCR, stringent complete response; PR, partial response; VGPR, very good partial response; SD, stable disease; PD, progressive disease						

Summary/Conclusions: Based on this phase 2 study, ICd is an active treatment regimen for pts with NDMM who are ineligible for transplant. This trial captured a population of pts that was elderly and with multiple comorbidities. In this context, the results with ICd, an all-oral triplet including a PI and alkylator, provide evidence of clinical efficacy with a manageable safety profile. With a median follow-up of ~18 months, median PFS was not reached and outcomes appear comparable to other regimens in elderly transplant-ineligible pts with NDMM. The preferred cyclophosphamide dose for ICd phase 3 studies is 300 mg/m^2, based on the similar PFS, higher response rate, and numerically lower rate of AEs *vs* 400mg/m^2. Updated PFS results will be presented at the meeting.

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THE ORAL PROTEASOME INHIBITOR IXAZOMIB IN COMBINATION WITH MELPHALAN-PREDNISONE FOR PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA: PHASE 1/2 DOSE-ESCALATION STUDY (NCT01335685)

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Background: Bortezomib-MP is a standard-of-care regimen for elderly NDMM pts. Whereas bortezomib is administered IV or SC, ixazomib is an oral proteasome inhibitor with a safety profile amenable to extended dosing that is approved in the US and EU, in combination with lenalidomide-dexamethasone, for the treatment of MM pts who have received at least 1 prior therapy. Based on the demonstrated feasibility and efficacy of a proteasome inhibitor-MP combination, the all-oral ixazomib-MP (IMP) regimen was evaluated in elderly, transplant-ineligible NDMM pts.
Aims: Primary phase 1 objectives were to determine the safety, MTD, and recommended phase 2 dose (RP2D) of ixazomib in combination with MP. The primary phase 2 objective was to determine the rate of CR+VGPR; secondary objectives included PFS and OS.
Methods: In phase 1, pts were enrolled to 4 arms – Arm A: ixazomib 3.0–3.7 mg (days 1, 4, 8, 11, 22, 25, 29, 32) plus M 9 mg/m^2 and P 60 mg/m^2 (days 1–4) in 42-day cycles (max 9 cycles); Arm B: ixazomib 3.0–4.0 mg (days 1, 8, 15) plus M 6 mg/m^2 and P 60 mg/m^2 (days 1–4) in 28-day cycles (max 13 cycles); Arm C/D: ixazomib 3.0–4.0 mg (days 1, 8, 15, 22, 29)/ixazomib 4.0 mg (days 1, 8, 22, 29) plus M 9 mg/m^2 and P 60 mg/m^2 (days 1–4) in 42-day cycles (max 9 cycles). In phase 2, an expansion cohort was enrolled at the RP2D. On all arms, after IMP induction, pts could receive maintenance with single-agent ixazomib (days 1, 8, 15; 28-day cycles).

Table 1.

	All patients	RP2D
Response rates, %	N=53	n=23
Overall response rate (ORR)	66%	65%
At least very good partial response (≥VGPR)	43%	43%
Complete response [CR] (stringent CR)	26% (19%)	22% (17%)
Median time to response, mos		
First response	1.7	1.9
≥VGPR	3.7	3.7
CR	11.6	9.5
Median time to progression (TTP), mos	24.6	18.4
Median progression-free survival (PFS), mos	23.5	18.4
Common AEs, all grades (grade ≥3), %	N=61	n=26
Thrombocytopenia	77% (49%)	62% (27%)
Neutropenia	62% (44%)	46% (23%)
Infections SOC	70% (13%)	69% (12%)
Diarrhea	62% (11%)	65% (15%)
Peripheral neuropathy NEC	39% (5%)	42% (4%)
Peripheral edema	30% (0%)	27% (0%)
Rash NEC	28% (7%)	23% (4%)
AE, adverse event; NEC, not elsewhere classified (high-level term); RP2D, recommended phase 2 dose; SOC, system organ class		

Results: 61 pts were enrolled, 11, 34, 11, and 5 to Arms A, B, C, and D (median age 74 yrs; 31% ISS stage III, 56% creatinine clearance ≤60 mL/min). Among

38 DLT-evaluable pts in phase 1, 10 had DLTs of Gr 3 rash (n=2, Arm A), Gr 3-4 thrombocytopenia (n=4, 1 pt in each arm), Gr 3-4 neutropenia (n=1, Arm A; n=4, Arm C, n=1, Arm D), Gr 4 hemorrhagic oesophageal ulcer (n=1, Arm B), Gr 3 ileus/neurogenic bladder (n=1, Arm B), Gr 3 vomiting/diarrhea (n=1, Arm B), and Gr 3 respiratory infection (n=1, Arm C). The RP2D was ixazomib 4.0 mg in Arm B, based on observed rates of toxicity; this cohort was expanded to 26 pts. Among all 61 pts, the median number of treatment cycles was 16; 36 pts (13 at RP2D) completed IMP induction and entered maintenance. Median number of maintenance cycles was 12. The maximum treatment duration was 1841 days (>5 yrs) at RP2D. Five pts remain on treatment (2 at RP2D); primary reasons for discontinuation were disease progression (48%) and adverse events (AEs, 21%). CR+VGPR rate was 43% (43% at RP2D), including 28% (22%) ≥CR and 19% (17%) sCR; median time to first response was 1.7 mos, and responses continued to mature over a long period (Table 1). Depth of response improved during ixazomib maintenance in 9/36 (25%) pts (VGPR to sCR in 5 pts; VGPR to CR in 2 pts; CR to sCR in 2 pts). Median TTP/PFS are shown in Table 1; median OS was not reached after median follow-up of 42.6/46.9 mos overall/at RP2D.

Summary/Conclusions: The RP2D was weekly ixazomib 4.0 mg plus M 6 mg/m^2 and P 60 mg/m^2 (days 1–4) in 28-day cycles, consistent with the ixazomib dose and schedule in TOURMALINE-MM1. AEs were mainly hematologic, infections, PN, and diarrhea. The all-oral IMP regimen is active in NDMM, with a 28% CR rate (19% sCR), a 43% ≥VGPR rate, and a median PFS of 23.5 mos; responses continued to improve over a prolonged period.

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FEASIBILITY AND EFFICACY OF DOSE ADJUSTED MELPHALAN – PREDNISONE – BORTEZOMIB IN PATIENTS ≥75 YEARS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA; PRELIMINARY RESULTS OF THE PHASE II HOVON 123 STUDY

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Background: There is a high rate of toxicity-related discontinuation in elderly patients with NDMM, negatively affecting outcome. In order to predict feasibility of treatment the IMWG developed the frailty score based on age, (instrumental) Activities of Daily Living and the Charlson comordity index.
Methods: Patients were treated with 9 cycles of MPV: Mel 6 mg/m^2, day 1-4; Pred 30 mg/m^2, day 1-4; and Bort 1.3 mg/m^2 day 1,8,15 and 22 of a 35-day cycle. This first planned analysis was restricted to the first 140 consecutive patients out of 240 planned patients.
Results: Of the 139/140 eligible patients none were fit (because of age ≥75 years), 30/139 (22%) were unfit, 100/139 (72%) were frail, and 9/139 (6%) unknown. The median follow up was 17.0 months. The discontinuation rate of MPV in the total population was 42%; 27% in unfit and 46% in frail patients (p=0.09). When also patients were included who discontinued bortezomib only these numbers were 27% in unfit and 52% in frail (p=0.02). Importantly, 6 cycles of MPV were found to be feasible in 70% of patients, both in unfit (80%) and frail (69%) patients. Age >80 years was associated with a significantly higher discontinuation rate of MPV or bortezomib only (70% *versus* 35% in patients aged 75-80 years, p=0.01). WHO performance was not associated with discontinuation rate. Response on protocol was ≥PR 73%, ≥VGPR 38% and ≥CR 11%, not significantly different in unfit *versus* frail patients. Response after 6 cycles was ≥PR 69%, ≥VGPR 35% and ≥CR 2%. Median progression free survival (PFS) was 17 months: 20 for unfit and 16 months for frail patients (p=0.13). Overall survival at 18 months was 76%: 89% for unfit and 72% for frail patients (p=0.22). Frail patients were found to have significantly less grip strength and lower walking speed as compared to unfit patients (Table 1).

Table 1.

	Unfit patients n=30	Frail patients n=100
Median age (range)	77 (75-80)	80 (75-91)
WHO (%)[1]		
0	57	22
1	37	41
2	3	23
3	3	14
ISS (%)[2]		
I	20	12
II	70	32
III	10	51
unknown		5
Grip strength (kg) *		
number of patients	n=29 (1 missing)	n=93 (7 missing)[3]
Low	17%	41%
Intermediate	21%	35%
High	62%	24%
Walking speed (m/s) **		
% of patients	n=29 (1 missing)	N=87 (13 missing)[4]
Low	10%	41%
Intermediate	24%	36%
High	66%	23%

[1] WHO performance significantly less in frail p=0.001 [2] ISS significantly higher in frail p<0.001[3] Grip strength significantly less in frail p=0.001 [4] Walking speed significantly less in frail p<0.001 *Average grip strength of maximum 3 attempts by dominant hand: low indicates ≤ 26.0 kg in men and ≤ 13.3 kg in women; high, ≥ 33.3 kg in men and≥ 21.1 kg in women; and intermediate otherwise, based on tertiles ** Average walking speed of maximum 2 "runs": low indicates ≤ 0.76 m/s in men and ≤ 0.6 m/s in women; high, > 1.0 m/s in men and women; and intermediate otherwise, based on tertiles

However, 58% and 59% of frail patients had an intermediate or high walking speed and grip strength respectively. Vice versa, 8% of patients with low

walking speed and 12% of patients with low grip strength, were not frail but unfit according to the IMWG frailty index. Discontinuation rate in patients with highest and lowest walking speed was 16/41 (39%) and 18/41 (44%). These numbers were 14/42 (33%) *versus* 17/43 (40%) for patients with the highest *versus* the lowest grip strength. PFS was not significantly different between patients with highest *versus* lowest walking speed (p=0.38). However, in contrast to comparable PFS in unfit and frail patients, there was a trend for better PFS in patients with highest *versus* lowest grip strength (20 *versus* 17 months, p=0.05).
Summary/Conclusions: Nine cycles of dose-adjusted MPV results in a high discontinuation rate of 42% in NDMM patients ≥75 years: 27% in unfit *versus* 46% in frail patients. Importantly, 6 cycles of MPV were found to be feasible with comparable response rate, also in frail. Preliminary analyses showed that functional geriatric assessments differed within IMWG frailty groups and that grip strength was associated with PFS, whereas frailty was not. Therefore, functional assesments will hopefully be complementory to the IMWG frailty score in guiding future therapy in unfit and frail patients.

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THE EUROPEAN MYELOMA NETWORK EMN09 STUDY: CARFILZOMIB, BENDAMUSTINE, AND DEXAMETHASONE IS EFFICIENT AND SAFE IN PATIENTS WITH ADVANCED MULTIPLE MYELOMA

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Background: Even after a prolonged treatment patients with multiple myeloma may have received little chemotherapy, however many may suffer from bortezomib induced peripheral neuropathy (PN). Bendamustine (Benda) leads to increased levels of defective ribosomale products (DRiPs). Carfilzomib (Carf), a proteasome inhibitor not inducing PN, inhibits degradation of DRiPs leading to plasma cell apoptosis.
Aims: With this scientific rationale a CBd combination of Carf and Benda and low dose dexamethasone (dex) was evaluated in a phase 1/2 trial in patients with relapsed/refractory multiple myeloma (RRMM).
Methods: Sixty-three patients with RRMM with ≥2 lines of prior therapy were enrolled with the last patient included in February 2017. Treatment consisted of 28-day cycles of Benda 70 mg/m^2 on day 1 and 8, Carf was given on day 1, 2, 8, 9, 15, 16 at 27 mg/m^2 after an initial dose of 20 mg/m^2. In 6 patients in the phase 1 part of the trial Carf was escalated to 36 mg/m^2. This was found to be the MTD. 20 mg dex was added on every treatment day and day 22 and 23. After 8 cycles, responding patients received maintenance only every 14 days with Carf for two days plus dex until progression.
Results: The phase 1 part of the trial suggested Carf at the 27 mg/m^2 level for the phase 2 part. Forty-one patients were evaluated for response and efficacy. At last data cut-off, the median follow-up was 5.95 months. Number of prior treatment lines ranged from 2 to 9, and ≥85% of patients had received previously transplantation, bortezomib and immunomodulatory drugs. The median time elapsed from diagnosis to treatment start was 5.8 years. Forty-three percent of patients achieved at least a PR including 28% ≥VGPR and an overall benefit of 92%. Median progression-free survival was 11.4 months and the 1-year overall survival was 75%. Hematological toxicity was well manageable. Non-hematological adverse events ≥grade 3 were infections, followed by dyspnea, cardiac events and thromboembolism. These data will be up-dated before the meeting.
Summary/Conclusions: In this elderly RRMM patients treated late in their disease, the combination of CBd provides effective outpatient therapy. Neither nausea, hair loss nor PN were an issue. Although cardiopulmonary as well as vascular signs need attention, CBd appears a very tolerable treatment option. In this heavily immunosuppressed patient population infection prophylaxis is mandatory.
Clinical trial information: NCT02056756.

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CHEMOTHERAPY BEFORE AND AFTER HEART TRANSPLANTATION FOR PATIENTS WITH ADVANCED CARDIAC AL AMYLOIDOSIS, SINGLE CENTER RESULTS WITH LONG-TERM FOLLOW-UP

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Background: Survival rates for patients with light-chain (AL) amyloidosis are gravely reduced by advanced cardiac involvement at Mayo cardiac stage IV with a median survival of 6,5 months. High-dose Melphalan (HDM) and autologous stem cell transplantation (ASCT) or other intense chemotherapy regimen cannot be applied to those patients due to the high risk of therapy-related mortality. One approach to improve the catastrophic prognosis of those patients is to perform a heart transplantation followed by intense chemotherapy.
Aims: Our aim was to examine the cases of cardiac AL patients treated with heart transplantation (HTx) at our center and to evaluate the clinical outcome of this treatment approach.
Methods: Data from 41 patients (21m, 20f) suffering from cardiac AL who were treated in our hospital between 2002 and 2017 were retrospectively analyzed. All patients were high-urgency listed for orthotopic HTx due to poor perspective of survival. Until 2009, 13 patients were listed, 8 of them with multiple organ involvement. Thereafter, we excluded patients with multiple organ involvement. All data are derived as medians with range or absolute numbers. Survival curves were calculated using the Kaplan-Meier method.
Results: Median age was 51 years (35-63) at diagnosis. Amyloidogenic lambda light-chains (LC) were detected in 35 and kappa LC in 6 patients. Median dFLC was 331 (69 – 2752) and median plasma cells in bone marrow were 13% (5-35). Median NT-proBNP was 6.332 ng/l (1.500 -53.194), median cTNT 0,11 µg/l (0,01-0,52) and median hsTNT was 60 ng/l (28-448) at diagnosis. Median NYHA stage was 3 (2-3) and median MAYO 2004 stage was 3 (2-3). Serum creatinine was at a median of 0,94 mg/dl (0,66-2,45), proteinuria at 0,1 g/day (range 0-10,7). Patients stayed on the high-urgency waiting list for a median of 26 (range 3-54) before 2009, and a median of 64 days (8-259) after 2009. 35 patients were treated with chemotherapy prior to HTx (mostly dexa w/o Bortezomib) to reduce dFLC during the waiting time. Eight patients died before receiving HTx with a median survival (start point: HU listing) of 26 days (6-177). With a median of 5 months after HTx (4-29), 18 patients received ASCT. HDM was used with either 200 mg/m2 (N=10) or reduced dosage (N=8) in patients with reduced kidney function (mostly due to renal complications after HTx). Complete remission (CR) was achieved in 7 patients (24% of all transplanted pts, n=29; 2 patients have not finished treatment yet), very good partial remission (VGPR) in 6 patients (21%) and partial remission (PR) in 7 patients (24%). Overall, 25 patients died. Cause of death was either progression of AL (N=16), sepsis (n=4), heart transplant rejection (n=3) or other (n=2). Patients that underwent HTx had a median survival of 46 months (2-177, 1-year survival: 77%).
Summary/Conclusions: HTx followed by chemotherapy is a feasible treatment approach in patients with advanced cardiac amyloidosis. Patients who reach HTx have a nearly 50% chance for a very good hematologic remission (VGPR or better) and consecutively a favorable survival probability with a median OS of nearly 6 years in our series.

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MM-013 PHASE 2 MULTICENTER STUDY OF POMALIDOMIDE PLUS LOW-DOSE DEXAMETHASONE IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA AND RENAL IMPAIRMENT

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Background: RI is a common comorbidity in pts with multiple myeloma (MM) that increases in incidence as the disease progresses and is associated with poor prognosis. Approximately 20% to 30% of pts with MM have RI at time of diagnosis; ≈ 2% to 13% of whom will require dialysis. The immunomodulatory agent, POM, in combination with LoDEX demonstrated comparable efficacy and tolerability in pivotal RRMM trials in pts with moderate RI. However, this regimen had not previously been fully studied in pts with severe RI or pts requiring hemodialysis.
Aims: To present updated safety and efficacy analyses from the multicenter, phase 2 MM-013 trial, in which pts with RRMM and moderate or severe RI, including those on hemodialysis, were treated with POM+LoDEX.
Methods: Three cohorts of pts with RRMM and RI were enrolled: (A) – moderate RI (estimated glomerular filtration rate [eGFR] ≥30 to <45 mL/min/1.73m^2), (B) – severe RI (eGFR <30 mL/min/1.73m^2) without hemodialysis, and (C) – severe RI requiring hemodialysis. All pts must have received ≥1 prior treatment including LEN and progressed during or after their last anti-myeloma treatment before entering the study. Pts received POM+LoDEX until disease progression

or unacceptable toxicity. Supportive care was allowed; thromboprophylaxis was required for all pts not on hemodialysis. The primary endpoint was overall response rate (ORR). Key secondary endpoints included safety, renal response, time to myeloma response, time to renal response, duration of response, progression-free survival (PFS), time to progression, and overall survival (OS). All pts provided informed consent.

Results: Enrollment has been completed with 81 pts (33 in cohort A; 34 in cohort B; 14 in cohort C), of which 13 (16.0%) were still on treatment as of January 28, 2017. Median follow-up for OS was 7.8 months. A total of 68 pts (84.0%) discontinued treatment; 39 (48.1%) due to PD. Median age was 72 yrs (range, 52-86 yrs), 60.5% of pts were male, and median time from diagnosis was 3.8 yrs (range, 0.03-19.44 yrs). Pts received a median of 4 (range, 1-10) prior anti-myeloma therapies. All pts had prior treatment with LEN (100%) and nearly all with BORT (97.5%). Median relative dose intensity of POM was 0.94 in both cohorts A and B, and 0.99 in cohort C. ORR was 39.4%, 29.4%, and 14.3% in cohorts A, B, and C respectively. PFS and OS results are presented in the Table 1. Grade 3/4 anemia and thrombocytopenia occurred more frequently in cohort C, likely due to severe RI requiring dialysis (Table 1). AEs leading to dose reductions were 18.2%, 14.7%, and 14.3% in cohorts A, B, and C, respectively.

Table 1.

	Cohort A (n = 33)	Cohort B (n = 34)	Cohort C (n = 14)
Grade 3/4 TEAEs, n (%)			
Hematologic			
Neutropenia	20 (60.6)	15 (44.1)	8 (57.1)
Anemia	9 (27.3)	12 (35.3)	8 (57.1)
Thrombocytopenia	9 (27.3)	6 (17.6)	6 (42.9)
Febrile neutropenia	1 (3.0)	1 (2.9)	0
Non-hematologic			
Infections	12 (36.4)	9 (26.5)	4 (28.6)
Pneumonia	4 (12.1)	2 (5.9)	1 (7.1)
Efficacy			
ORR (≥ PR), n (%)	13 (39.4)	10 (29.4)	2 (14.3)
CBR (≥ MR), n (%)	16 (48.5)	13 (38.2)	3 (21.4)
PFS, median (95% CI), mos	6.5 (4.6, 10.6)	4.2 (2.8, 6.5)	2.4 (1.0, 6.4)
OS, median (95% CI), mos	17.7 (7.8, NE)	11.8 (6.4, 14.3)	5.2 (1.8, 9.7)
Renal response, n (%)	7 (21.2)	12 (35.3)	1 (7.1)

CBR, clinical benefit rate; MR, minimal response; NE, not estimable; PR, partial response; TEAE, treatment-emergent adverse event.

Summary/Conclusions: POM+LoDEX is efficacious in pts with RRMM with moderate or severe RI, including those on hemodialysis, who had more advanced disease due to worse renal function. The safety profile was acceptable among the three groups and no new safety signals were observed. This study demonstrates that POM+LoDEX can be administered in pts with moderate or severe RI, including those on hemodialysis.

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PEMBROLIZUMAB MONOTHERAPY FOR RELAPSED/REFRACTORY MULTIPLE MYELOMA: PHASE 1B KEYNOTE-013 STUDY

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Background: Treatment options are needed for patients with RRMM. PD-L1 is expressed in patients with multiple myeloma, and blocking the programmed death 1 (PD-1) pathway may provide antitumor activity. Pembrolizumab is a humanized, high-affinity monoclonal antibody directed against PD-1 with robust antitumor activity and favorable safety in several solid and hematologic malignancies. KEYNOTE-013 (NCT01953692) is a multicohort phase 1b study of pembrolizumab monotherapy in patients with hematologic malignancies; results are reported for patients with RRMM.

Aims: To determine the safety, tolerability, and antitumor activity of pembrolizumab monotherapy in patients with RRMM.

Methods: Patients with RRMM who have failed ≥2 prior lines of therapy including a proteasome inhibitor and immunomodulatory drug (IMiD) received pembrolizumab 10 mg/kg every 2 weeks or 200 mg fixed dose every 3 weeks. Primary end points were safety, tolerability, and objective response rate (ORR) as determined by investigators, per International Myeloma Working Group 2006 criteria.

Results: At data cutoff of January 2, 2017, 30 patients were treated. The median (range) duration of follow-up was 15 (1-32) months. 28 (93%) patients discontinued the study; the most common reason was disease progression in 14 (47%) patients and clinical progression in 9 (30%) patients. 2 (7%) patients are still on treatment. Median (range) age was 70 (56-81) years. 21 (70%) patients had an ECOG performance status of 1. Patients received a median (range) of 4 (2-10) prior lines of therapy. 20 (67%) patients were lenalidomide refractory, 10 (33%) were double-refractory, 9 (30%) were triple refractory, and 1 (3%) patient was quadruple refractory. Among patients who received pembrolizumab at 10 mg/kg, the median (range) of pembrolizumab exposure was 6 (2-15) cycles; among those who received 200-mg fixed dose of pembrolizumab, the exposure was 3 (2-6) cycles. No patient experienced a response. Seventeen (57%; 95% CI, 37-75%) patients had stable disease. 13 (43%; 95% CI, 26-63%) patients had progressive disease as their best response. Treatment-related adverse events (TRAEs) occurred in 12 (40%) patients. The most common TRAE was asthenia (n=5, 17%); arthralgia, aspartate aminotransferase increased, fatigue, hyperglycemia, hypothyroidism, myalgia, pruritus, and blurred vision occurred in 1 patient each. A grade 3 TRAE (myalgia) occurred in 1 (3%) patient. There were no grade 4 TRAEs and no deaths due to TRAEs. 1 (3%) patient had an immune-related adverse event (grade 1 pruritus).

Summary/Conclusions: The safety profile of pembrolizumab in RRMM was consistent with that observed with other cancers. Best response observed while on pembrolizumab monotherapy was stable disease. Recent results of ongoing studies, such as KEYNOTE-023 (NCT02036502), demonstrate promising efficacy of pembrolizumab in combination with IMiDs (lenalidomide) and dexamethasone in patients with RRMM.

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ASSESSMENT OF MOBILIZATION COST FOR MULTIPLE MYELOMA USING 2 DIFFERENT STRATEGIES: HIGH-DOSE CYCLOPHOSPHAMIDE VERSUS PLERIXAFOR. ON BEHALF OF IFM

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Background: Treatment with autologous transplantation (ASCT) remains the standard of care upfront for Multiple Myeloma patients considered eligible for transplant. Peripheral blood stem cell (PBSC) collection, also called mobilisation, is needed prior to ASCT. The optimal methodology for mobilizing PBSC has yet to be defined, with either G-CSF alone, also called steady state procedure, or use of Plerixafor, a CXCR4 antagonist (Mozobil®)+G-CSF or high dose cyclophosphamide (usually administered at a dose of 1.5 to 6g/m^2 IV for one to two days)+G-CSF. The former is rarely used with the most recent induction regimens, and the 2 latter have demonstrated similar PBSC collection rates. Because of the intense competition for hospital resources and the staff required to manage patients preparing for mobilization and transplantation, it is important to quantify the total impact of mobilization on staff resource and cost to the hospital.

Aims: We aimed at better evaluate the respective cost of the 2 techniques of mobilization for the French health care system, high dose cyclophosphamide (n=57) *versus* plerixafor (n=55).

Methods: This is an observational cohort database analysis of 112 consecutive patients with MM treated upfront with ASCT between 2009 and 2013 and that had been mobilized with either high dose cyclophosphamide or plerixafor from 15 IFM centers. Patients must have successfully underwent ASCT. This study was not aimed at evaluating the suitability or advisability of one therapy *versus* another. A cost-consequences analysis of the different regimens of mobilization will be performed. Cost will be quantified using "microcosting" approach. Only direct medical costs are included in this economic analysis. Hospital resources will be calculated using two different approaches: per diem hospitalization costs (excluding direct medical costs) *versus* French public diagnosis-related group

(DRG). The point of view of the French Public Health System is adopted for this study. Monetary values for 2012 euros prices will be used for all components.
Results: Median (range) age was 59.5 (24-72), sex ratio was 1.5, ISS 3 was 26% in either group, all patients were collected to allow the number of graft requested by the hematologist of reference. The median CD34 collected was 8.9 (4-30) for HD cyclophosphamide and 5.8 (2-15) for plerixafor. The analysis demonstrated that days in the hospital were the primary cause for cost difference across the 2 mobilization techniques. In that regards, plerixafor appeared very cost effective compared to HD cyclophosphamide. All data will be updated at EHA 2017 including cost comparison.
Summary/Conclusions: For a long time HD cyclophosphamide was recommended for mobilization upfront in Myeloma therapy, as it was needed to improve response rate and depth of response, despite only 10% of the patients improving. With the progress made recently with the induction regimens, the choice for the mobilization regimen is now based more on safety and cost saving. In that regards, one must acknowledge that plerixafor has become one, if not the, most attractive option for Myeloma.

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SYSTEMATIC LITERATURE REVIEW AND NETWORK META-ANALYSIS OF INDUCTION TREATMENT FOR NEWLY DIAGNOSED TRANSPLANT-ELIGIBLE MULTIPLE MYELOMA PATIENTS

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Background: Based on the current guideline, bortezomib-based two or three drug regimens are mainly listed as a category 1 primary treatment option for transplant-eligible patients with myeloma. However, to date there are few direct head-to-head randomized controlled trials (RCTs) comparing effects of these recommended regimens, which makes it difficult to assess which treatment is most favorable to obtaining high response rates.
Aims: to determine the ranking of the currently recommended induction regimens and to compare efficacy of all available treatments.
Methods: We conducted a systematic literature review to identify all eligible RCTs that include at least one currently recommended regimen by searching PubMed, Web of Science, ASH, ASCO, EHA, and ESMO databases. A Bayesian network meta-analysis (NMA) with a fixed-effect model was performed to rank the regimens of their very good partial response rates or better (≥VGPR) and provide a comparison of treatment options. One of the most commonly utilized regimens, lenalidomide-dexamethasone, was used as reference treatment.
Results: Ten RCTs were identified including nine treatment regimens : vincristine-doxorubicin-dexamethasone (VAD), thalidomide-dexamethasone (TD), bortezomib-dexamethasone (VD), bortezomib-doxorubicin-dexamethasone (PAD), bortezomib-cyclophosphamide-dexamethasone (VCD), bortezomib-thalidomide-dexamethasone (VTD), bortezomib-thalidomide-dexamethasone-cyclophosphamide (VTDC), lenalidomide- dexamethasone (RD) and bortezomib-lenalidomide-dexamethasone (VRD). Figure 1 shows the probability of being the best induction treatment. The Figure 1 also presents the postinduction odds ratios for achieving a ≥VGPR and 95% credible intervals (CrI) *versus* RD. This analysis showed a significantly higher ≥VGPR with VRD compared with most of the other investigated interventions, including VD, PAD, VCD and RD (OR [95% CrI]): VD 4.4 [1.2–19.6]; PAD 3.8 [1.1–16.2]; VCD 3.4 [1.1–14.2]; RD 1.7 [1.1–2.5]. The similar result was also seen in comparing with VTD and VTDC, but the difference did not reached statistically significant level : VTD 2.4 [0.7–10.4]; VTDC 2.4 [0.5-13.7]. VRD was identified as the best induction treatment since it was the most favorable in terms of i) only treatment that was significantly better than RD, and ii) probability of being best regimen (84% of the simulations).

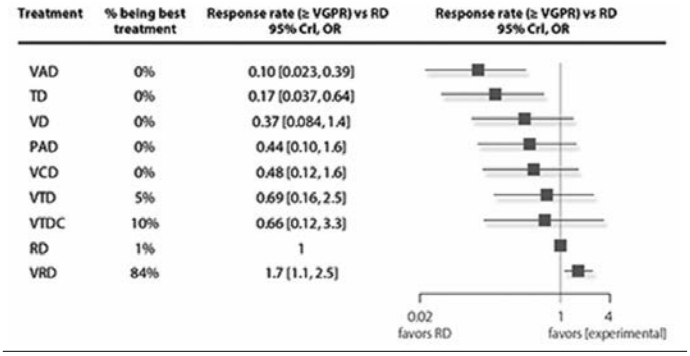

Figure 1.

Summary/Conclusions: Our systematic review and NMA included most of the recommended induction treatments for transplant-eligible myeloma patients and identified VRD as being most effective in achievement of ≥VGPR. NMAs can provide an overview of the best treatment and each regimen's relative efficacy in case of lacking head-to-head RCTs, thereby supporting clinical decision-making.

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A STUDY OF UTILITY OR FUTILITY OF PERFORMING SKELETAL SURVEYS IN PARAPROTEINAEMIA: A MULTICENTER EXPERIENCE FROM UK

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Background: Recent International Myeloma Working Group(IMWG) guidelines recommend that conventional skeletal surveys should be supplanted by low dose whole body computed tomography (CT),whole body magnetic resonance imaging (MRI) and or ^{18}fluoro-deoxyglucose (^{18}F-FDG/PET)[1] .However, resource, funding and radiology capacity issues, have posed significant challenges to implementing these recommendations. The risk of progression of Monoclonal Gammopathy of Undetermined Significance (MGUS) to a neoplastic plasma cell disorder is approximately 1% per year[2] and even lower in low risk MGUS. It is thus not necessary to perform imaging in unselected MGUS patients.
Aims: To look at all skeletal surveys requested across 3 large hospitals in UK over a year and analyze their justification, effectiveness and utility.
To decide if a rational clinico–biochemical algorithm could be used to reduce the number of imaging requests, thereby avoiding unnecessary radiation exposure, and make a possible switch to modern imaging methods cost effective
Methods: A total of 397 skeletal surveys were performed across three hospitals over one year. The data set was analyzed for clinical indications, paraprotein level, rationale for requesting the skeletal survey, the diagnostic yield and also the number of follow up CT/ PET or MRI required.
A pragmatic algorithm was developed and applied to see if the requests were justified and could have been safely reduced. (Figure1).
Results: Of the 397 analyzable skeletal surveys performed, 266 were on myeloma, 81 for MUGS, 48 were for non-paraprotein related indications. Of the 266 myelomas, 30% of skeletal surveys were reported as positive according to IMWG criteria[1]. A detailed analysis of 130 myeloma patients revealed a significant proportion of false negatives (6%) and false positives (7%), highlighting the insensitivity and poor specificity of this imaging modality. More importantly more than a third (38%) of myeloma patients required follow up imaging with MRI, PET or WBLDCT irrespective of the initial skeletal survey result, indicating a significant duplication rate and waste of resources. In the MGUS group, majority of skeletal surveys were negative (91%) but 9% were reported as positive. Follow up imaging with CT and MRI was performed in 23% of the MGUS group. However none these were positive. When the clinico-biochemical algorithm was applied, the number of requests was reduced by at least a quarter (24%), avoiding unnecessary radiation exposure and precious resources.

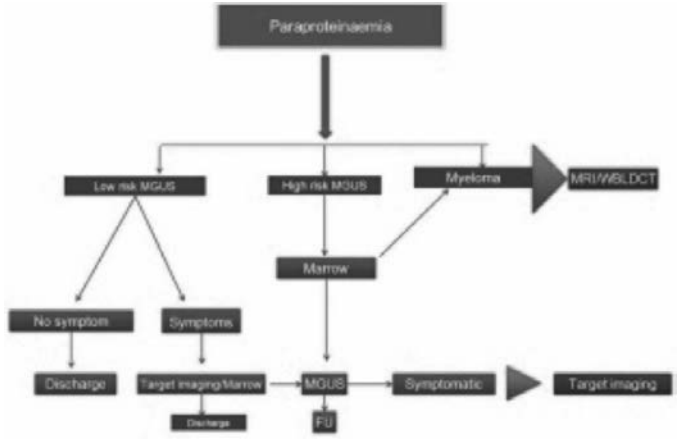

Figure 1.

Summary/Conclusions: - Skeletal survey has very limited role in investigation of paraproteinaemia and should be abandoned. - Our pragmatic clinic-biochemical imaging algorithm reduced imaging requests significantly(24%) allowing the preferred imaging modalities to be performed productively in a cost effective way in face of ever increasing health care cost and demands.

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SERUM FLC MEASUREMENTS COMPLEMENT BONE MARROW ASSESSMENT TO DETERMINE PROGNOSIS IN MYELOMA PATIENTS ACHIEVING DEEP RESPONSES

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Background: In multiple myeloma (MM), abnormal serum free light chain ratios (FLCr) after therapy associate with poor prognosis, independent of depth of response. However the value of FLCr in the context of minimal residual disease (MRD) remains unclear. A proportion of MRD-negative patients experience early relapses and conversely, some MRD-positive patients can endure long-term survival; which may result from improved immunosurveillance following normal plasma-cell recovery.
Aims: We hypothesised that serum FLC levels and ratios add clinical value at the time of MRD assessment.
Methods: The study included 275 intact immunoglobulin MM patients from the IFM2009 clinical trial who achieved at least a very good partial response (VGPR) after consolidation therapy. Median PFS from the end of consolidation was 38.3 months; median OS was not reached. Serum FLCs were measured using Freelite immunoassays (The Binding Site). Normal range for k/l FLCr was 0.26-1.25. We defined immunosuppression as levels of both the uninvolved (polyclonal) FLC+uninvolved heavy+light chain (HLC; measured with Hevylite) below their normal range. MRD assessment in bone marrow samples was based on 4-colour multiparametric flow cytometry (MFC).
Results: At the end of consolidation, 79/275 (29%) patients were MRD-positive, 79/275 (29%) had abnormal FLCr, 16/275 (6%) had elevated iFLC, with immunosuppression identified in 52/275 (19%). Using Cox regression all the variables associated with shorter PFS (p<0.001 for all) and OS (p<0.050 for all; except elevated iFLC which showed a trend towards shorter OS (p=0.070)). Among 196 MRD-negative patients, 37/196 (19%) had abnormal FLCr, 2/196 (1%) had elevated iFLC with immunosuppression identified in 23/196 (12%). Median PFS for MRD-negative patients was not reached; however both an abnormal FLCr (median PFS: 31.4 months; p<0.001) and immunosuppression (median PFS: 31.4 months; p=0.005) identified a group of patients with poorer outcomes. On the other hand, median PFS for MRD-positive patients was 21.3 months; 42(53%) of these patients had abnormal FLCr and dismal outcomes (median PFS 12.6 *vs* 30.7 months for abnormal *vs* normal FLCr, respectively; p=0.004). Absolute FLC measurements did not reach statistical significance for PFS in these patients.
Summary/Conclusions: Serum FLC measurements in combination with low-sensitivity MFC bone marrow assessment at the end of consolidation therapy render the most powerful prognostic information in MM patients achieving deep responses. In those where disease is no longer detected using MFC, abnormal FLCr confer poor prognosis, which may partly be due to inefficient immune recovery. Absolute FLC measurements were not informative, supporting the rationale of evaluating biomarkers of the tumour and immune system recovery. Our results warrant further studies to validate the clinical utility of FLC measurements in combination with next-generation (8-colours) flow cytometry.

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THE CONNECT MM REGISTRY: IMPACT OF THE CYTOGENETIC ABNORMALITY T(11;14) ON SURVIVAL OUTCOMES IN AFRICAN AMERICAN AND NON-AFRICAN AMERICAN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA

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Background: The cytogenetic abnormality t(11;14) is common, occurring in approximately 20% of patients with newly diagnosed multiple myeloma (NDMM) (Avet-Loiseau, *Leukemia*, 2013). Historically, t(11;14) has been associated with standard-risk multiple myeloma (MM) and generally favorable outcomes (Avet-Loiseau, *Leukemia*, 2013). However, some retrospective analyses have reported the presence of t(11;14) to be a poor prognostic factor (Kaufman, *Leukemia*, 2016). Connect MM is a largely community-based, US prospective observational cohort study that collects data on management and natural history of patients with NDMM in clinical practice.
Aims: This analysis assessed the impact of t(11;14) on survival outcomes in African American and non-African American patients in a mostly community-based setting.
Methods: Adult patients with NDMM within 60 days of diagnosis were eligible for enrollment in the registry. Patients who completed induction and were tested for t(11;14) by fluorescence *in situ* hybridization or cytogenetics were grouped by race (African American and non-African American). Endpoints were progression-free survival (PFS) and overall survival (OS). Kaplan-Meier analyses were adjusted for cohort, age, International Staging System stage, transplant intent, presence of t(4;14), diabetes history, and baseline levels of hemoglobin, platelets, calcium, and creatinine. Data cutoff was Jul 7, 2016.
Results: 3011 patients were enrolled in 2 cohorts. Cohort 1 enrolled 1493 patients from Sep 2009–Dec 2011; median follow-up was 39.3 months. Cohort 2 enrolled 1518 patients from Dec 2012–Apr 2016; median follow-up was 16.4 months. A total of 1539 (52%) patients were tested for t(11;14). Of these, 363 (24%) were positive for t(11;14). By race, 53 (26%) of 205 African American and 310 (23%) of 1334 non-African American patients were positive for t(11;14). First-line bortezomib exposure was similar across all groups. In African American patients, the presence of t(11;14) resulted in a trend toward shorter PFS compared to those without t(11;14) (Table 1. Additionally, African American patients with t(11;14) had significantly higher risk of death compared to African American patients without t(11;14). A higher rate of early mortality was observed *vs* non-African American patients. In non-African American patients, no differences in PFS or OS were noted based on the presence or absence of t(11;14). For OS, the interaction between race and t(11;14) status was statistically significant (*P*=0.004).

Table 1.

	African American (n=205)			Non-African American (n=1334)		
	t(11;14)			t(11;14)		
	Yes (n = 53)	No (n = 152)	Adjusted Hazard Ratio (95% CI)	Yes (n = 310)	No (n = 1024)	Adjusted Hazard Ratio (95% CI)
Median PFS, months	19.1	45.2	0.66 (0.41, 1.04) *P* = .076	33.1	38.1	1.03 (0.84, 1.25) *P* = NS
Median OS, months	not reached	not reached	0.44 (0.23, 0.84) *P* = .012	69.3	71.0	1.17 (0.90, 1.54) *P* = NS

NS, not significant.

Summary/Conclusions: In Connect MM, the effect of t(11;14) on OS was significantly different between African American and non-African American patients. Specifically, t(11;14) was associated with poorer survival outcomes in African American patients, and not in non-African American patients. Thus, the presence of t(11;14) may be a risk factor for poor prognosis in African American patients. Additional analyses will be conducted to elucidate the role of induction treatment, transplant and maintenance in African American and non-African American patients with t(11;14).

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RAS-PATHWAY MUTATION PATTERNS DEFINE EPIGENETIC SUBCLASSES IN JUVENILE MYELOMONOCYTIC LEUKEMIA

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Background: Juvenile myelomonocytic leukemia (JMML) is an aggressive myeloproliferative disorder of early childhood. While some cases show spontaneous remission, allogeneic hematopoietic stem cell transplantation (HSCT) remains the only curative treatment option for the majority of patients, however, the 5-year event-free survival reaches only about 50%. Hyperactive RAS signaling is assumed to be the main driving event in JMML. It is caused by genetic alterations in *CBL*, *KRAS*, *NF1*, *NRAS,* or *PTPN11* in about 90% of patients. So far, there is no clear understanding of how RAS pathway mutations relate to the heterogeneous disease biology and variable clinical outcome seen in JMML patients. As a consequence, established clinical and genetic markers fail to fully represent the observed disease heterogeneity.
Aims: We hypothesized that DNA methylation profiling, either alone or in combination with genetic alterations, might provide a molecular basis for disease classification.
Methods: Genome wide DNA methylation analysis using the HumanMethylation450 Bead Chip array was performed in a discovery cohort of 20 JMML patients. We developed a strategy to eliminate methylation events that attribute to epigenetic changes in normal hematopoiesis. The clinical relevance of our findings was assessed in an unselected sample set consisting of 148 consecutive patients with JMML (n=130) or Noonan syndrome associated myeloproliferative disorder (n=18) registered in the EWOG-MDS 1998 & 2006 trials. Data integration was performed in a subset of patients with available exome sequencing (n=50) and expression profiling (n=15) data.
Results: Systematic DNA methylome analysis of JMML samples identified three subgroups with low, intermediate and high methylation levels (LM, IM, and HM). Detailed analysis of the validation cohort not excluding the Noonan patients identified an association of methylation groups with clinical features. The HM subgroup (n=41) was enriched for high-risk characteristics: All HM cases had elevated levels of HbF, 88% were older than 2 years at diagnosis, 74% had low platelets (<70/nl), and 66% carried somatic *PTPN11* mutations. In contrast, the LM subgroup (n=62) was enriched for patients with low-risk disease: All 18 patients with Noonan syndrome, 13/14 patients with CBL syndrome, and 15/19 patients with *NRAS* mutations were assigned to the LM group. The IM group (n=45) was enriched for cases with monosomy 7 and somatic *KRAS* mutations. The unfavorable risk profile in the HM group translated into poor 5-year survival (HM 57%, LM 87%; log rank p<0.01) and a high incidence of relapse after HSCT (HM 48%, LM 9%; Gray's test p=0.01). In a multivariate Cox regression model, only methylation group (HM *vs* LM: RR 10.9 [1.8-66.2]) and *PTPN11* mutation status (PTPN11-mutant *vs* other: RR 3.3 [1.2-8.9]) remained as independent prognostic factors for CIR (p=0.01).
JMML-specific differentially methylated probes were significantly enriched for regions decorated with H3K27me3 or PRC2 components and for genes associated with oncogenic RAS-signaling. Integrative analysis of genetic and epigenetic events in these cases identified additional gene alterations leading to frequent co-occurrence of ≥2 mutations activating the RAS-RAF-MEK-ERK pathway in the HM and IM subgroups. This finding was paralleled by a significant up-regulation of *DNMT1* and *DNMT3B* expression suggesting aberrant activation of the DNA methylation machinery in this context.
Summary/Conclusions: Our integrated approach identified three JMML subgroups characterized by distinct clinical and biological features. We provide evidence for a molecular mechanism by which additional genetic events, presumably further activating the RAS-RAF-MEK-ERK pathway, mediate DNA hypermethylation via up-regulation of DNMTs in more aggressive JMML cases.

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CYTOGENETIC ABNORMALITIES IN POST-POLYCYTHEMIA VERA AND POST-ESSENTIAL THROMBOCYTHEMIA MYELOFIBROSIS: CORRELATIONS WITH GENOTYPE AND PHENOTYPE IN THE MYSEC STUDY

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Background: The molecular and phenotypic correlates of cytogenetic abnormalities in primary myelofibrosis (PMF) have been widely investigated. This information in post-polycythemia vera (post-PV) and post-essential thrombocythemia (post-ET) myelofibrosis (referred to as secondary myelofibrosis, SMF) is scant. The MYSEC project (Myelofibrosis Secondary to PV and ET Collaboration) collected 781 SMF patients in Europe and United States and recently disclosed phenotype-genotype associations in SMF (Leukemia, 2017).
Aims: The primary objective of this study is to report cytogenetic abnormalities in SMF on a large scale and to assess molecular and phenotypic correlations of cytogenetic abnormalities. In addition, prognostic relevance of different cytogenetic patterns is investigated.
Methods: Diagnosis of SMF was performed according to the IWG-MRT criteria (2008). The MYSEC study was approved by the Review Board of each Institution and conducted in accordance with the Declaration of Helsinki. Bone marrow cytogenetic analysis was made at time of SMF diagnosis and considered evaluable if at least 20 metaphases were available. Results were described according to the International System for Human Cytogenetic Nomenclature. Karyotype was defined abnormal if a structural or numeric chromosomic alteration was present in at least two metaphases. The presence of three or more aberrations defined a complex karyotype; two or more distinct autosomal monosomies or single autosomal monosomy associated with at least one structural abnormality defined monosomal karyotype (MK). Continuous values were compared via non-parametric Mann-Whitney U tests, with Holm corrections for multiple testing; categorical feature counts were compared with Fisher's exact tests. Time-to-event analysis used Kaplan-Meier estimators and Cox models for regression.
Results: Within the whole cohort of 781 SMF patients, 376 had cytogenetic data. Cytogenetic abnormalities were reported in 128 (34.1%) cases: 72 (60%) were sole, 22 (18.3%) double, 26 (21.7%) complex, 11 (9.2%) MK (all included in complex karyotype) and eight unknown. The most prevalent individual abnormalities were 20q- (25%), 13q- (20.8%), +8 (8.3%) and +9 (5.6%). Patients with post-PV MF had significantly higher frequency of abnormal karyotype than those with post-ET MF (*P*=.012). Chromosomal abnormalities did not cluster differently among the different genotypes (*JAK2, CALR*, *MPL* and triple negativity*)*. Abnormal karyotype was significantly associated with lower platelet count (*P*=.004), larger spleen size (*P*=.016), higher circulating blasts (*P* <.001) and presence of constitutional symptoms (*P*=.014) at the time of SMF diagnosis. Within abnormal karyotypes, we found that patients with MK tended to have lower platelet count (*P*=.04) with respect to those with sole aberrations. Overall survival (OS) was significantly inferior in patients with abnormal karyotype (*P*=.012), even adjusting for SMF diagnosis type (*P*=.02). When investigating OS according to different abnormalities, we found that patients with MK have inferior OS respect to those with sole abnormality (*P* <.0001) (Figure 1).

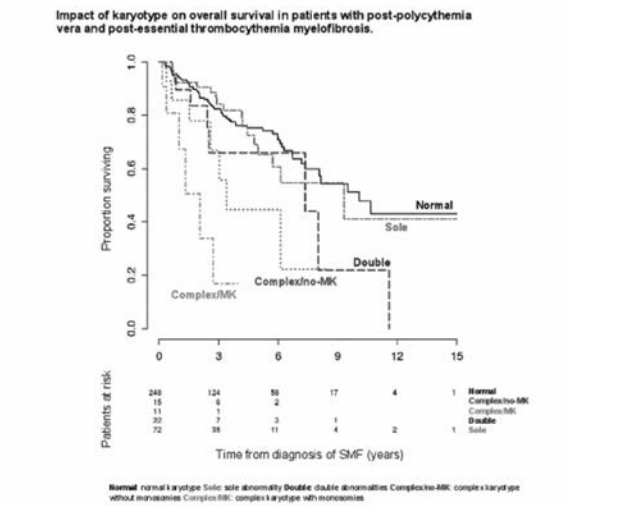

Figure 1.

Summary/Conclusions: Abnormal karyotype was found in 34.1% of SMF patients at diagnosis and was over-represented in post-PV MF. No different distribution was detected among genotypes. Abnormal karyotype was associated with lower platelet count, larger splenomegaly, higher circulating blast cells and presence of constitutional symptoms. Concerning outcome, the presence of abnormal karyotype implied inferior survival and, among subtypes, MK remained the most powerful predictor.

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MUTATIONAL LANDSCAPE OF MYELODYSPLASTIC SYNDROME/ MYELOPROLIFERATIVE NEOPLASM - UNCLASSIFIABLE

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Background: MDS/MPN-U is a rare, poorly characterized myeloid neoplasm within the MDS/MPN category in the World Health Organization (WHO) classification. A median survival of 12.4 months from time of referral was previously reported for a cohort of 85 patients with MDS/MPN-U seen at the MD Anderson Cancer Center (MDACC, DiNardo *et al. Leukemia* 2014). The International Prognostic Scoring System (IPSS) for MDS (Greenberg *et al. Blood* 1997) discriminated amongst prognostically distinct categories in that cohort, while neither the IPSS for primary myelofibrosis (PMF, Cervantes *et al. Blood* 2009) nor the revised IPSS (IPPS-R) for MDS (Greenberg *et al. Blood* 2012) did. Median survival of 21.4 months from the time of diagnosis was reported in a multi-institutional cohort (n=69, Wang *et al. Blood* 2014). Information on the genomic landscape of MDS/MPN-U is limited to one report on the frequency of *SETBP1* mutations (8.3%, Meggendorfer *et al. Leukemia* 2013).
Aims: To describe the mutational landscape of MDS/MPN-U using targeted multi-gene sequencing.
Methods: Targeted sequencing was performed on DNA from 97 patients with MDS/MPN-U (diagnosed per WHO 2008 criteria but excluding refractory anemia with ringed sideroblasts and thrombocytosis) seen across 4 US institutions (MDACC, 43; Cleveland Clinic, 29; Moffitt Cancer Center, 16; Vanderbilt University, 9). Gene panels used varied between institutions, with 20 genes (*ASXL1, CBL, DNMT3A, ETV6, EZH2, IDH1, IDH2, JAK2, KIT, NPM1, NRAS, PHF6, RUNX1, SETBP1, SF3B1, SRSF2, TET2, TP53, U2AF1, ZRSR2*) in common.
Results: Mutational frequencies for the 20 genes tested in all 97 patients were as follows: *TET2,* 28%; *ASXL1,* 27%; *JAK2,* 25%; *SRSF2,* 22%; *EZH2,* 15%; *SF3B1,* 12%; *RUNX1,* 12%; *ZRSR2,* 11%; *SETBP1,* 11%; *U2AF1,* 11%; *NRAS,* 10%; *DNMT3A,* 9%; *TP53,* 8%; *CBL,* 4%; *ETV6,* 4%; *NPM1,* 4%; *IDH2,* 2%; *KIT,* 2%; *PHF6,* 1% and *IDH1,* 0%. In addition, the frequency of mutations in ten other genes of interest in hematologic malignancies was assessed: *BRAF*, 0% (n=52); *CSF3R*, 4% (n=52); *CALR*, 4% (n=53); *MPL*, 3% (n=68); *MLL*, 1% (n=72); *STAG2*, 6% (n=72); *CEBPA*, 4% (n=73); *KRAS*, 4% (n=81); *PTPN11*, 4% (n=82) and *FLT3*, 2% (n=82). Median survival for the whole cohort (n=97) was 12.4 months (range, 1-173). The 43 MDACC patients in this analysis were included in the cohort of 85 previously reported by DiNardo *et al.* Median age was 70 years (21-89). Median (range) values for leukocytes, neutrophils, hemoglobin, platelets and bone marrow blasts at the time of sample collection for sequencing were 13.4 (1-179) x 10^9/L, 7.9 (0.4-152.4) x 10^9/L, 9.1 (3.1-15) g/dL, 123 (6-1168) x 10^9/L and 2% (0-17), respectively. On univariate analysis (n=97), only the presence of *EZH2* and *ZRSR2* mutations were associated with trends towards statistical significance for survival. Mutated *EZH2* adversely impacted survival (p=0.056), while mutated *ZRSR2* had a favorable impact on survival (p=0.074). The IPSS-R for MDS was useful to differentiate between risk groups with different survival times (p=0.065) while the dynamic IPSS for PMF (Passamonti *et al.* Blood 2010) was not (p=0.39). On multivariate analysis, only *EZH2* mutations and IPSS-R very low risk (*versus* all other categories combined) were statistically significantly associated with inferior and superior survival, respectively.
Summary/Conclusions: In this cohort of 97 patients with WHO-defined MDS/MPN-U, mutations in genes encoding epigenetic regulators (*e.g.*, *TET2, ASXL1, EZH2*), spliceosome components (*e.g.*, *SRSF2, SF3B1, ZRSR2, U2AF1*), signaling molecules (*JAK2, NRAS*), the transcription factor *RUNX1* and *SETBP1* were found at frequencies ≥10%. Although the analysis is limited by small numbers, *EZH2* mutations were independently associated with poor survival. This represents the largest cohort of patients with MDS/MPN-U interrogated for mutations in multiple genes to date.

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GENOME WIDE DNA METHYLATION PROFILING IS PREDICTIVE OF OUTCOME IN JUVENILE MYELOMONOCYTIC LEUKEMIA

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Background: Juvenile myelomonocytic leukemia (JMML) is a myeloproliferative disorder of childhood that is initiated by mutations in the Ras pathway. Outcomes in this disease vary dramatically from resolution with minimal or no therapy to relapse despite hematopoietic stem cell transplantation. Identifying biomarkers to distinguish patients with aggressive disease courses from those who can receive minimal therapy remains a priority for clinicians.
Aims: We utilized an unbiased screening approach to investigate genome-wide DNA methylation in newly diagnosed JMML patients. We then sought to determine whether a specific DNA methylation signature was capable of predicting outcomes in this heterogeneous disease, with a particular emphasis on identifying a biomarker predictive of spontaneous resolution.
Methods: Genome wide DNA methylation analysis was carried out using the Illumina 450k BeadChip platform in a discovery cohort of 39 well-characterized patients with JMML enriched for those who experienced spontaneous resolution without chemotherapy. A separate cohort of 40 patients with JMML was then used for validation. Of note, patients with Noonan syndrome were excluded from both cohorts. All 79 patients were then compared to 22 healthy controls between 1 and 5 years of age using peripheral blood derived DNA.
Results: JMML patients with aggressive disease have a distinctly hypermethylated DNA profile at the most variable CpG sites compared to patients with less aggressive disease as well as healthy controls. Methylation patterns did not differ based on the tissue of origin (peripheral blood, splenic tissue, or bone marrow) and were similar between monocyte enriched cell populations and unsorted mononuclear cells. Unsupervised clustering of the discovery cohort based on the most highly variable CpG sites (top 0.5% ranked by standard deviation, 1527 CpG sites) identified three clusters. For patients in the cluster with the lowest levels of methylation, only one patient out of 15 (7%) had an event at 4 years (95% confidence interval [CI], 2-32%). This compared to 45% (5/11) (CI, 17-77%) for patients in the cluster of intermediate levels of methylation and 61% (8/13) (CI, 32-86%) in those patients with the highest level of methylation. The proportion of patients with events differed significantly by cluster (p=0.0039) and remained independently prognostic in multivariable analysis (p=0.033) in the context of age and the number of somatic mutations at diagnosis. We next sought to validate our findings in an independent cohort of 40 patients. We classified each patient in the validation cohort into one of the three clusters defined by the discovery cohort. The proportion of patients having an event at four years was 8% (1/12) (CI, 0-38%) in those with the lowest level of methylation. This compared to 36% (4/11) (CI, 11-69%) for patients with intermediate levels of methylation and 76% (13/17) (CI, 50-93%) for those with the highest levels of methylation. We then compared our combined cohort of 79 JMML patients with 22 healthy, age-appropriate controls. Remarkably, using the same set of CpG sites defined in the discovery cohort, 27/79 JMML patients clustered more closely with the controls than with other patients. Of these 27 patients, 14 (52%)

experienced spontaneous resolution and only two (7%) experienced an event at 4 years.
Summary/Conclusions: Using a genome-wide methylation analysis, patients with the lowest degree of DNA methylation within the most variable CpG sites had improved event free survival compared to patients with higher degrees of methylation. Interestingly, patients who experienced spontaneous resolution of their disease clustered more closely with healthy controls than to patients with aggressive JMML. DNA methylation is a robust biomarker independently capable of predicting outcome and should be tested prospectively in the context of clinical trials.

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LEUKEMIC TRANSFORMATION OF MYELOPROLIFERATIVE NEOPLASMS: IS NGS PROFILE THE BEST PROGNOSTIC BIOMARKER?

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Background: Leukemic transformation occurs in 8% to 23% of myelofibrosis patients in the first 10 years after diagnosis and in 4% to 8% of polycythemia vera and essential thrombocytosis patients within 18 years of diagnosis and is almost always fatal.
Aims: We retrospectively analyzed the survival outcome of patients with myeloproliferative neoplasms (MPNs) who progressed to acute myeloid leukemia (AML) based on the treatments received, response, different prognosis groups according to the (ELN) and based on a next-generation DNA sequencing profile (NGS).
Methods: A total of 72 patients diagnosed in our institute with AML secondary to MPNs between 2000 and 2016 were retrospectively analyzed. NGS was performed in 44 cases. Mutations found by NGS were classified according three different cellular functions of interest (Tumors suppressor (*TP53*), ADN/Histones' epigenetic (*DNMT3A, EZH2,IDH1/2,ASXL1*) and alternative splicing (*SRSF2,U2AF1,ZRSR2,PRPF8,SF3B1*)) and three groups were determined: Group A: patients without altered cellular function; Group B: patient with one altered function; Group C: patients with more one altered functions. AML treatment response was evaluated according Mascarenhas' proposed criteria for response assessment of AML secondary to MPNs. Overall survival (OS) was calculated according the different treatments, treatment response and NGS profiles.
Results: 72 patients who developed AML secondary to MPNs were included in the study.43.6% (N=31) had prior ET, 25% (N= 18) PV, 20.8% (N=15) PMF and 11.1% (N=8) secondary myelofibrosis. The median age at AML transformation was 70 (range: 38-89). The median time to AML transformation from MPNs diagnosis was 108 months (range: 2.4-408). Among these 72 AML, 5.6% (N=4) belonged to the favorable risk category according to ELN 2017, 13.9% (N= 10) belonged to the intermediate risk category and 55.6% (N=40) to the adverse risk category. 45.8% (N=33) patients were treated with intensive chemotherapy (IC), 15.3% (N=11) with azacitidine (AZA) and 38.9 (N=28) with supportive care (BSC). Median OS was 4.5 months (range, 0.1-65), with no significant difference between the three ELN 2017 risk categories (respectively 2.5 months (range: 1-9), 5.5 months (range: 1-60) and 5 months (range: 1-36) in the favorable, intermediate and adverse risk categories). Patients who received IC ($p<0.01$) or AZA ($p<0.05$) have a significant better OS (median OS of 7 months (range: 0.5-65) and 8.5 months (range: 3-24) respectively) than patients who received BSC (median OS of 2 months, range: 0.1-36). However, there is no significant difference between the IC and HMA groups ($p=0.44$). 7 Patients in Complete Cytogenetic Response (CCR) or Acute Leukemia Response-Complete (ALR-C) received an alloSCT had a better median OS than the 9 patients who did not (23 *vs* 6.5 months, $p=0.063$). Patients with group A and B NGS profiles have a significant better median OS (respectively 14 and 8 months) than Group C (3 months) ($p <0.05$).
Summary/Conclusions: Our results confirm the poor outcome of patients with secondary AML treated with IC and suggest that AZA provides comparable OS. ELN2017 risk stratification predicted poorly patients outcome although a NGS-based classification performed better.

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INCIDENCE AND OUTCOME OF SECONDARY NON HEMATOLOGICAL CANCERS IN ADULT PATIENTS WITH MASTOCYTOSIS

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Background: Mastocytosis is a clonal disease characterized by heterogeneous manifestations and a normal life expectancy in the majority of cases. In such a condition, it is important to ascertain if other diseases, and particularly solid malignancies, can worsen the prognosis.
Aims: To assess incidence and outcome of secondary primary malignancies (SPM) in adult mastocytosis patients.
Methods: We performed a retrospective analysis of 826 adult (>18 years at diagnosis) mastocytosis patients diagnosed and regularly followed in 6 Italian Institutions. SPM were defined as *de novo* cancers diagnosed after mastocytosis. We excluded from the analysis non-melanoma skin cancers due to the possible under-reporting of such neoplasms by patients themselves. Also, we did not consider newly hematological neoplasms, as they mainly represent a progression from Systemic Mastocytosis (SM) to SM with an Associated Hematological Neoplasm (AHN). Standardized Incidence Ratio (SIR) was calculated as the ratio between the observed cases in our cohort and the expected cases in the sex- and age-matched general Italian population in the same time period (these data were retrieved from http://www.registri-tumori.it). Times to event (patient-years) were calculated from the diagnosis of mastocytosis to the date of SPM diagnosis, death, or last contact, whichever comes first. Survival curves were estimated according to the Kaplan-Meier method.
Results: Males were 450 (54%). Median age at diagnosis was 49.3 years (range 19-84). Median follow-up was 2.3 years (range 0-41). Subtype diagnoses were: Cutaneous Mastocytosis (n=46), Indolent SM (n=633), Smoldering SM (n=10), SM-AHN (n=34), Aggressive SM (n=47) and Mast cell leukemia (n=2). Fifty-four patients were classified as having mastocytosis in the skin. Overall, 42 patients had a history of malignancies prior to the diagnosis of mastocytosis: in these patients we did not detect any relapse of their prior malignancy after the diagnosis of mastocytosis. A total of 35 SPM were diagnosed in 34 patients (4.1%). Median age at SPM was 56.4 years (range 37-76). Median time from mastocytosis to SPM was 22 months. The overall rate of SPM was 12.8 per 1,000 person-years (95%CI: 9.1-17.6) while the rate in the general adult population was 7.6 per 1,000 person-years (95%CI: 7.5-7.7) resulting in an increased hazard ratio of 1.7 (95%CI: 1.2-2.3). The risk for SPM was higher than expected in females (SIR 1.93, 95%CI: 1.2-3.1) while it was not significantly increased in males (SIR 1.46, 95%CI: 0.9-2.4). We found a clearly increased risk for melanoma (n=8, SIR 15.9, 95%CI: 7.9-31.9) and thyroid cancer (n=3, SIR 9, 95%CI: 2.9-27.9) while a non-significant increased risk was found for prostate cancer in males (n=5, SIR 2.06, 95%CI: 0.8-4.9) and breast cancer in females (n=3, SIR 1.7, 95%CI: 0.5-5.3). All the other malignancies were sporadic (one or two cases for each cancer type) and comparison to the general population was not significant. The death rate in patients with SPM was 14.7% with a median overall survival (OS) from SPM diagnosis of 38 months. OS was significantly inferior in patients with SPM as compared to patients without secondary neoplasia (5-year OS 77.6% *vs* 93.7% respectively, $p=.019$) (Figure 1).

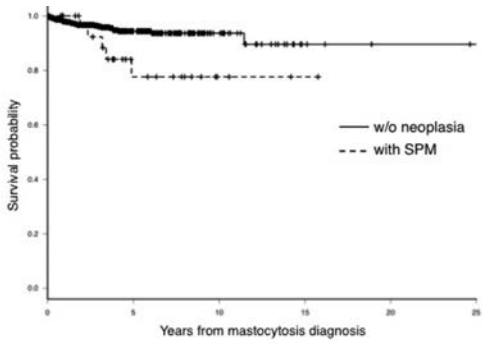

Figure 1.

Summary/Conclusions: Patients with mastocytosis may have a significantly higher risk of developing a secondary non hematological cancer as compared to the matched general population. Careful follow-up of these patients is warranted as the rate of malignancies may increase over time and reduce life expectancy.

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HIGH WBC COUNT WAS THE RISK FACTOR FOR SURVIVAL IN PATIENTS WITH CSF3R- MUTATED CHRONIC NEUTROPHILIC LEUKEMIA

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Background: Colony stimulating factor 3 receptor gene (CSF3R)-mutated chronic neutrophilic leukemia (CNL) is a rare chronic myeloproliferative neoplasm. There is the limited information on the clinical course of CNL.
Aims: To explore the clinical course of patients with *CSF3R*-mutated CNL and identify risk factor(s) associated with survival.
Methods: A retrospective study was conducted to assess natural history and identify risk factor(s) for survival in patients with *CSF3R*-mutated CNL. Survival analysis was performed by the Kaplan-Meier method taking the interval from the date of diagnosis to death or last contact. The log-rank test was used to compare survival data. Cox regression model was used for multivariable analysis.
Results: Data of 47 patients with CSF3R-mutated CNL were collected and analyzed. 35 (76%) patients were male. Median age was 62 years (range, 16-92 years). At diagnosis, 17 (36%) patients had fatigue, 2 (4%) had a fever, 8 (17%) experienced diarrhea or abdominal discomfort, 20 (43%) were asymptomatic and leukocytosis had been mostly an incidental laboratory finding. 20 (43%) patients had palpable splenomegaly, and 4 (9%), palpable hepatomegaly. PB parameters, median and (range), were WBC 42.4×10^9/L (14.4-217.0), hemoglobin 100g/L (42-157), platelets 165×10^9/L (17-570), blast percentage 0% (0-10), neutrophil percentage 82% (70-99). The median of blast cells in bone marrow were 1% (range, 0-12%). 46 (98%) patients were in the chronic phase and 1 (2%) in the accelerated phase at diagnosis. Most of the CSF3R mutations was T618I (n=45, 96%), others were T568M (n=1, 2%) and O706T (n=1, 2%). 34 (72.3%) patients and 41 (87.2%) patients were screened for ASXL1 and SETBP1 mutations, respectively. 21 (61.8%) patients harbored ASXL1 mutation and 22 (53.7%) harbored SETBP1 mutation. All patients were BCR-ABL1, PDGFR and FGR mutation negative, 2 were CALR mutation and JAK2V617F mutation positive, respectively. Hydroxyurea was the most frequently used therapy (n=46). Other therapies included interferon-a (n=7), hypomethylating agents (n=4), thalidomide (n=2), ruxolitinib (n=1), imatinib (n=3), dasatinib (n=1), chemotherapy (n=6), and transplant (n=2). With a median follow up of 17 months (range, 2-103 months), 7 patients progressed to blastic phase or acute myeloid leukemia (n=6) or myelodysplastic syndrome (n=1), 17 patients died. Survival rate at 30 months was 55%. Median survival was 39 months (95% CI 8.5-69.5). Multivariate analysis showed that WBC >40×10^9/L (HR=3.26, 95% CI 1.14-9.30, p= 0.027) was the sole risk factor for survival. However, SETBP1 or ASXL1 mutation was not associated with survival.
Summary/Conclusions: High WBC count was independently predictive of shortened survival in patients with CSR3R-mutated CNL.

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CLINICAL PHENOTYPE AND OUTCOME OF ESSENTIAL THROMBOCYTHEMIA AND PREFIBROTIC MYELOFIBROSIS DIAGNOSED ACCORDING TO THE REVISED 2016 WHO DIAGNOSTIC CRITERIA

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Background: The World Health Organization (WHO) classification system for myeloid neoplasms was recently revised in 2016. The revised WHO criteria underscore the importance of differentiating prefibrotic PMF (prePMF) from "true" essential thrombocythemia (ET) as these two entities have different clinical outcomes. For these reasons, standardization of morphologic findings in the bone marrow biopsy and an explicit definition of minor clinical criteria for the diagnosis of prePMF have been added (anemia, leukocytosis >11 x10^9/L, palpable splenomegaly, increased LDH).
Aims: To compare the clinical phenotype at diagnosis and the outcome of ET and prePMF diagnosed according to the new 2016 WHO criteria.
Methods: We identified in our database all patients affected with ET, prePMF and PMF diagnosed according to 2008 WHO criteria who satisfied these two requirements: a bone marrow fibrosis grade 0-1 at diagnosis and at least one DNA sample to define the mutational status. Firstly, the bone marrow morphology of all 404 identified patients was reviewed by an expert pathologist. Then, we reclassified patients according to the new 2016 WHO criteria as follows: patient with ET morphology were classified as ET, patients with PMF morphology and at least one clinical criteria (leukocytes, anemia, increased LDH, splenomegaly) were classified as prePMF, patients with PMF morphology but without clinical criteria were classified as myeloproliferative neoplasms unclassifiable (MPNu).
Results: According to the new criteria our cohort included 269 patients with ET, 109 patients with prePMF and 26 with MPNu. By comparing clinical phenotype at diagnosis in prePMF, MPNu, and ET respectively, we observed that prePMF showed higher leukocyte count, lower hemoglobin levels, higher platelet count, higher LDH values, higher number of circulating CD34-positive cells, and showed more frequently splenomegaly (Table 1). The higher frequency of *CALR* mutations in prePMF compared to ET might contribute to the high level of platelet count observed in prePMF. ET and MPNu did not differ in terms of leukocyte count, hemoglobin, platelet count, LDH, circulating CD34-positive cells and splenomegaly (Table 1). The 26 patients with MPNu were not further considered in the analysis of disease complications and overall survival due to the low number. PrePMF patients had lower overall survival (overall survival at 10 years 86.4% *vs* 96.6%, *P* <.001) and a trend to a higher incidence of leukemic evolution (cumulative incidence of acute myeloid leukemia at 10 years 2.3% *vs* 1.9%, *P* .067) compared to ET patients, while they did not differ in terms of thrombotic complications (cumulative incidence of thrombosis at 10 years 18.5% *vs* 18%, P>.90). Finally, we analyzed the subgroup of "old" ET diagnosed according to 2008 WHO criteria. Of 358 "old" ET, 268 were reclassified as ET, 25 as MPNu and 65 as prePMF. The "old" ET reclassified as prePMF had a higher risk of overt myelofibrotic evolution compared to the "old" ET reclassified as ET (cumulative incidence of overt myelofibrosis at 10 years 9.7% *vs* 0%, *P*=.033).

Table 1.

Clinical features of patients according to the new 2016 WHO criteria

	ET (A)	MPNu (B)	prePMF (C)	*P*		
N°	269	26	109	A vs B	B vs C	A vs C
Sex (male/female)	105/164 (39%/61%)	7/19 (27%/73%)	55/54 (51%/49%)	.291	.047	.051
Age at onset, years, median (range)	53.1 (17.4-88.5)	44.3 (18.2-79.4)	54.7 (15.6-83)	.015	.019	.938
Hemoglobin, g/dL, median (range)	14.2 (8.4-17.7)	13.5 (12.1-15.8)	13.5 (8.5-17.1)	.101	.390	<.001
WBC count, x 10^9/L, median (range)	8.3 (4.2-28)	8.1 (5.3-10.6)	10.3 (4.7-23.5)	.117	<.001	<.001
PLT count, x 10^9/L, median (range)	677 (450-2810)	765 (414-1825)	823 (97.8-3000)	.057	.643	<.001
Splenomegaly, no. (%)	12 (4.5%)	0 (0%)	31 (29%)	.609	.001	<.001
LDH, mU/mL, median(range)	200 (77-472)	194 (100-220)	265 (66-935)	.133	<.001	<.001
Circulating CD34+ cells, x 10^9/L, median(range)	3.6 (0.4-13.2)	3.6 (0.6-26.5)	6.6 (0.2-94.1)	>.900	.001	<.001
Mutational status, no. (%)				.419	.774	<.001
JAK2 V617F	179 (66.5%)	17 (65.3%)	57 (52.3%)			
CALR	48 (17.8%)	7 (26.9%)	39 (35.8%)			
MPL	9 (3.4%)	1 (3.9%)	7 (6.4%)			
Triple neg	33 (12.3%)	1 (3.9%)	6 (5.5%)			

Summary/Conclusions: ET and prePMF diagnosed according to 2016 WHO criteria are two entities with a different clinical phenotype at diagnosis and a different outcome. The clinical phenotype at disease onset of MPNu and ET is similar.

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VALIDATION OF THE REVISED IPSET-THROMBOSIS SCORE IN 734 PATIENTS WITH WHO 2016-DEFINED ESSENTIAL THROMBOCYTHEMIA. REPORT OF THE REGISTRO ITALIANO TROMBOCITEMIE

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Background: The minimal effective treatment in Essential Thrombocythemia (ET) patients is tailored mainly on the basis of thrombotic risk scores (*primum non nocere).*The Revised International Prognostic Score for Thrombosis in ET (R-IPSET-Th) is based on different combinations of Age >60 yrs (Age >60), JAK2 V617F mutation (JAK2+) and Prior Thrombosis (PrTh+).
Aims: To validate the R-IPSET-Th in a cohort of ET patients reclassified according to the WHO 2016 criteria.
Methods: The web-based Registro Italiano Trombocitemie (RIT) recruited since 2005 patients with thrombocythemic bcr/abl negative chronic myeloproliferative neoplasms (MPN). ET patients (reclassified according to WHO 2016 criteria) with complete information (characteristics at diagnosis, antiplatelet and/or cytoreductive treatment, date and description of thrombotic events during the follow-up) were considered for this analysis. According to the R-IPSET-Th score, the patients were divided in 4 thrombotic risk groups: Very Low Risk (VLR: Age <60, absence of JAK2 mutations, no PrTh+), Low risk (LR: only JAK2+), Intermediate Risk (IR: only Age >60), High Risk (HR: PrTh+, or Age >60 with JAK2+). The rates (n and%, n/100 pt-yrs) of first thrombotic events during the follow-up (Th-FUP) were reported for each risk group. The thrombosis free survival (TFS, time from diagnosis to the first thrombosis) was determined for each risk group (Kaplan Meier analysis), and the curves were compared with the log-rank test. The concordance between the R-IPSET-Th score and the IPSET-Th score was evaluated (Harrell C concordance index).
Results: Overall, 734 ET patients were analyzed (females 62%). Data at diagnosis were: Age >60 in 286 (39%), JAK2+ in 417 (57%), and PrTh in 126 (17%) patients. Moreover: CVRFs in 66%, PLT >1000 x 10^9/L in 17%, and WBC >10 x 10^9/L in 21% of patients. The patients in the 4 R-IPSET-Th score risk groups were: VLR 193 (26%), LR 197 (27%), IR 79 (11%), HR 265 (36%). The median follow-up was 12, 12, 9, and 11 years, respectively (whole cohort, 11 years). The rates of treatment were: 88%, 94%, 92%, 91%, respectively (whole cohort, 91%), with anti-platelet drugs (mainly low dose aspirin); 71%, 62%, 95%, 95%, respectively (whole cohort, 80%), with cytoreductive drugs (mainly hydroxycarbamide). The Th-FUP were 103 (14.0%), with a rate increasing with the risk score (p <0.001): in VLR (n 15, 8%), in LR (n 20, 10%), in IR (n 12, 15%), in HR (n 56, 21%). The Th-FUP/100 pt-yrs increased (p <0.01) as follows: 0.60%, 0.79%, 1.61%, and 1.91%, respectively. TFS progressively decreased (p <0.001) from VLR group to HR group (Figure 1). In detail, the probability of TFS was 0.98, 0.97, 0.94, 0.88 at 5 years, and 0.85, 0.87, 0.78, 0.54 at 20years. The patient stratification according to the R-IPSET-Th and the IPSET-Th scores showed a concordance of 0.82 (Harrell C index).

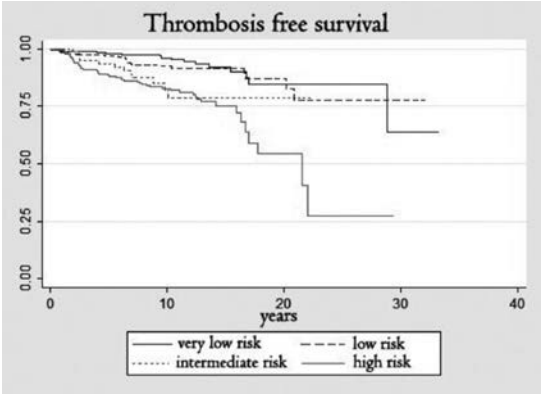

Figure 1.

Summary/Conclusions: In this study of the Registro Italiano Trombocitemie (RIT), we confirmed that the Revised International Prognostic Score for Thrombosis in ET (R-IPSET-Th) separated ET patients in 4 groups with increasing risk of thrombosis during the follow-up (p <0.001). According to the R-IPSET-Th score, an over-treatment seems to have occurred in this cohort of ET patients (anti-platelets in almost all cases, and cytoreduction in around 2/3 of VLR and LR cases), probably because other adjunctive risk factors have been considered.

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CORRELATIONS BETWEEN INFLAMMATORY BIOMARKERS AND INDIVIDUAL SYMPTOMS EXPRESSED BY MYELOFIBROSIS PATIENTS IN THE COMFORT-I TRIAL: ANALYSIS OF BASELINE ASSOCIATIONS AND CHANGES OVER TIME

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Background: Myelofibrosis (MF) is characterized by significant inflammation driven by clonal dysregulation and subsequent disruption of cellular signaling cascades. Studies have confirmed a close relationship between circulating inflammatory BMKs and baseline symptom burdens, along with the potential to mitigate symptoms upon improvement of these proteins (Dueck Blood, 2013). To date, no study has evaluated the correlations between elevated biomarkers (BMKs) and specific MF symptoms.
Aims: In this analysis of the phase-III placebo controlled COMFORT-I study we investigated the relationships between blood BMKs and individual MF symptoms at baseline and post-treatment with ruxolitinib.
Methods: Biomarker levels at baseline, week 4 and 24 were measured along with MF symptoms (MFSAF 2.0-Mesa JCO 2013; collected during blinded phase of COMFORT-I). Patients were randomized to ruxolitinib *vs* placebo. BMKs were assessed using Rules-Based Medicine, Inc. (Austin, TX) Human MAP panel. Associations between the individual symptoms measured within the MFSAF and log2-transformed biomarker data were investigated at baseline using Spearman correlations. Mixed models were used to assess symptom and BMKs changes over time. Models included terms for visit, arm, visit-by-arm interaction, age, sex, and body mass index (BMI).
Results: *Study Population*. A total of 309 subjects were randomized in COMFORT-I with median age of 68 (range 40-91). Approximately 46% of patients were female and 50% had primary myelofibrosis (61% high risk). All 309 subjects had BMKs measured at one or more of the three visits included in this analysis, with 308 having biomarker values paired with MFSAF symptom scores at the same visit. *Correlations of Baseline Biomarkers and Symptoms*. Total symptom score (TSS) statistically significantly (p<0.05) correlated with 20 BMKs at baseline (Table 1). For individual symptoms, spleen-related symptoms appeared to statistically significantly correlate more frequently with BMKs at baseline: abdominal discomfort (23 BMKs), feeling full (20 BMKs), and pain under left ribs (19 BMKs). Night sweats, itchiness, and bone or muscle pain significantly correlated with 15, 14, and 10 BMKs each. The BMKs with the strongest correlations (absolute Spearman correlation of at least 0.20 with p<0.001) with at least one symptom included APOA1, EPO, FERRITIN, MIP1A, and PSAF. *Associations with Symptom and Biomarker Change Over the Trial Course.* Twenty BMKs were significantly associated with TSS over time. Like at baseline, BMKs appeared to be more often statistically significantly (p<0.05) associated with spleen-related symptoms over time including 25 and 24 BMKs for abdominal discomfort and feeling full, respectively. Night sweats, pain under left ribs, bone or muscle pain, and itchiness were associated with 20, 12, 12, and 9 BMKs each. Strongest associations (p<0.001) between symptoms and BMKs over time included VCAM1 (4/6 symptoms+TSS), B2MICG (3/6 symptoms+TSS), LEPTIN (3/6 symptoms+TSS), TIMP1 (2/6 symptoms+TSS), TNFRII (2/6 symptoms+TSS), INTLK18 (2/6 symptoms+TSS), and VWD (1/6 symptoms).

Table 1.

Biomarkers	Total Symptom Score	Worst Abdominal Discomfort	Worst Feeling Full/Early	Worst Pain Under Rib On Left	Worst Bone Or Muscle Pain	Worst Itchiness	Worst Night Sweats
ATM/TRYP	0.14	0.13	0.12	0.12	0.17		0.14
ASPECTIN				**0.15**	**0.17**		
AFP	-0.13	-0.13	-0.13	-0.13	-0.19		
AMYLOID*		**0.12**					
APOA1	-0.21*	-0.22*	-0.25*	-0.20*	-0.17		
BDNF						**0.12**	
CA125AG						-0.13	-0.13
CEA							**0.12**
CKM8	-0.17	-0.18	-0.14	-0.19*			-0.16
CLCN3N		**0.15**	**0.14**				**0.12**
CRP					0.13		
ENA78			**0.12**			**0.12**	
ENDOTH1					-0.11		
EOTAXIN							**0.12**
EPO	-0.20*	-0.21*	-0.17	-0.17	-0.14	-0.12	-0.16
FERRITIN	**-0.20***	**-0.20***	**-0.17**	**0.15**	**-0.12**	**-0.17**	**-0.15**
FGF2B*				0.17			
GMCSF				**0.12**			
IGF1							0.15
IHUK15	**-0.13**			**-0.12**		**-0.13**	
INTLK1A	-0.16	-0.14	-0.13			-0.15	
INTLK18A	**0.13**	**0.14**					
INTLK2	-0.17	-0.18	-0.16	-0.16		-0.13	
INTLK3	**-0.12**	**-0.14**					
INTLK5	-0.17	-0.16	-0.15	-0.14		-0.16	-0.13
INTLK7	**-0.15**	**-0.15**	**-0.14**	**-0.13**		**-0.12**	
MCP1	-0.14	-0.15		-0.15			-0.12
MCP3	**-0.16**	**-0.17**	**-0.15**	**-0.14**		**-0.12**	**-0.15**
MIP1A	-0.17	-0.15	-0.14	-0.20*	-0.12		-0.20*
MMP3	**-0.17**	**-0.18**	**-0.18**	**-0.15**		**-0.11**	**-0.17**
PAI1						0.12	
PSAF	**-0.15**	**-0.15**	**-0.11**	**-0.13**		**-0.13**	**-0.20***
SHBG					-0.12		
TEC	**0.15**	**0.15**	**0.15**	**0.14**			
TIMP1		0.12	0.12				
TNFRII		**0.13**					
VCAM1	0.14	0.18	0.17				
VWD			**0.12**				

* Spearman p-value <0.001

Summary/Conclusions: Spleen-related symptoms demonstrate close association with BMKs at baseline as well as BMKs over time, after taking into account age, sex, BMI, and treatment. At baseline and over time, abdominal symptoms remained the most frequently correlated or associated symptoms with biomarker levels. More research is needed to determine the role of some of these key BMKs in altered cellular signaling and potentially targetable pathways for symptom reduction.

Platelet disorders: Basic

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NOVEL HETEROZYGOUS ITGB3 P.T746DEL MUTATION INDUCING SPONTANEOUS ACTIVATION OF INTEGRIN AIIBB3 CAUSES AUTOSOMAL DOMINANT MACROTHROMBOCYTOPENIA WITH ABNORMAL AIIBB3 LOCALIZATION

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Background: Congenital macrothrombocytopenia is a rare platelet disorder and its cause is genetically heterogeneous. Recently, integrin αIIb and β3 mutations have been identified in congenital macrothrombocytopenia patients with platelet aggregation dysfunction. Here, we found a novel, heterozygous *ITGB3* mutation in a pedigree and examined how this mutation contributed congenital macrothrombocytopenia.
Aims: To detect gene mutations responsible for the congenital macrothrombocytopenia in this pedigree and reveal the molecular pathophysiology.
Methods: Whole exome sequencing (WES) was performed to detect gene mutations. Expression and activation state of αIIbβ3 in platelets was evaluated by flow cytometry (FCM) and western blotting (WB). The effects of mutations on αIIbβ3 activation state, phosphorylation of FAK, and morphological changes were analyzed in transfected cells by WB and immunofluorescence staining.
Results: The patients were 56-year-old Japanese woman and 2 of her 3 sons. They had no bleeding tendencies and near-normal bleeding time (Duke's method). Hematological examination revealed their decreased platelet counts (58-86 x 10^9/l) with increase of mean platelet volume (12.8-14.5 fl). In all affected family members, giant platelets were observed on the peripheral blood smears. Platelet aggregation induced by ADP (1-10 μmol/l) and collagen (2 μg/ml) was obviously reduced although that induced by ristocetin (1.5 mg/ml) was within normal limit. The family pedigree indicates that the inheritance pattern is autosomal dominant. Common congenital macrothrombocytopenias, such as *MYH9* disorders, Bernard-Soulier syndrome and type 2B von Willebrand disease were excluded by the absent leukocyte inclusion bodies, normal ristocetin-induced platelet aggregation and normal platelet GPIb/IX expression, normal von Willebrand factor assays, respectively. WES revealed that all affected family members had a heterozygous *ITGB3* p.T746del mutation. FCM showed decreased surface expression level of αIIbβ3 in the affected member's platelets. However WB of platelet lysates showed that there was no difference in the total amount of αIIbβ3 among the affected and unaffected members and normal controls. FCM showed a constitutive activation of αIIbβ3 on the patient's platelets as reflected by the spontaneous binding of PAC-1 antibody. Immunofluorescence staining using CHO cells showed membrane localization of αIIbβ3 in wild-type αIIb/β3-expressing cells and cytoplasmic localization in αIIb/β3 (p.T746del)-expressing cells. *ITGB3* p.T746del led to spontaneous tyrosine phosphorylation of FAK and morphological changes, such as rhomboidal changes, elongated changes, abnormal cytoplasmic protrusions, and membrane ruffling, in transfected cells. FAK inhibitor (1,2,4,5-Benzenetetraamine tetrahydrochloride) hindered the localization change of αIIbβ3 and the morphological changes in transfected cells by dose dependent manner.
Summary/Conclusions: The autosomal dominant heterozygous *ITGB3* p.T746del mutation was found to be responsible for constitutive activation of αIIbβ3 in the patients' platelets as well as transfected cells. It is considered that *ITGB3* p.T746del mutation unclasps the highly conserved membrane proximal complex of αIIb and β3 cytoplasmic tails and renders the activated form. Activation of αIIbβ3 leads to phosphorylation of FAK causing morphological changes in transfected cells, which is considered to reflect abnormal thrombopoiesis leading to the production of giant platelets. We conclude that platelet aggregation dysfunction is due to decrease of αIIbβ3 expression on the platelet membrane surface due to cytoplasmic localization. These results suggest that the gain-of-function mutation around membrane region of αIIbβ3 leads to macrothrombocytopenia with impaired surface αIIbβ3 expression.

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CHANGES IN THE GENE EXPRESSION PROFILE OF IMMUNE THROMBOCYTOPENIA PATIENTS TREATED WITH ELTROMBOPAG

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Background: Eltrombopag (ETP) is an orally bioavailable, small non-peptide molecule thrombopoietin receptor agonist that stimulates platelet production by a mechanism similar, but not identical to, endogenous thrombopoietin. ETP interacts with the transmembrane domain of thrombopoietin receptor, initiating a JAK/STAT signaling pathway inducing the proliferation and differentiation of the megakaryocytes to increase platelets production.
Aims: To assess the gene expression profile (GEP) and the underlying signaling pathways modified before and during the ETP treatment in chronic immune thrombocytopenia (ITPc) patients.
Methods: ITPc patients (n=14) treated with ETP were evaluated. Complete response (CR) was defined as a platelet count of ≥100x10^3/mm^3 and treatment failure was defined as a platelet count of≤20 x10^3/$mm^3$for 4 consecutive weeks at the highest recommended dose of ETP, a major bleeding event, or the need to change therapy. RNA was isolated from mononucleated cells pre/post ETP treatment. The "paired" GEP of the ITPc patients included the semi-supervised analysis cluster samples before and after (28 day) the treatment with ETP to detect changes attributed to ETP. This paired GEP was showed in Figure 1. The GEP workflow consisted of the following steps: 28-paired samples were hybridized to GeneChip® Human Gene 2.0 ST Array (Affymetrix's). The robust microarray analysis (RMA) algorithm was used for background correction and normalization, while signal expression was calculated by significance analysis of each microarray to provide a robust statistical inference by a permutation method. P-values were provided and adjusted by multiples testing using a false discovery rate (FDR). The pathways and upstream regulators related with the most differentially expressed genes were analyzed by *in silico* analysis tools: Advaita Bio's iPathwayGuide (http://www.advaitabio.com/ipathwayguide)" and DAVID Bioinformatics Resources.
Results: The median age of the 14 ITPc patients enrolled in the study was 77 years (range, 35-87y). 64% patients (n=9) were treated with ETP after ≥2 lines of treatments. Only 3 patients were splenectomized. Median platelet (P) and white blood cell counts (WBC) increased after treated by ETP at day 28. (P and WBC pre: 14,15x10^3/mm^3 and 6,85x10^3/mm^3 *vs* P and WBC post: 132x10^3/mm^3 and 9,1x10^3/mm^3). All but two patients achieved CR (85,7%) and other 2 were considered failure of treatment. Regarding the gene expression profile, *in silico* analysis showed that the expression of 147 genes was modified after ETP treatment; all of them were overexpressed after treatment. Semi-supervised cluster analysis showed 2 groups: pre and post ETP treatment (Figure 1). Pathway analysis revealed that 38 genes were involved in the maintenance of hemostasis, most of them related to platelet activation (*PTGS1, GP1BA* or *GP6*). Interestingly, the paired GEP pointed out *E2F1 and GFI1B* as possible leaders of the increase of the megakaryopoiesis. Other signaling pathways overexpressed by ETP treatment are downstream routes of PI3K/Akt (*GFI1B, JAM3, ITGB3 and ITGA2B*) and platelet activation (*GP6, GP9, GP1BA* or *PTGS1*).

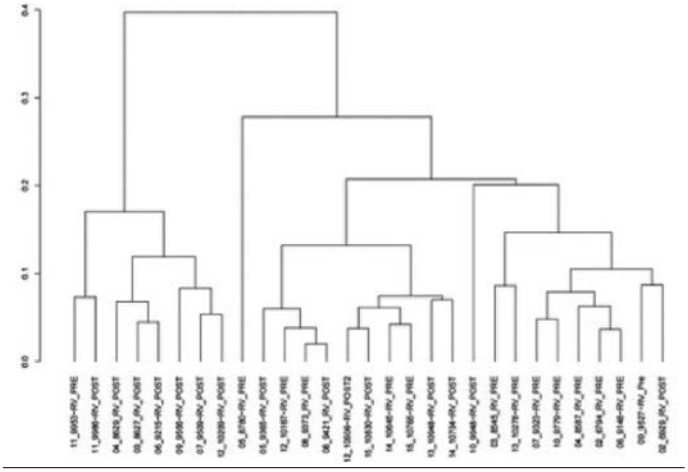

Figure 1.

Summary/Conclusions: In ITPc patients, ETP can induce overexpression of genes involved in platelet activation and megakaryopoiesis and also alter key/relevant/important-signaling pathways such as JAK/STAT and PI3K/Akt.

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DEFECTIVE PTEN REGULATION CONTRIBUTES TO B CELL HYPERRESPONSIVENESS IN CHRONIC IMMUNE THROMBOCYTOPENIA

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Background: Immune thrombocytopenia (ITP) is a complex autoimmune disease characterized by low platelet counts. The autoantibodies produced by autoreactive B cells against self-antigens, specifically immunoglobulin G (IgG)

antibodies against glycoprotein IIb (GPIIb)/IIIa and/or GPIb/IX, are considered to play a crucial role. B cell homeostasis and function are controlled by cell surface receptor-ligand interactions. The activation of PI3K is initiated by engagement of the pre-B cell receptor (BCR) and the BCR. The phosphatase and tensin homolog (PTEN) suppress the activity of the PI3K pathway. As a consequence, loss of PTEN function leads to excessive PI (3, 4, 5) P 3 at the plasma membrane and to recruitment and activation of Akt family members that potently drive cell survival and proliferation .PTEN regulates normal signaling through the B cell receptor (BCR). In immune thrombocytopenia (ITP), enhanced BCR signaling contributes to increased B cell activity, but the role of PTEN in human ITP has remained unclear. Both IL-21/IL-21R signaling and PI3K-PTEN molecules are involved in maintaining normal humoral immunity and deletion of autoreactive B cells. In this study, we want to determine whether abnormalities in PTEN might contribute to increase B cell responsiveness in this disease and IL-21 mediated PTEN induction was defective. Meanwhile, we want to evaluate the relation between the expression of PTEN in B cells and the prognosis of ITP, which will provide a theoretical basis of new treatment strategy for the ITP patient.

Aims: PTEN is involved in maintaining normal B cell function. Since B cell overactivity is characteristic ofImmune thrombocytopenia(ITP) we sought to determine whether abnormalities in PTEN might contribute to increase B cell responsiveness in this disease.

Methods: 1. This study recruited 28 newly-diagnosed CITP patients and 26 sex and age matched health volunteers as health controls(HC)Peripheral blood mononuclear cells were isolated from collected anti-coagulated blood. 2. Flow cytometry and real time quantitative PCR were used for detecting the level of PTEN from PBMC cells of HC and CITP patients. 3. The relationship between PTEN levels and the disease severity of CITP was analyzed. 4. PBMC cells were incubated with human rIL-2 rIL-21r CD40L or anti-IgM alone or in combination for 72h and after that the PTEN level was detected by flow cytometry .The proportion and surface activated marker of B cells were determined by flow cytometry

Results: 1. Compared to HC the expression of PTEN was diminished in each CITP B cell population except IgD-CD38low/-memory B cells. In addition PTEN mRNA was also decreased in ITP B cells. 2. The level of PTEN in B cells was slightly correlated with blood platelet count (p=0.008) and also directly correlated with the positive serum platelet-specific antibody (P=0.03). 3. The capacity of IL-21 to induce PTEN expression in B cells of HC was found by flow cytometry. Importantly.We found that CD40L and anti-IgM were the most potent inducers of PTEN expression in normal B cells, followed by IL-21 and IL-2. Neither IL-21 alone nor CD40L plus anti-IgM nor the three in combination stimulated PTEN protein up-regulation in B cells in CITP patients. 4. These immature B cells in CITP patients had a greater expression of CD95 but less PTEN compared to HC suggesting that down-regulation of PTEN was associated with an increasing proportion of immature B cells with a more activated phenotype in CITP patients (Figure 1).

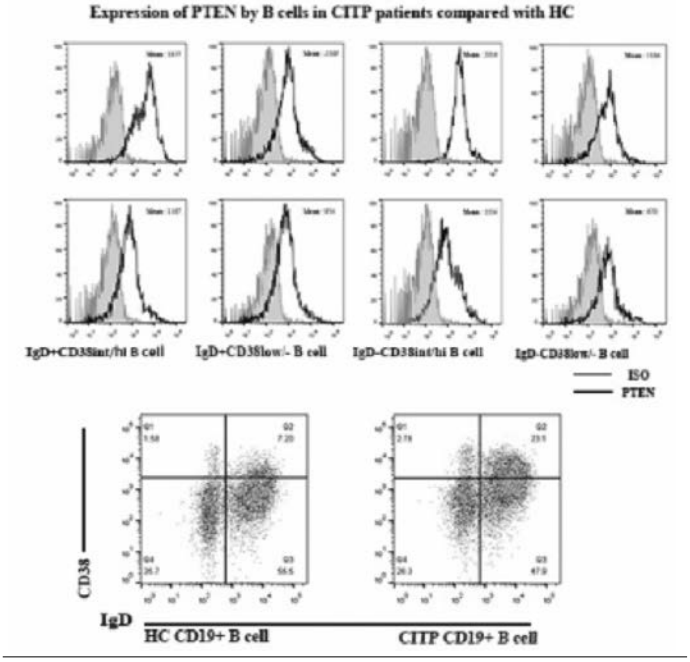

Figure 1.

Summary/Conclusions: Immune thrombocytopenia B cell showed decreased levels of PTEN and the decrease was associated with low platelet count and positive serum platelet-specific antibody.The capacity of IL-21 to induce PTEN was defect in CITP.Together,these data suggesting that the defective PTEN expression,regulation and function contribute to B cell hyper-responsiveness in CITP.

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A DECREASED INTRACELLULAR S1P LEVEL AND S1P RECEPTORS EXPRESSED ON MEGAKARYOCYTES POSSIBLY CONTRIBUTE TO DEFECTIVE PROPLATELETS FORMATION IN IMMUNE THROMBOCYTOPENIA

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Background: Immune thrombocytopenia (ITP) is a common autoimmune disorder characterized by increased bleeding tendency and isolated thrombocytopenia. The precise pathogenesis of the decreased thrombopoiesis in ITP remains unknown. It has been demonstrated that megakaryocytes (MKs) in ITP show impaired proplatelets formation (PPF) (Br J Haematol.2014;165:854-64). However, the pathogenesis of the impaired PPF in ITP is not entirely understood. Additionally, the lipid mediator sphingosine 1-phosphate (S1P) plays a critical role in megakaryocytic PPF in the bone marrow (BM) niche (J Exp Med. 2012;209:2137-40). It has been demonstrated that cell-surface S1P receptors (S1PR) on MKs trigger the activation of Gi/Rac GTPase signaling. Sphingosine kinase 2 (Sphk2) is the major isoenzyme regulating intracellular synthesis of S1P in MKs. Additionally, intracellular S1P influences the expression of Src family kinase (SFK) in MKs, including 6 members (Fyn, Lyn, Src, Yes, Fgr, and Hck), which mutually regulate each other with Rac GTPase.

Aims: To determine whether the S1P levels in both the BM niche and within MKs, as well as S1PR expression of MKs contribute to the defective thrombopoiesis in ITP through impaired PPF.

Methods: The PPF of ITP-MKs was measured by an *in vitro* PPF assay using HSCs from the BM. (FASEB J. 2010;24:4701-10). Additionally, all-trans-retinoic acid (ATRA), the S1pr1-specific agonist SEW2871, and extracellular S1P were used as interference factors. The concentration of S1P in the plasma and BM was analysed by ELISA. The concentration of intracellular S1P was measured using liquid chromatography mass spectrometry (LC/MS) analysis. Intracellular Sphk2 , SFKs and cell-surface S1PR were measured using PCR and western blotting. The location of Sphk2 was analysed by immunofluorescence using an anti-human Sphk2 antibody. The activities of Rac-GTP were quantified by pull-down assay.

Results: Significantly fewer numbers of proplatelet-forming MKs were observed in ITP cultures. The concentration of S1P in the plasma and in BM of patients with ITP was measured, of which the results showed no significant difference in the plasma/BM S1P ratio. Decreased expression of S1PR1 and S1PR4 was observed in ITP MKs. We found that downstream Gi/Rac GTPase signalling activated by S1PR1 was down-regulated. ITP-MKs exhibited decreased intracellular Sphk2, indicating less biosynthesis of intracellular S1P. Immunostaining of Sphk2 in ITP MKs was performed, showing that less Sphk2 was primarily localized to the nucleus of ITP MKs. Intracellular S1P of ITP MKs was further explored showing a decrease of megakaryocytic S1P production ascribed to significantly reduced Sphk2. Furthermore, the gene expression and protein levels of family of SFKs were examined in ITP MKs, and the overall mRNA expression was significantly limited in ITP MKs. The total Src protein levels and p-SFKs (phospho-tyrosine-418) were significantly reduced in ITP MKs, indicating significant reduction of SFK activity. ATRA, SEW2871 and extracellular S1P enhanced Rac GTPase activity and SFK expression, which rescued the defect of PPF in ITP.

Summary/Conclusions: Decreased intracellular S1P ascribed to significantly reduced Sphk2, results in down-regulated SFK expression and activity, and decreased S1PR1 and S1PR4 down-regulate Gi/Rac GTPase signalling in ITP-MKs. Therefore, abnormal S1P/S1PR possibly plays a role in the pathogenesis of impaired PPF in ITP, which may be therapeutically regulated by ATRA.

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ANTIBODY MEDIATED GLYCAN MODIFICATION: A POTENTIAL ROLE IN PLATELET DESTRUCTION IN AUTOIMMUNE THROMBOCYTOPENIA

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Background: Immune thrombocytopenia (ITP) is a bleeding disease caused by autoantibodies (AAbs) directed against platelet (PLT) glycoproteins (GP). A novel mechanism of antibody-mediated PLT destruction based on Fc-independent PLT clearance via Ashwell-Morell receptors (AMRs), which recognize glycan modifications on the surface of PLTs, has been suggested in mice.

Aims: In this study we investigated the effects of human AAbs from ITP patients on the glycan pattern of human PLTs and the consequent impact on their survival *in vivo*.

Methods: Monoclonal platelet antigen capture assay (MAIPA) and lectin binding assay (LBA) were used to analyzed sera from ITP patients and healthy donors. In LBA, after incubation with sera and PLTs from healthy donors, the modification in glycan pattern was investigated by flow cytometry using lectins; Ricinus communis agglutinin (RCA), Erythrina cristagalli lectin (ECL) and Peanut agglu-

tinin (PNA) that bind to galactose, N-acetyllactosamine and N-acetylgalactosamine residues, respectively. The NOD/SCID mouse model was used to study the impact of different glycan patterns on the survival of human PLTs.
Results: In this work 37 sera from ITP patients and 25 sera from healthy donors were analyzed. In the LBA, after incubation with AAbs, different patterns of glycan modification were observed. 17/37 sera caused a significant increase in PNA binding compared to heathy donors: (median fold increase (FI): 1.21, range: 1.08 – 1.40). 9/37 sera induced higher ECL binding (median FI: 1.02, range: 1.08 – 1.15). In contrast, 8/37 sera showed strong decrease in RCA binding (median FI: 0.52, range: 0.50 – 0.59). Sera from healthy donors did not induced significant change. Interestingly, not only GP-Ib/IX AAbs but also GPIIb/IIIa AAbs were able to modify glycan pattern. In NOD/SCID mice the administration of AAbs induced an accelerated clearance of human PLTs from the circulation. The destruction of human PLTs by ITP-AAbs was decreased but not completely prevented by a specific neuraminidase inhibitor that blocks glycan changes on PLT surface (survival of human PLTs after 5h: 48%, range 41-53% *vs* 29%, range 22-40%).
Summary/Conclusions: Our results demonstrate that AAbs from ITP patients are able to induce cleavage of glycan moieties on the PLT surface in distinct manners. Antibody-mediated modification of glycan patterns seems to contribute to AAb-mediated PLT destruction.

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NOVEL RUNX1 MUTATIONS IN FAMILIES WITH INHERITED THROMBOCYTOPENIA

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Background: Familial platelet disorder with propensity to acute myeloid leukemia (FPD/AML) is a rare autosomal dominant inherited thrombocytopenia (IT) caused by mutations in the hematopoietic transcription factor RUNX1; an important hallmark of this IT is the increased risk of developing myeloid neoplasms, such as AML and myelodysplastic syndromes (MDS). FPD/AML is caused by different mutations of *RUNX1* encoding the DNA binding subunit (known as core binding factor-alpha, CBF-alpha) of the CBF transcription complex. The N-terminus domain of RUNX1 (runt-homologous domain) mediates DNA binding and heterodimerization to CBF-beta, the other subunit of the CBF complex. The C-terminus of RUNX1 includes domains that are involved in transcription activation and repression. This IT is characterized by impaired megakaryopoiesis and moderate thrombocytopenia, with normal-sized and dysfunctional platelets.
Aims: To unravel the molecular basis of ITs and to improve our knowledge on the molecular basis and clinical-laboratory picture of FPD/AML.
Methods: Whole exome sequencing (WES) was performed in 86 propositi with an unknown IT after the diagnostic workup based on the most updated diagnostic algorithm for ITs (Clin Genet 2016;89:141). *RUNX1* variants detected by WES were confirmed by Sanger sequencing in the propositi and all available family members, which also undergo clinical-laboratory characterization. The study was approved by the Institutional Review Board of the IRCCS Policlinico S. Matteo Foundation; all patients gave written informed consent.
Results: We identified three pedigrees (families 1-3) with different *RUNX1* heterozygous mutations, all segregating with thrombocytopenia in the respective families: the novel variants c.578T>A and c.967+2_5del, and the known c.351+1G>A. The thirteen individuals carrying the *RUNX1* mutations had mild thrombocytopenia (platelet count ranging from 70 to 130 x 10^9/L) with mild bleeding tendency. Platelet sizes were within the normal range in all the six patients analyzed, and the serum level of thrombopoietin was normal or moderately increased. No specific morphological alteration of platelets was detected, except for moderate reduction in the alpha-granule content in family 1, confirmed by immunofluorescence analysis. The surface expression of the major platelet glycoprotein (GP) complexes GPIIb-IIIa and GPIb-IX-V was normal. In family 1 a moderate reduction of GPIa-IIa was detected, regardless of genotypes at the *ITGA2* locus. A defective aggregation was detected after platelet stimulation with collagen 4 mcg/ml and ADP 2 mcM in the five patients investigated; normal responses were obtained using collagen 20 mcg/mL, ADP 20 mcM and ristocetin 1.5 mg/mL, suggesting mild functional platelets defects. Of note, three patients from two families developed AML, with a prevalence lower than reported in literature, probably because of a different criteria of enrolment (*RUNX1* germline mutations are usually searched in ITs associated with AML). No solid/hematological cancer was reported in family 1.
Summary/Conclusions: FPD/AML is an IT lacking pathognomonic laboratory criteria: it is characterized by a mild functional defect and, much more importantly, by a normal platelet size, similarly to the other ITs predisposing to hematological malignancies (*ANKRD26* and *ETV6*-related thrombocytopenias). Given the importance of recognizing these diseases for patients counseling, follow-up, and therapeutic approach, we recommend a systematic screening for *RUNX1*, *ANKRD26*, and *ETV6* mutations in all patients with an autosomal dominant IT and normal platelet size.

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Abstract withdrawn.

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A SINGLE-ARM, OPEN-LABEL, LONG-TERM EFFICACY AND SAFETY STUDY OF SUBCUTANEOUS ROMIPLOSTIM IN CHILDREN WITH IMMUNE THROMBOCYTOPENIA

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Background: The use of romiplostim in children with ITP has been evaluated in phase 1/2 and 3 studies. Here we describe children with ITP who will receive open-label SC romiplostim for up to 3 years (y).
Aims: To assess platelet responses in children with ITP receiving romiplostim.
Methods: Eligible children, recruited in 16 countries worldwide, had ITP for ≥6 months, ≥1 prior ITP therapy, and platelet (plt) counts ≤30×10^9/L. Weekly SC dosing started at 1 µg/kg and was titrated in 1 µg/kg increments up to 10 µg/kg to target plt counts of 50-200×10^9/L. The primary endpoint was the% of time in the first 6 months with a plt response (plt count ≥50×10^9/L without rescue medication use in the past 4 weeks).

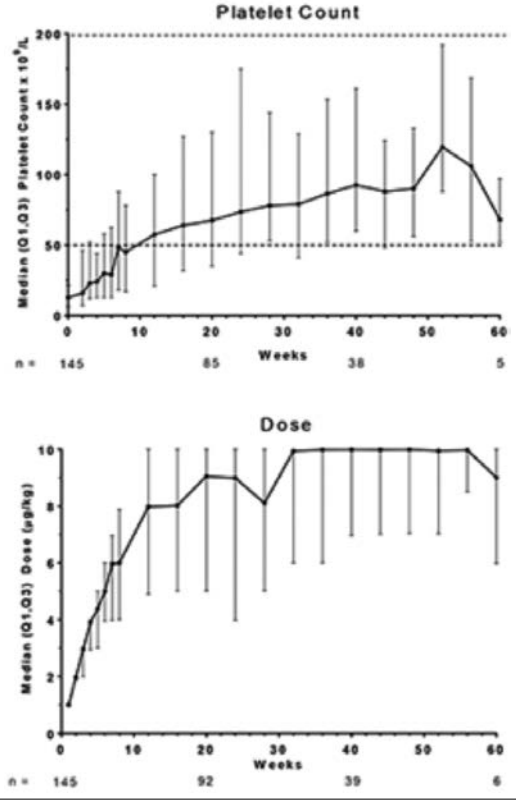

Figure 1.

Results: As of 15 Mar 2016, 145 patients received ≥1 dose. At baseline, median (min-max) age was 10 (2-17) y; 51% were female; 4% had prior splenectomy. Median (min-max) ITP duration was 1.9 (0.5-12.3) y and plt count was 13 (2-168)×10^9/L. The median (Q1, Q3)% of time with a plt response in the

first 6 months was 50% (0%, 83.3%); that of months 7-12 was 92% (33%, 100%). Overall, 80% (114/143) of patients had a plt response. The median (Q1, Q3)% of time with an increase in plt counts ≥20×10^9/L above baseline was 60% (25%, 84%). The median dose increased to 10 µg/kg by week 32. Median (min-max) treatment duration to date was 25 (1-67) weeks for a total exposure to date of 79 patient-years. Median (min-max) average weekly romiplostim dose over the course of the study was 6.1 (0.4-9.0) µg/kg. 32 patients (22%) discontinued treatment for lack of efficacy (n=17), required other therapy (n=5), patient request (n=4), noncompliance (n=2), adverse event (AE) (n=2) (interstitial lung disease in a 15 y old boy and abdominal pain, vomiting, and headache related to treatment per investigator in a 9 y old girl), administrative decision (n=1), and investigator decision (n=1). 34 (23%) patients received rescue medications. 15 (10.3%) patients had serious AEs (SAEs) including epistaxis (n=4), petechiae (n=2), decreased plt count (n=2), and thrombocytopenia (n=2). A case of abdominal pain was the only SAE deemed treatment-related by the investigator. CTCAE grade 3 bleeding was seen in 8 patients (6%) and included epistaxis (n=5), ecchymosis (n=2), petechiae (n=2), and 1 case each of hematemesis, hematoma, SC hemorrhage, injection site hemorrhage, and mouth hemorrhage. No grade 4 or 5 bleeding was observed. No neutralizing antibodies against romiplostim or TPO were identified. Of 30 patients with baseline bone marrow biopsies (bone marrow biopsies were obtained at European sites), all had modified Bauermeister scores of grade 0 (no reticulin) or 1 (fine fibers) and bone marrows typical for ITP. Of these 30 patients, 21 had evaluable on-study biopsies obtained after ~1 year of treatment, with no increases in 2 or more grades, findings of collagen, or bone marrow abnormalities (Figure 1).

Summary/Conclusions:
In this year 1 datacut of an ongoing open-label study of romiplostim in children with ITP, the% of time in the first 6 months with a platelet response was 50%, with 80% of children having a platelet response at some point on study. The median romiplostim dose reached 10 µg/kg and there were no new safety signals. No effects of romiplostim were observed on the bone marrow in the subset of patients with bone marrow biopsies. Future datacuts for years 2 and 3 in this study, the largest of romiplostim in children with ITP with 79 patient-years of exposure to date, will provide more information on platelet response, dose requirements, and safety.

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NOVEL THIENOPYRIDINES AS POTENT PLATELET INHIBITORS: FUTURE TREATMENTS FOR PLATELET HYPERACTIVITY DISORDERS?

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Background: Platelet hyperactivity is associated with a number of disorders including Acute Coronary Syndromes (ACS) and manifests as increased platelet activation and often inappropriate thrombus formation. The thienopyridine class of anti-platelet drugs, of which clopidogrel and prasurgrel are the most well known, target the $P2Y_{12}$ receptor on platelets, blocking the effects of the platelet agonist ADP. However, the effect of these drugs is variable amongst patients, with some patients responding well and some remaining at risk of thrombosis. This variability highlights a need for a refinement of this class of $P2Y_{12}$ inhibitor.
Aims: The aim of this study was to assess the efficacy of six novel thienopyridine derivatives synthesized by our group by examining their potential as *in-vitro* inhibitors of platelet function.
Methods: Healthy human platelets were isolated and incubated with novel thienopyridine compounds (DJ0081, DJ0199, DJ0021, DJ0206, DJ0171, DJ0097) (10µM, 30min) prior to stimulation with ADP (10µM) and analysis of alpha granule secretion (CD62P expression), GPIIbIIIa activation (PAC1 expression) and platelet leukocyte aggregate (PLA) formation using flow cytometry. Furthermore, light transmission aggregometry (LTA) was used to assess ADP-stimulated aggregation after these treatments. As clopidogrel is usually prescribed in combination with the COX-1 inhibitor acetylsalicylic acid (ASA), synergy of the novel compounds with ASA (30µM) was also analysed by LTA. All results were compared to ADP-stimulated samples and samples treated with clopidogrel (10µM, 30min) prior to ADP stimulation.
Results: All six novel compounds demonstrated a significant reduction in ADP-mediated platelet aggregation (P<0.001), CD62P expression (p<0.001), PAC1 expression (p<0.01) and PLA formation (p<0.05). These compounds were also shown to enhance the inhibitory effects of ASA. DJ0171 and DJ0199 were particularly potent, displaying greater inhibitory effect than clopidogrel.
Summary/Conclusions: The study demonstrates the potential for new thienopyridine compounds as modulators of platelet function and points to the possibility of future use in patients at risk of platelet hyperactivity and thrombosis.

Quality of life, palliative care, ethics and health economics 1

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PATIENT-REPORTED OUTCOMES AND HEALTHCARE RESOURCE UTILIZATION BEFORE AND DURING TREATMENT WITH ECULIZUMAB: RESULTS FROM THE INTERNATIONAL PAROXYSMAL NOCTURNAL HEMOGLOBINURIA REGISTRY

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare disease caused by somatic phosphatidylinositol glycan class A (*PIGA)* gene mutation in bone marrow stem cells. Clinical manifestations may include fatigue, abdominal pain, dyspnea, dysphagia, erectile dysfunction, anemia, sudden hemoglobin level reductions due to complement-induced hemolysis, and PNH-related complications such as thrombosis, chronic kidney disease, and pulmonary hypertension, all of which impair quality of life (QoL) and could impact survival. Eculizumab, a humanized monoclonal antibody approved for treatment of PNH, reduces intravascular hemolysis, thrombosis rates, and other PNH-associated comorbidities. The International PNH Registry (NCT01374360) is an ongoing prospective, multinational, observational study established to record the natural history of patients with PNH and collect data on long-term efficacy and safety of eculizumab treatment.
Aims: Analyze patient-reported outcomes (PRO) and healthcare resource utilization (HRU) before and during eculizumab treatment.
Methods: Patient assessment questionnaire (PAQ) data for patients with PNH who commenced eculizumab after Registry enrollment and had data available as of August 1, 2016, were analyzed. Patients had to have non-missing data on demographics, ≥1 recorded PAQ within 12 months prior to eculizumab initiation, and ≥1 PAQ recorded ≥6 months after initiation. Outcomes of interest included changes in QoL assessments (Functional Assessment of Chronic Illness Therapy [FACIT]-Fatigue score; EORTC QLQ-C30 score, disease symptoms, Karnofsky Performance scale, HRU, and missed work days.
Results: Of 4082 enrolled patients, 649 had non-missing data on demographics and initiated treatment with eculizumab as of August 1, 2016; 229 patients (55% female; 86% white; 74% from Europe) of the 649 met inclusion criteria for the current analysis. Median (min, max) interval between PNH disease start and initiation of treatment was 4.4 (0.1, 44.9) years. Clinically meaningful improvement in FACIT-Fatigue score (≥4-point increase) was reported by 53% of patients after initiating eculizumab (mean change, 5.2 points, Figure 1). Clinically meaningful improvement (≥10-point increase) was also observed in EORTC QLQ-C30 mean scores for global health/QoL (mean change, 15.1), role functioning (16.3), emotional functioning (12.1), and social functioning (13.9) subscales. PNH-related symptoms disappeared in 19–44% of patients who reported the symptom prior to eculizumab across all assessed symptoms except erectile dysfunction, which did not disappear in any of the 21 patients who answered this question both before initiation and during eculizumab treatment. Mean Karnofsky scale scores improved by 8.4 points after eculizumab initiation. HRU decreased for emergency room visits and number of missed work days while patients received eculizumab (incidence rate ratio [IRR] [95% confidence interval (CI)], 0.33 [0.20, 0.54] and 0.48 [0.25, 0.93], respectively) and increased for healthcare provider visits and hospital admissions (IRR [95% CI], 1.47 [1.22, 1.77] and 1.17 [0.60, 2.27], respectively).

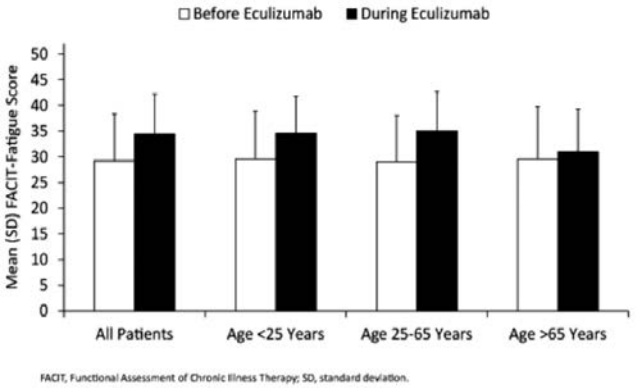

Figure 1.

Summary/Conclusions: In this cohort of patients from the International PNH Registry, treatment with eculizumab was associated with clinically meaningful improvements in PROs, including assessments of fatigue, global health status, patient functioning, and disease-related symptoms, as well as a decrease in emergency room visits and number of missed work days.

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ECONOMIC IMPACT OF INTRODUCING AGE-ADJUSTED D-DIMER CUT-OFF LEVELS IN THE DIAGNOSIS STRATEGY OF VENOUS THROMBOEMBOLISM

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Background: The diagnosis of venous thromboembolism (VTE) can be safely excluded in the case of D-dimer levels below a well defined cut-off level in patients with a low or intermediate pre-test probability (PTP), as the test negative predictive value (NPV) is close to 100%. As ageing is associated with increased D-dimer levels, the question arose whether D-dimer measurement was useful to rule out VTE in elderly patients.
Aims: The aim of the present study was to evaluate the clinical performance of a diagnosis strategy based on age-adjusted cut-off values calculated by multiplying the patient's age by 10 in patients aged over 50, and to evaluate its economic impact.
Methods: We included 1255 consecutive outpatients with non-high PTP of VTE referred to the emergency departments at 5 French centres (2 university hospitals, and 3 general hospitals, in whom D-dimer testing was prescribed. The same standardized procedure was used in the 5 centres *i.e.* D-dimer measurement in patients with a non-high PTP, and imaging techniques (usually computed pulmonary angiography in case of suspected PE and Doppler ultrasonography in case of suspected DVT) in the case of D-dimer above the cut-off level. D-dimer levels were evaluated using the same fully automated latex-based assay (HemosIL D-dimer HS500, Instrumentation Laboratory), the usual cut-off level for VTE exclusion being 500 ng/mL (fibrinogen equivalent units, FEU).
Results: VTE diagnosis was established by objective testing in 115 patients (9.2%): 88 of the 1082 patients referred for suspected PE (8.1%) and 27 of the 173 patents referred for suspected DVT (15.6%). D-dimer levels were above 500 ng/mL in all patients with VTE and in 521 of the 1140 patients without VTE (45.7%), leading to test NPV and sensitivity of 100%. The overall test specificity was 54.3%, even though it significantly decreased in an age-dependent manner over 60 years old. This is due to increased D-dimer levels in older patients particularly in those above 80 years. Using age-adjusted cut-off levels, calculated by multiplying the patient's age by 10, significantly improved the overall test specificity (60.2%). The NPV remained high (99.9%), even though a 78 y-old female with a low PTP of PE would have been misdiagnosed as her D-dimer level (540 ng/mL) was above 500 ng/mL but below the age-adjusted cut-off value. Such an improvement in test performance was found both in patients with suspected PE and DVT (Table). As such an increase in test specificity would have led to exclude VTE in a higher percentage of patients in the studied population, we evaluated the cost-effectiveness of both strategies, taking into account the local reimbursement rates of D-dimer testing, angiography and Doppler ultrasonography (16.20, 58.72, and 75.60 Euros respectively). The economic impact of the proposed diagnosis strategy was a decreased of 6.9% of total costs (45,023.4 *vs* 48,356.4 Euros) for PE diagnosis and 5.1% (9,909 *vs* 10,438.2 Euros) for DVT. If such an analysis was used in the US, where angiography and Doppler US were more expensive (648 and 226 US$ respectively), and D-dimer less costly (14 US$), the cost savings would have been even higher (-11.0% for PE, and -6.3% for DVT).
Summary/Conclusions: The use of age-adjusted cut-off levels for D-dimer, in patients aged over 50 years old, led to a significant increase in the test specificity, but correlatively to slightly decreased NPV and sensitivity. Even though some patients with D-dimer levels above 500 ng/mL but below the age-adjusted cut-off value could be misdiagnosed, such a strategy was found to be safe in our studied population with a high NPV (99.9%) and sensitivity (99.1%), and cost-effective.

P371

IMPACT OF CELLULAR THERAPY ON THE ECONOMIC BURDEN AND SURVIVAL FOLLOWING RELAPSE AFTER HLA IDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ACUTE LEUKEMIA AND MYELODYSPLASTIC SYNDROME

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Background: Relapse following allogeneic hematopoietic stem cell transplant (aHSCT) is associated to a very poor outcome and remains an unmet medical needs. The impact of treatment approach on costs and survival remains unknown. The development of innovative cellular therapy for the treatment of relapse following aHSCT may change its dismal outcome but the cost of such intervention has prohibited its large-scale development.
Aims: The objective of this study was to measure the economic burden associated with the management of relapse following aHSCT and to evaluate the impact of treatment choice on survival and health care costs.
Methods: A retrospective medical chart review was conducted at Maisonneuve-Rosemont Hospital (HMR) after research and ethic committee approval. Patients were selected using the Hematopoietic Stem Cell Transplant (HSCT) program database. Eligible patients were diagnosed with acute leukemia (AL) or MDS and relapsed following a HLA identical aHSCT between January 1st 2011 and December 31st 2014. Patients' and disease characteristics and relapse-related health care resource utilization were collected from the date of post transplant relapse until death or last follow-up. Canadian unit costs for each intervention/treatment were obtained from literature and governmental publications.
Results: During the study period, 645 HSCT were performed at HMR, 303 were allogeneic. A total of 36 patients met the inclusion criteria and were included in the analysis. 32 recipients were diagnosed with AL and 4 with MDS. Treatment approaches following aHSCT relapse were divided in three groups according to patient and physician choices: group 1 received supportive care (n=9), group 2 received chemotherapy or tyrosine kinase inhibitors (n=21) and group 3 received a cellular based therapy, either donor lymphocyte infusion (DLI) or a second aHSCT (n=6). The mean cost of care per patient per month was C$20,239 (SD=17,079). The median survival following relapse for the entire cohort was 12.4 months (SD=2.8). For group 1, 2 and 3, the mean cost of care per patient per month was C$17,436 (SD=16,447), C$22,914 (SD=18,474) and C$15,082 (SD=12,954), respectively. The median survival was 4.0 months (SD=2.0), 7.2 months (SD=1.6), and 44.6 months (SD=8.4), for treatment group 1,2 and 3 respectively (Figure1).

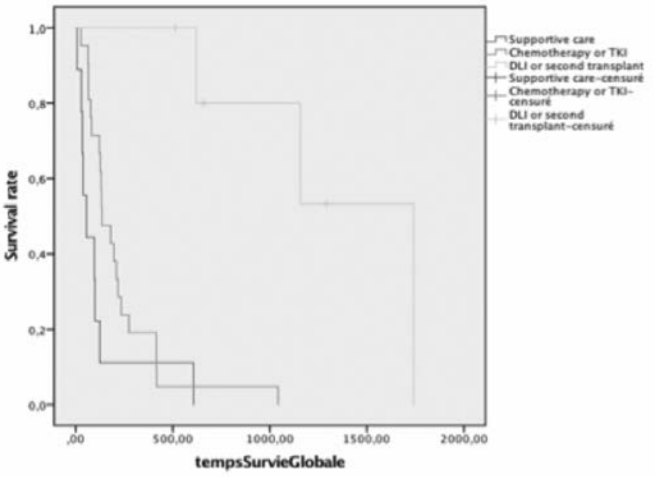

Figure 1. Survival according to treatment group.

Summary/Conclusions: Relapse following AHSCT is associated to a poor prognosis and survival and to significant use of health care resources. Despite the selection bias, only patients who received cellular based therapy, either DLI or another HSCT, enjoyed a prolonged survival. Healthcare resources devoted to the care of patients in relapse post AHSCT provide a comparative basis for cost efficiency analysis in the development of innovative cellular therapy.

P372

ACUTE MYELOID LEUKEMIA TREATMENT PRACTICE PATTERNS, HEALTHCARE RESOURCE UTILIZATION (HRU) AND COSTS IN A US COMMERCIALLY-INSURED POPULATION

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Background: AML is a rapidly progressive hematologic malignancy that accounts for 25% of adult leukemias in the Western world, with an estimated 5-year survival of 26%, and is associated with high HRU and costs.
Aims: To estimate HRU and costs among newly-diagnosed AML patients (pts) in a US commercially insured population by receipt of chemotherapy (CT) or stem cell transplant (SCT).
Methods: This was a retrospective observational study using the PharMetrics Plus® database. Pts were adults with AML (ICD-9-CM code 205.0x and corresponding ICD-10-CM codes) diagnosed between Jan 2007 and Jun 2016 (study period). Pts were excluded if: first AML claim was for remission/relapse;

not continuously enrolled for 12-months (mos) before the first AML claim (index date); evidence of acute promyelocytic leukemia anytime during the study period; missing enrollment information; or ≥1 hospitalizations during follow-up (FU) with missing cost. Pts were classified as treated or untreated, with treatment defined based on receipt of CT (inpatient or outpatient) or SCT. For treated pts, FU was partitioned into 2 periods: index date to 6 mos and >6 mos post index date. Mean HRU and costs over the FU period were calculated by receipt of treatment and, for treated pts, by time since index date.
Results: 10,197 pts met study criteria including 6,862 treated pts (67%) and 3,335 untreated pts (33%). Mean age was 55 and 60 years in treated and untreated pts, respectively. Mean follow-up was 19.3 mos in treated pts and 18.1 mos in untreated pts. Mean total costs were higher for treated pts ($386,711) *vs* untreated pts ($83,274). In treated pts, mean total costs were $166,156 during the first 6 mos (mean duration 3.9 mos), and $220, 555 during the remaining follow-up period (mean duration 19 mos). 26% of treated pts had SCT. Costs of inpatient and outpatient CT during the first 6 mos were $86,188, representing 22% of the total cost for treated pts (Table 1).

Table 1.

	Treated Pts			Untreated Pts
	All (n = 6,862)	Index date to 6 mos (n = 6,862)	6 mos post index date onward (n = 5,627)	All (n = 3,335)
SCT / RBC transfusions / platelet transfusions / GCSF, %	26.5 / 57.0 / 38.7 / 39.8	8.4 / 43.8 / 26.3 / 22.9	22.8 / 40.1 / 25.9 / 31.4	0.0 / 18.2 / 7.8 / 2.9
Office or outpatient visits, mean	108	36	72	39
Outpatient pharmacy claims, mean	72	16	56	46
Emergency department visits, mean	1.7	0.6	1.0	1.1
Hospitalizations, mean	3.6	1.6	2.0	1.1
Inpatient days, mean	49	25	24	11
Total costs, mean, $	386,711	166,156	220,555	83,274
SCT	54,526	16,699	37,827	0
Inpatient CT	102,999	67,849	35,150	0
Outpatient CT	35,827	18,339	17,488	0
Other inpatient	70,362	26,605	43,758	38,024
Other outpatient	102,058	31,939	70,119	21,816
Pharmacy	20,938	4,725	16,213	23,434

Summary/Conclusions: HRU and costs of managing AML pts are considerable, with greatest HRU and cost in pts receiving CT or SCT.

P373

HEALTH-RELATED QUALITY OF LIFE IN AL AMYLOIDOSIS PATIENTS WITH NERVOUS SYSTEM INVOLVEMENT

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Background: In light chain (AL) amyloidosis, misfolded light chains accumulate and cause progressive peripheral neuropathy (PN) and failure of critical organs such as the heart and kidneys. Consequently, a progressive, ascending sensorimotor neuropathy is often a related clinical finding.
Aims: This study describes disease characteristics and health-related quality of life (HRQoL) in AL amyloidosis patients with peripheral nerve involvement (AL-PN).
Methods: An online survey was administered to AL-PN (n=126) and non-nerve–affected (n=215) patients to assess patient characteristics and HRQoL (based on the SF-36v2® Health Survey [SF-36v2]). The survey measures eight domains including physical functioning (PF), role physical (RP) due to health-related physical limitations, bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role emotional (RE), mental health (MH), in addition to physical (PCS) and mental component summary (MCS) measures. Patient characteristics were compared using chi-square tests. Differences in symptomatic and HRQoL burden were tested with multivariable logistic and linear models, respectively. Differences in mean HRQoL between AL-PN and non-AL-PN patients were compared to established minimally important differences (MIDs).
Results: Compared to non-nerve–affected patients, greater proportions of AL-PN patients visited ≥6 doctors (42.1 *vs* 19.5%, p <0.001) and ≥6 specialists (24.6 *vs* 9.9%, p <0.001). AL-PN patients also had symptoms for ≥1 year prior to receiving a diagnosis (50.8 *vs* 39.1%, p=0.035), relative to non-nerve–affected patients. Nearly all AL-PN patients (97.6%) reported multi-system involvement. Gastrointestinal involvement was more prevalent in AL-PN patients *versus* non-AL-PN patients (68.3 *vs* 28.8%, p <0.001). There were greater odds of experiencing numbness (OR=4.23, 95% CI: 2.45–7.30, p <0.001) and fatigue (OR=3.09, 95% CI: 1.36–7.02, p <0.01) among AL-PN patients as compared to non-AL-PN patients, even after controlling for other types of organ involvement. Similar findings were observed for gastrointestinal symptoms, such as alternating bouts of constipation or diarrhea (OR=1.92, 95% CI: 1.12–3.34, p=0.019) and early satiety/feeling fullness in the stomach (OR=1.80, 95% CI: 1.03–3.16, p=0.04). With the exception of RE, MH, and MCS, there were significant differences in SF-36v2 scores among AL-PN patients as compared to non-AL-PN patients (p <0.05 for all). These significant differences also exceeded the thresholds for clinically meaningful differences between the two groups.
Summary/Conclusions: This study suggests that the burden of illness from AL amyloidosis may be greater for those with PN involvement *versus* those without. AL-PN patients also experienced more complicated journeys to diagnosis and significantly worse symptoms related to nervous systems and physical HRQoL. The SF36v2, a reliable and valid assessment of HRQoL in AL amyloidosis studies, was sensitive to differences in HRQoL between AL-PN and non-AL-PN patients. Future research should examine whether improvements in neuropathy symptoms following treatment subsequently lead to improvements in HRQoL among patients with AL-PN. These findings are helpful for patient-focused drug development and supportive treatments.

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ACCESS TO COMMUNITY CHEMOTHERAPY IMPROVES PATIENT QUALITY OF LIFE

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Background: Deciding how services for people with haematological cancers are to be delivered is going to be an important challenge in the coming years. Clinics have limited clinic capacity in terms of staff and bed space to cope with increased demand. In Wales many day units are already at capacity, overcrowded and have long waits for treatment. Ambulatory care, including diagnosis, observation, consultation, intervention, and rehabilitation, has the potential to improve the patient experience, if traditionally-based hospital services are moved into the community. We used a Mobile Unit – a 34-tonne articulated lorry which opens out to become a bespoke clinical space - to deliver treatments in a community setting to a range of haematology patients for a period of 12 months in South Wales.
Aims: We aimed to explore whether the administration of cytotoxic therapy on a Mobile Unit in a community setting for patients with haematological cancers could prove to be a safe and efficient alternative to hospital therapy, and in particular whether this model of service delivery would be acceptable to patients. Our target group was patients with myeloma, aiming for up to 20 a day once or twice a week.
Methods: The first drugs administered on the Mobile Unit were zoledronate infusions, followed by bortezomib. When twice weekly doses were required, patients collected an additional injection pack which they could self-administer in their own homes, thereby saving another trip into hospital. Immunoglobulin infusions, taking between 1-2 hours, were also administered. There was a consultant review clinic on board for patients receiving bortezomib which further reduced the numbers of hospital visits for patients and also a nurse-led Quality of Life clinic.
Results: In one year 548 treatments were administered on 91 days to a total of 54 individual patients. All 54 patients had a diagnosis of myeloma. 56% are female and 44% are male with an age range of 46 to 90 years of age, with 48% over 70 years of age. 37 patients are married and all but 4 classed themselves as White British. The greatest number of patients treated in a single day was 16. Patient experience was measured using self-administered questionnaires which 50 patients completed. 100% of patients thought it was convenient or extremely convenient having their treatment on a Mobile Unit. 98% felt safe having their treatment outside hospital and 92% said their experience was better than hospital. Patients could drive right up to the door of the Mobile Unit and the average time waiting from arrival to treatment chair was 2 minutes, with many people not having to wait at all. Uptake of the psychosocial support services was lower than expected with only 10 people opting for additional support. Any criticisms received focused on the locations we chose to site the Mobile Unit in relation to accessibility via public transport.
Summary/Conclusions: Treatment in the community alleviates the stress of treatment and with minimal waiting times it gives some patients the ability to maintain family life and where possible to continue to work. It is both feasible and acceptable to begin to ambulate many different sorts of treatments. The possibilities opening up for haematology include rituximab maintenance; community blood transfusions; delivering pentamidine for patients at risk of pneumocystis infection; late effects clinics for teenage and young adult cancer patients; and myeloproliferative neoplasm clinics, possibly near community pharmacies to facilitate dispensing medicines such as hydroxycarbamide.

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THE BUDGET IMPACT OF TREATMENT-FREE REMISSION FOR FIRST-LINE NILOTINIB OR GENERIC IMATINIB IN TREATING CHRONIC PHASE PHILADELPHIA-POSITIVE CHRONIC MYELOID LEUKEMIA

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Background: The 2013 European LeukemiaNet (ELN) guidelines recommend switching from first-line therapy as early as 6 months with poor molecular response (MR). They also recognize the possibility of treatment-free remission (TFR), requiring deep MR (MR^4 or $MR^{4.5}$). These emerging shifts in practice will dramatically change chronic myeloid leukemia (CML) treatment patterns. Occurring in parallel to this is the introduction of generic imatinib in Europe,

which will have a substantial price difference compared with nilotinib. However, given the possible changes in switching patterns and use of TFR, this price difference may not translate into a similar magnitude of difference in drug budget for first-line nilotinib *vs* imatinib due to better MR with nilotinib.
Aims: To estimate the budget impact for first-line nilotinib *vs* imatinib when considering generic imatinib pricing, early treatment-switching, and TFR.
Methods: A state transition model was developed to project the impact of ELN switching and TFR use on clinical outcomes and treatment costs. Analyses were run for 1000 patients with newly diagnosed CML, starting either nilotinib or imatinib, over a 5-year time horizon and using French drug pricing. It was assumed that all patients in the model would switch therapy (imatinib to nilotinib, and nilotinib to dasatinib) based on the failure criteria of the ELN guidelines. As such, ENESTnd trial data were re-analyzed to estimate switching based on MR. The model assumed that patients could enter first-line or second-line TFR after 36 months of continuous therapy where the last 12 months were at $MR^{4.5}$. Duration of first-line or second-line TFR was based on an extrapolation of ENESTfreedom and ENESTop treatment-free survival curves, respectively. Monthly drug costs were €2,952 for first-line nilotinib and €1,063 for generic imatinib, assuming a 50% discount to brand pricing.
Results: A greater number of patients in the first-line nilotinib arm remained on first-line therapy (690 *vs* 479 at 15 mos., and 542 *vs* 368 at 60 mos.); achieved $MR^{4.5}$ on first-line therapy (442 *vs* 248 by 60 mos.); entered TFR on first-line therapy (347 *vs* 183 by 60 mos.); entered TFR on either first- or second-line (494 *vs* 400 by 60 mos.); and was in any TFR at 60 months (293 *vs* 200). The incremental budget impact per patient for first-line nilotinib *vs* imatinib decreased each year from €16,482 in Year 1 to €377 in Year 5. Overall, the 64% lower drug acquisition costs per month of imatinib (€1,063) *vs* nilotinib (€2,952) provided only a 17% lower total budget impact over five years (€141,204 *vs* €170,002) per patient.
Summary/Conclusions: Results from the model considered more switching as per 2013 ELN guidelines, which resulted in greater and quicker switching than observed in ENESTnd. Overall, it was projected that compared with imatinib, patients who receive first-line nilotinib would have earlier and more sustained molecular response-requirements for TFR eligibility—and be subject to less treatment-switching. The model projected that less than 50% of patients would remain on first-line imatinib at 15 months. This would significantly reduce the budget benefits of a lower imatinib acquisition price. The budget impact between first-line imatinib and nilotinib would be further reduced by TFR, which occurred in the model more frequently in the nilotinib group. The superior efficacy of nilotinib and the associated differences in switching and TFR eligibility are predicted to substantially offset the lower unit cost for generic imatinib.

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GAH SCALE PREDICTS TREATMENT TOLERABILITY IN OLDER PATIENTS (>65 YEARS) DIAGNOSED WITH HEMATOLOGICAL MALIGNANCIES

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Background: The Geriatric Assessment in Hematology (GAH) scale is a newly developed tool that is intended to be an ancillary questionnaire to better categorize older patients diagnosed with common hematological neoplasms for intensive treatment in routine clinical practice. It is a brief (<12 min) and easy instrument, which takes into account 8 dimensions of geriatric assessment that were initially dichotomized into 0 or 1. The GAH scale has recently been shown to be psychometrically valid, responsive to clinical change, and able to predict survival.
Aims: To determine the weights for each dimension of the GAH scale and the cut-off points for the scale to be used as a tool to predict treatment tolerability in older patients diagnosed with myelodysplastic syndrome / acute myeloblastic leukemia, multiple myeloma, or chronic lymphocytic leukemia.
Methods: This was a multicenter, retrospective, observational study conducted at 14 Spanish sites. Prior participants of the GAH study were given treatment within 3 months after having completed the GAH scale were eligible for inclusion after giving informed consent.
A logistic regression model and a full multiple linear regression model were calculated to determine the weights for each dimension to find out its contribution to the final score; the ROC curve analysis was used to calculate the cut-off points that defined three groups: "go-on" (low probability to develop toxicity regardless of intensive or attenuated therapy), "slow-go" (high probability to develop toxicity with intensive therapy but low probability with attenuated therapy), and "no-go" (high probability to develop toxicity regardless of therapy).
Results: A total of 108 patients (women, 53.7%; median age [IQR], 78 [73-83] years) out of 360 included in the main study were evaluated. During treatment administration, 61 (56.5%) patients developed treatment-related toxicities, requiring discontinuation/modification of the initial therapy. The coefficients for the dimensions are: 7 for number of drugs, -10 for gait speed, 2 for mood, 23 for activities of daily living, 6 for subjective health status, 27 for nutrition, -5 for mental status, and 1 for comorbidities. The sum of the GAH scale score, ranging from 0 to 66, plus a factor derived from the treatment intensity (34 points for intensive therapy or 0 for attenuated) leads to a maximum score of 100 points, with a cut-off point set at 47. Figure 1 shows the GAH scale score for treatment toxicity prediction and the classification of patients according to their score. Among the 97 (89.8%) patients that received intensive therapy, 26 patients were classified as go-on, 48 as slow-go, and 23 as no-go. The proportion of patients that developed toxicities for each group was 34.6%, 56.3%, and 78.3%, respectively with a statistically significant difference (*P*=0.002).

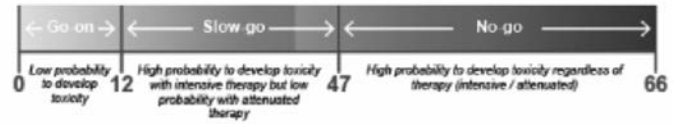

Figure 1.

Summary/Conclusions: The GAH scale appears to have the potential to give guidance for election of individual treatment regimens. By identifying elderly patients at high risk to develop toxicities, it may help to choose low-toxicity combinations, to avoid harmful therapies and to identify those patients that could benefit from more intensive treatment. Nonetheless prospective studies with larger populations should be performed to confirm these findings and to try to determine particular cut-off points for different diseases.

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NUTRITIONAL NEEDS AND PREFERENCES OF MYELOPROLIFERATIVE NEOPLASM PATIENTS: PHASE IA OF THE NUTRIENT STUDY

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Background: Cachexia, weight loss, and malnutrition in cancer patients are important contributors of adverse outcomes of cancer patients. MPN patients have abnormal cytokine expression (*e.g.*, IL-1, IL-6, IL-8, and TNF-a) that contributes to symptom burden (*e.g.*, fatigue, pruritus, night sweats, bone pain) and nutritional deficiencies (e,g., hypocholesterolemia, hypoalbuminemia, weight loss). Diets rich in fruits, vegetables, legumes, whole grains, fish, nuts, and low-fat dairy products are associated with a decrease in inflammatory (*e.g.*, TNF-a, IL-6, and CRP) and thrombotic markers (*e.g.*, homocysteine, fibrinogen; Chrysohoou 2004, Smidowicz 2015). To date, no studies have evaluated the nutritional needs or preferences of MPN patients in regards to dietary change.
Aims: The aim of this project was to determine the nutritional needs and preferences that will help inform the creation of a tailored MPN dietary intervention.
Methods: An internet-based survey was hosted by the Mayo Clinic Survey Research Center and promoted on multiple MPN-based forums, Facebook pages and websites during February of 2017. The survey included data on demographics, MPN characteristics, nutritional habits, supplement use, and symptom burden using the MPN-SAF TSS/MPN-10 (Emanuel 2012).
Results: *Demographics and symptom burden*: 919 international MPN patients responded to the online survey of which 22.5% were diagnosed with MF, 37.1% with PV, and 37.4% with ET. Respondents represented MPN patients from 37 countries, although the highest proportion of respondents were from the United States (48.8%), United Kingdom (32.7%), Australia (6%), and Canada (3.6%). Average MPN-SAF TSS score was 33.6 (SD=17). *Dietary Habits:* 22.5% of patients endorsed having food allergies or intolerances (Table 1). 31.4% of individuals followed a specific diet or had dietary restrictions. On average, MPN patients ate 2.1 meals outside the home per week. Among those taking supplements, 16.6% took supplements with the intent of reducing inflammation. Half (47.5%) of these individuals felt that the supplements they used made them feel better. Approximately 15% of respondents had tried alternative medicine to help treat their MPN. Among these, 44.8% were under the care of a naturopath and 60.2% endorsed that their treatment plan included dietary change. *Nutritional Education Preferences*: Overall, 34.4% of patients endorsed

using diet to help control their symptoms or MPN disease. Patients most often utilized books (28.2%), websites (27.1%), health care providers such as physicians, NPs or naturopaths (28.2%), online forums (23.2%), friends (12.2%), nutritionists(9.5%), phone or tablet applications (9.1%), or videos (4.2%) for nutritional education. The vast majority (95.9%) of MPN patients endorsed being willing to eat only certain foods if it helped to control symptom burden and or could help their MPN to stabilize or reduce the risk of their MPN getting worse (98.0%).

Table 1.

Frequency of dietary allergies, intolerances, restrictions and supplement use among a large international cohort of MPN patients (N=919).

Food Allergies and Intolerances	Frequency among all respondents
Milk	8.3%
Wheat	6.9%
Fruit	4.1%
Shellfish	2.8%
Soy	2.3%
Peanuts	1.7%
Egg	1.4%
Tree Nuts	1.4%
Fish	1.3%
Dietary Restrictions	
Low salt	6.6%
Gluten-free	6.5%
Mediterranean diet	6.0%
Vegetarian	5.7%
Low fat	5.3%
Anti-inflammatory	5.1%
Lactose intolerant	3.9%
Supplement Use	
Vitamin D	28.7%
Multivitamin/mineral	22.7%
Calcium	16.5%
Vitamin B12	13.8%
Curcumin	8.8%
CoQ 10	7.6%
Digestive enzymes	7.2%
Antioxidants	6.4%

Summary/Conclusions: There remains an unmet need for symptom burden improvement in low-risk MPN patients or among those who have reoccurrence of symptoms while on JAK inhibitor therapy. Nutritional interventions for MPN patients have not previously been investigated and have the potential to be paired with traditional interventions to allow MPN patients to self-manage symptom burden. This study represents the first evaluation of MPN-related nutritional habits and preferences. These results will be used to inform the creation of an MPN nutritional intervention with the goal of improving symptom burden and reducing inflammation.

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DO PHYSICIANS NEED HELP TO ADEQUATELY INFORM AND SUPPORT PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA? RESULTS FROM A QUALITATIVE STUDY IN GREECE

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Background: Despite recent progress in prognostication and management, chronic lymphocytic leukemia (CLL) remains unpredictable at diagnosis, while virtually incurable, posing challenges to physicians on how to properly communicate the actual nature of the disease. Moreover, the great majority (~85%) of patients do not need treatment at diagnosis, creating a major cognitive dissonance between the perception of leukemia diagnosis and the "wait & watch" strategy usually applied, that may become a major reason of anxiety and quality of life (QoL) impairment for patients and frustration for physicians. Evidently, both patients and physicians need parameters that would allow co-decision making tailored to each particular case.
Aims: To identify physicians' needs in order to improve their communication skills and thus facilitate CLL patient empowerment through a patient-centeredness model.
Methods: An in-depth qualitative study with semi-structured interviews was conducted within hematologists (n=30) all over Greece. Data collection was considered as completed when saturation was reached *i.e.* no new themes emerged as assessed by the investigators. Content analysis was performed separately by a hematologist and a health psychologist with 98% inter-rater reliability score.
Results: None of the participants had ever received formal communication training but rather adopted the techniques of senior physicians or developed their own through experience alone, thus frequently doubting their approaches (n=12/30, 40%). The most popular communication technique mentioned was adaptation of the quality and quantity of information provided according to each patient's characteristics (n=29/30, 96.7%); followed by the use of caregivers as mediators for the communication of difficult issues (n=24/30, 80%); balance of realism and hope (n=21/30, 70%); careful choice of wording (*e.g.* lymphocytosis instead of leukemia) (n=18/30, 60%); gradual disclosure (n=17/30, 56.7%); and, descriptions through pictorial representations or metaphors (n=16/30, 53.3%). Even though physicians did not systematically assess patients' anxiety and depression levels, they often found themselves dealing with patients' emotions (n=29/30, 96.7%) through lengthy discussions. With regards to decision making, some mentioned that physicians should make all the decisions (n=9/30, 30%) and that patients are not always willing to take part in the decision-making process (n=8/30, 26.7%), while others were keener on stirring patients towards a decision (n=15/30, 50%), taking into account patients' preferences (n=10/30, 33.3%). Most physicians felt uncomfortable delivering bad news such as initial diagnosis, relapse and poor prognosis (n=25/30, 83.3%). Self-reported needs included (i) communication skills training (n=20/30, 66.7%); (ii) psychological support (n=7/30, 23.3%); and, (iii) working in a multidisciplinary team (n=8/30, 26.7%).
Summary/Conclusions: In the absence of structured communication guidance there is great uncertainty among physicians concerning their skills on communicating CLL nature and handling difficult situations, leading to distress endangering their engagement in a healthy relationship with the patient. Additional studies are warranted at European level for identifying physician needs in different countries aiming at improving their communication skills to support and empower CLL patients for participating in their own care and enhance their QoL.

Stem cell transplantation - Clinical 1

P379

OUTCOME OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR PATIENTS WITH ACUTE LEUKEMIA ABOVE 70 YEARS OF AGE: ON BEHALF OF THE ACUTE LEUKEMIA WORKING PARTY OF THE EBMT

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Background: The average age of patients (pts) with AML is about 67 years. Historically, many of these pts were not considered as viable candidates for allogeneic transplantation (HCT) because of concerns about increased transplantation-related toxicity and excessive non-relapse mortality (NRM), a challenging problem especially in older individuals. However the development of reduced-intensity conditioning (RIC) regimens and the improvement in HCT supporting care allowed the successful application of HCT in older pts with AML.
Aims: Compare outcome of allo SCT in acute myeloid leukemia AML patients aged above 70 years with that of younger patients.
Methods: AML patients aged between 50 and 90 years old receiving a first or second allo SCT between 2004 and 2014 with MSD or UD donor were included in the study. Comparison of outcomes of patients aged above 70 with that of patients between 50-70 years were performed for the whole group and separately according to disease status at SCT (CR1, CR2, above).
Results: Altogether N=16874 pts were included in the study, N=713 were aged above 70 years old (median 72, IQR 71-73) and N=16161 between 50 and 70 (median 59, IQR 55-63) . Older pts were more often male (62 *vs* 55%, p<0.001), had more often secondary AML (42% *vs* 28%, p<0.001), more advanced disease (42% *vs* 27%, p<0.001), more often peripheral blood stem cell grafts (96 *vs* 91%, p<0.001), more often unrelated donors (79% *vs* 59%, p<0.001) and poorer Karnofsky score (36% below 90 *vs* 29%, p<0.001), received more often reduced intensity conditioning (80 *vs* 63%, p<0.001). Incidence of acute GVHD II/IV, III/IV, chronic GVHD and relapse were the same in the two groups in multivariate analysis. Non-relapse mortality (NRM) at two years was 34% (95%CI 31%>38%) in pts above and 24% (25%>32%) in those below 70 years of age (p<0.001). Overall survival and leukemia-free survival (LFS) at 2 years was 38% (95%CI 34-42) *vs* 50% (95%CI 49-50) p<0.001 and 33% (95%CI 29-37) *vs* 44% (95%CI 43-45) in the two groups, respectively (p<0.001). Among pts in CR1, 2 years survival was 43%(95%CI 37-51) *vs* 57%(95%CI 56-58) (p<0.001), in CR2 it was 36% (95%CI 27-27) *vs* 52% (95%CI 50-54) (p=0.002) and in advanced disease 35% (95%CI 29-41) *vs* 33% (95%CI 31-34) (p=0.36) in pts above and below 70 years of age, respectively. Among pts older than 70 years of age a Karnofsky score <80% was associated with improved survival and LFS in multivariate analysis (HR 0.7 95%CI 0.5-0.9 , p=0.005 and HR 0.7 95%CI 0.5-0.9 , p=0.003 respectively).
Summary/Conclusions: In AML with CR1, CR2 status at allo SCT, pts above 70 years of age have worse NRM, survival and LFS compared to pts 50-70 years of age. In pts above 70 years of age Karnofsky score is of significant importance for outcome.

P380

BLOOD BAALC AND MN1 COPY NUMBER ASSESSMENT BY DIGITAL DROPLET PCR PRIOR TO ALLOGENEIC TRANSPLANTATION PREDICTS RELAPSE IN ACUTE MYELOID LEUKEMIA PATIENTS

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Background: Acute myeloid leukemia (AML) patients (pts) that relapse after allogeneic stem cell transplantation (HSCT) have a dismal prognosis. Identification of pts at high risk of relapse may allow preemptive therapy & improve outcomes. At diagnosis high expression of the AML associated genes *BAALC* (brain and acute leukemia, cytoplasmic) & *MN1* (meningioma 1) adversely impact on AML pts outcomes, but little is known about their usability for residual disease detection. Recently, we demonstrated a higher cumulative incidence of relapse (CIR) for pts with high pre-HSCT *BAALC* copy numbers in 82 AML pts (ASH 2016, #517). Until today no study assessed the prognostic impact of *MN1* copy numbers prior to HSCT.
Aims: To assess the prognostic impact of peripheral blood (PB) pre-HSCT *BAALC* & *MN1* copy numbers in an expanded set of AML pts in hematologic CR using digital droplet (dd) PCR.
Methods: We identified 118 AML pts (median age at HSCT 64 [range 31-76] years [y]) in first (55%) or second complete remission (CR; 23%) or CR with incomplete recovery (22%) with PB prior to HSCT (median 7, range 0-29 days) available. All pts received non-myeloablative (NMA) conditioning (fludarabine 3x30 mg & 200 cGy total body irradiation). At diagnosis karyotypes & *NPM1*, *CEBPA* gene mutations (mut) & presence of *FLT3*-TKD & *FLT3*-ITD were assessed. Quantification of *BAALC* & *MN1* normalized to *ABL1* copy numbers in pre-HSCT PB of the AML pts & in PB of healthy controls (n=7, median age 63 [range 40-82]y) was performed by ddPCR. Median follow up after HSCT for pts alive was 1.8y.
Results: European LeukemiaNet (ELN) 2010 classification was 20% favorable, 25% intermediate-I, 24% intermediate-II, 31% adverse. AML pts & healthy controls did not differ in age (*P*=1), sex (*P*=1) or mean *BAALC* (*P*=.37, Figure 1A) or *MN1* (*P*=.96, Figure 1B) copy numbers. *BAALC* & *MN1* copy numbers correlated well in pts (R=.80) & healthy controls (R=.75). The previously determined cut-off of 0.14 *BAALC* copy numbers (in 82 pts; ASH 2016, #517) defined pts with high (27%) & low (73%) pre-HSCT *BAALC* copy numbers. A cut-off of 0.74 *MN1* copy numbers was determined using the R package 'OptimalCutpoints' & defined pts with high (12%) & low (88%) pre-HSCT *MN1* copy numbers. Applying these cut-offs, 71% of the pts had low *BAALC* & *MN1* copy numbers & 10% had high *BAALC* & *MN1* copy numbers, 2% had high *MN1* but low *BAALC* & 17% had high *BAALC* but low *MN1* copy numbers. Pts with high & low pre-HSCT *MN1* copy numbers did not differ significantly in pre-treatment characteristics or remission status at HSCT (CR *vs* CRi) while pts with high pre-HSCT *BAALC* copy numbers were less often in CRi at HSCT (*P*=.02). Both high pre-HSCT *BAALC* & *MN1* copy numbers significantly associated with higher CIR (*P*=.02, Figure 1C & *P*<.001, Figure 1D, respectively). In multivariate analyses, high pre-HSCT *BAALC* (Hazard Ratio [HR] 2.5, Confidence Interval [CI] 1.1-5.7, *P*<.001) & high pre-HSCT *MN1* copy numbers (HR 5.6, CI 2.6-12.2, *P*<.001) retained their prognostic impact on CIR after adjustment for ELN 2010 genetic risk groups.

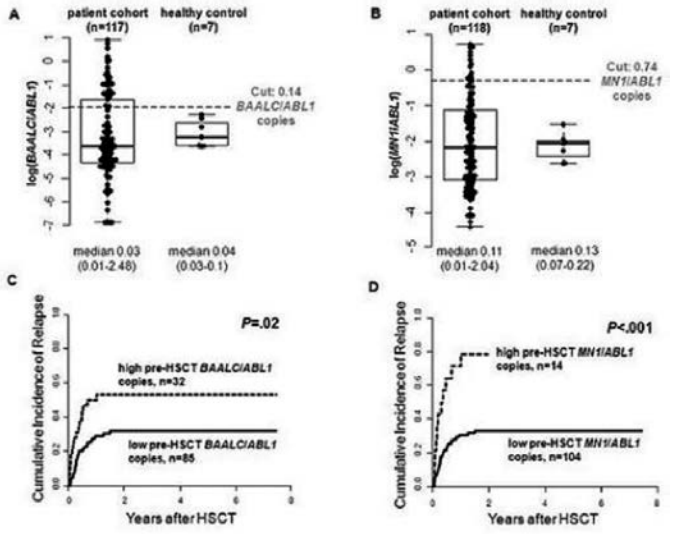

Figure 1.

Summary/Conclusions: High pre-HSCT copy numbers of *BAALC* & *MN1* associated with higher CIR in univariate & multivariate models and might indicate residual disease burden in these AML pts. High copy number pts should be closely monitored for relapse in the post-transplant period. Prospective clinical trials are needed to validate the determined cut-offs, to evaluate if *BAALC* or *MN1* copy numbers or a combination of the genes represents the most suitable prognosticator pre-HSCT and whether AML pts with high pre-HSCT *BAALC* or *MN1* copy numbers benefit from additional pre- or post-HSCT treatment.

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THE USE OF BPX-501 DONOR T CELL INFUSION (WITH INDUCIBLE CASPASE 9 SUICIDE GENE) TOGETHER WITH HLA-HAPLOIDENTICAL STEM CELL TRANSPLANT TO TREAT CHILDREN WITH HEMOGLOBINOPATHIES AND ERYTHROID DISORDERS

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Background: Allogeneic HSCT from either an HLA-identical sibling or an unrelated donor is a potentially curative treatment for patients with hemoglobinopathies and erythroid disorders (ED), such as Thalassemia Major (TM),

Sickle Cell Disease (SCD) and Diamond-Blackfan Anemia (DBA). Bertaina et al (Blood, 2014) have previously shown that abTCR depleted haplo-transplantation in children with multiple types of non-malignant disorders was feasible. An ongoing Phase I/II trial evaluates the safety and efficacy of post-transplant infusion of donor T-cells transduced with the iC9 suicide gene (BPX-501 cells). (ClinicalTrials.gov identifier: NCT02065869). The iC9 vector contains the sequence for the CD19 marker, so that the BPX-501 cells (CD3+/CD19+) can be tracked in peripheral blood. We report on 15 children with hemoglobinopathies and ED.

Aims: This study was performed to determine the clinical impact of infusing BPX-501 T cells post αβ T-cell depleted haplo-identical HSCT in pediatric patients with hemoglobinopathies.

Methods: Fourteen patients were transplanted from a parent and one patient was transplanted from a sibling. Conditioning regimen included busulfan, thiotepa and fludarabine. Low dose ATG was administered to prevent graft-*versus*-host disease (GvHD) and graft failure. No post-transplantation GvHD prophylaxis was given. Median follow-up is 387 days (range 126-631 days). Six patients were males and nine females, and median age at diagnosis and at HSCT was 0.8 and 8.9 years (range 2.5-19.2), respectively. Two patients had DBA and four with SCD. All 9 TM patients were βo/βo, and among the those with TM, 4 patients belonged to class I and 3 to class II of the Pesaro classification. All 15 patients were transfusion-dependent and receiving iron-chelation therapy before haplo-HSCT. 13/15 patients maintained full donor chimerism. The patients with secondary graft failure were re-transplanted from the same donor and maintained full donor chimerism.

Results: All patients are alive and well with no Treatment Related Mortality (TRM). Initial discharge was at a median of 23.5 days(range 14-55) and there were two patients re-hospitalized at 30, 163 days respectively. Grade I/II skin acute GvHD occurred in four patients and one patient had acute skin GvHD Grade IV. No chronic GVHD was observed. Median time to neutrophil recovery was 14 days (range 10-32 days), while median time to platelet recovery was 11 days (range 8-12 days). Median time to last RBC transfusion was 8 days (5 – 34 days). See Figure 1 for individual Hemoglobin levels. Median time of infusion of $1x10^6$ BPX-501 T cells/kg was 14 days after HSCT (range 10-26). BPX-501 cells expanded after infusion and still persist in all patients. Immune reconstitution with normal cellular and humoral immunity present at 180 days post HSCT. All patients remain transfusion-free with a median hemoglobin of 11 or greater after 6 months.

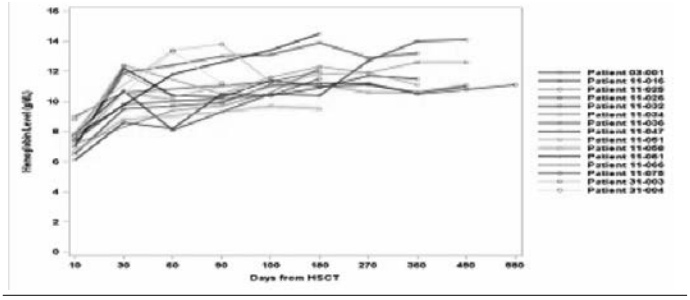

Figure 1.

Summary/Conclusions: These data suggest that Haplo-HSCT combined with infusion of BPX-501 T cells with a suicide gene may be a safe and curative option for children with hemoglobinopathies and ED who lack a matched donor. Infusion of gene modified T cells with an inducible suicide mechanism, combined with selective αβ T-cell depletion, offers the potential to rapidly reverse GvHD and eliminate the need for the use of GvHD prophylaxis. Additionally, this approach results in rapid hematological and immune reconstitution for Haplo-HSCT recipients.

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EXCELLENT RESPONSE, LOW TRM AND GOOD SURVIVAL IN PATIENTS WITH THERAPY-REFRACTORY AGVHD AFTER TREATMENT WITH EQUIPOTENT MSCS OF A SERUM-FREE MSC-BANK GENERATED FROM POOLED BM-MNCS OF MULTIPLE DONORS

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Background: All clinical data published thus far on the use of MSCs were generated using cells expanded from individual bone marrow donors hence suffer from huge inter-donor differences in MSC generation, expansion and immunomodulatory potential. To control these variables and to be able to administer to all patients highly similar MSC products, we established a proprietary pooling procedure and generated a large bank of MSC end-of-passage-1 vials from which end-of-passage-2 MSC products are expanded for clinical use. The manufacturing process is fully GMP-compliant and generates an animal serum-free MSC product with near-identical phenotype and in-vitro immunomodulatory potency. Importantly, they showed a significantly higher allosuppressive potential than the mean allosuppressive potential of MSCs generated from individual donors. All tested individual MSC doses were equipotent in suppression of the alloantigen-driven reaction in mixed lymphocyte reactions (Kuçi *et al.* Haematologica 2016: 101 (8); 985-994).

Aims: A "hospital exemption" issued by the national regulatory authority Pau-Ehrlich-Institute (Number: PEI: A.11748.01.1) licenses the clinical use of these products for patients with steroid refractory GVHD. On the basis of this licence patients were with severe GVHD were treated who were either non responsive to any other treatment or who were resistant steroids after 7 days.

Methods: Using these standardized MSC products altogether 52 patients were treated between December 2014 and December 2016. Patients were male (n=31, 60%) or female (n=21, 40%) and were transplanted for leukemia (n=38, 73%) or non-malignant (n=14, 27%) diseases. Median age was 8y (range: 0.5-52 years). Stem cell source MSD (n=9, 17%), MUD (n=33, 63%) or MMFD (n=10, 19%) and derived from BM (n=27, 52%), peripheral blood (n=24, 46%) or cord blood (n=1, 2%). Patients were suffering from aGVHD grade II (n=3, 5.5%), III (n=14, 27%), or IV (n=31, 60%) or extensive cGVHD (n=4, 7.5%). Acute GVHD occurred at a median time of 52 days (5-280 days) after transplant. Patients received in weekly intervals up to four MSC infusions after having failed to respond to the treatment with either two lines (n=10, 19%), three lines (n=20, 38%), four lines (n=10, 19%), 5 lines (n=7, 13%), six lines (n=4, 8%), or 7 lines (n=1, 2%) of immune suppressive drugs.

Results: Response was defined as either complete response (CR) in patients who showed complete resolution of all signs of GVHD, partial response (PR) in patients who showed one overall GVHD grade less according to the Glucksberg criteria, or non response (NR) at day 28 after first MSC transfusion.

At day +28, 12 patients (23%) achieved CR, 29 patients (57%) PR (overall response= 80%), 9 patients (17%) NR, and in 2 patients (4%) no data were available at day +28. At the last follow up of GVHD**,** 29 patients (56%) were in CR, 13 patents (25%) in PR, 9 patients (17%) in NR, and for 1 patient (2%) no data were available. At 2 years these response rates resulted in a non-relapse mortality rate (NRM) of 27±6%, cumulative relapse incidence (CIR) of 14±4%, and an overall survival rate (OS) of 59±9%. Patients with aGVHD III and IV had an OS survival probability at 2 years of 77±12% and 59±35%, respectively thus dramatically in excess of expected survival rates for patients with such severe aGvHD. There was no difference between younger (n=40) and older patients (n=12) than 16 years.

Summary/Conclusions: Treatment with standardized equipotent MSCs from the "FRANKFURT MSC-BANK" offers an excellent chance to overcome treatment-resistant and steroid-refractory acute GVHD.

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HIGHER PEAK TACROLIMUS CONCENTRATIONS AFTER ALLOGENEIC TRANSPLANTATION INCREASE THE RISK OF ENDOTHELIAL CELL DAMAGE COMPLICATIONS

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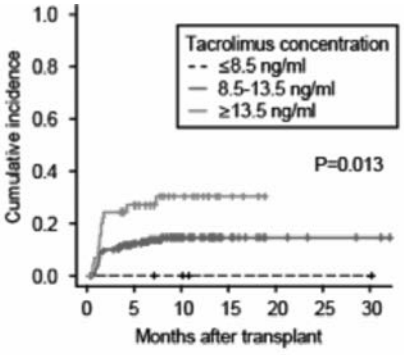
Background: Noninfectious transplantation-related complications (TRC) such as GVHD and endothelial cell damage (TRC-EC) including sinusoidal obstructive syndrome (SOS), transplant-associated microangiopathy (TAM), idiopathic pneumonia syndrome (IIP) are dismal complications after allogeneic hematopoietic stem cell transplantation (allo-HSCT).Calcineurin inhibitors such as tacrolimus (TAC) have been broadly utilized to manage post-transplant complications. Higher blood levels of TAC were expected to reduce the risk of GVHD, but may increase the risk of endothelial damage. Since TRC-EC often developed in patients with GVHD, it is difficult to judge appropriateness of immunosuppression clinically.

Aims: Here we evaluated the impact of TAC blood levels upon TRC-EC occurrence and prognosis after allo-HSCT.

Methods: Two hundred sixty-one consecutive patients (pts) who received TAC as a GVHD prophylaxis after allo-HSCT at our institute from 2009 to 2015 were candidates for this retrospective study. Pts who received haploidentical allo-HSCT

and pts with unavailable TAC concentration data were excluded. A total of 253 pts was eligible. All pts received standard GVHD prophylaxis by continuous intravenous (iv) TAC with starting dose of 0.02 mg/kg/day from 1 day before allo-HSCT (day -1) and iv methotrexate on day 1, 3, 6 at dose of 10 mg/m2, 7mg/m2, 7 mg/m2, respectively. TAC dosage was adjusted to target the serum concentration of 8-12 ng/ml until at least day 30 and then tapered. TAC was rapidly tapered in case of the pathological diagnosis of TAM.TAC serum concentration was sequentially examined triad weekly until day 35 at least. The primary endpoint of this study was to evaluate the cumulative incidence of TRC-EC in relation to weekly mean/peak TAC concentration. Secondary endpoint was OS.

Results: Median patient age was 45 years (16-68). The risks of disease were standard in 168 and high in 85 pts. Forty pts were diagnosed of TRC-EC; SOS: 7 pts (median onset: day 24 (17-40)), TAM: 27 pts (median onset: day 40 (25-128)), IIP: 6 pts (median onset: day 161.5 (46-223)).The cumulative incidence of TRC-EC at day 250 was 0.16 (95%CI, 0.12-0.21). Univariate analysis showed that higher peak TAC concentrations (PTC) during day 22-28 (P=0.013), male pts (P=0.018) and grade 3-4 acute GVHD (P<0.01) were significantly associated with the development of TRC-EC. Higher mean TAC concentrations (MTC) during day 0-7 was correlated with higher incidence of TRC-EC, but not significant (P=0.069). In multivariate Fine-Gray analysis, high PTC during day 22-28 (HR: 1.92, 95%CI, 1.07-3.45, P=0.028) and grade 3-4 acute GVHD (HR: 8.33, 95%CI, 4.18-16.59, P<0.01) remained associated with TRC-EC occurrence. The probability of OS at 15-months was 0.56 (95%CI, 0.47-0.64). Univariate analysis showed that pts diagnosed TRC-EC (P<0.01), pts older than 50 (P<0.01), pts with high disease risk (P<0.01) and pts who received reduced intensity conditioning regimens (P=0.010) were significantly associated with poor OS. PTC and MTC at any time-period were not significant factors for OS. By Cox proportional-hazards regression models, TRC-EC diagnosis (HR: 1.90, 95%CI, 1.16-3.11, P=0.011) and high disease risk at transplant (HR: 1.76, 95%CI, 1.14-2.73, P=0.011) were significantly associated with poor OS (Figure 1).



The cumulative incidence curves of TRC-EC.
The peak tacrolimus concentrations (PTC) during 22-28 days after allo-HSCT were divided into low (≤8.5ng/ml), intermediate (8.5-13.5 ng/ml) and high (≥13.5 ng/ml). The incidence of TRC-EC was proportionally increased with the PTC.

Figure 1.

Summary/Conclusions: Higher peak TAC concentrations during 22-28 days after allo-HSCT increased the risk of TRC-EC. And the development of TRC-EC was associated with poor OS.

P384

IMPACT OF CONDITIONING REGIMEN ON OUTCOMES OF T-REPLETE HAPLO-IDENTICAL TRANSPLANTATION FOR PATIENTS OVER 45 YEARS-OLD WITH AML: A STUDY ON BEHALF OF THE ACUTE LEUKEMIA WORKING PARTY OF THE EBMT

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Background: T-cell replete haplo-identical stem cell transplantation (haploSCT) is a valid therapeutic option for adult patients (pts) with high risk acute myeloid leukemia (AML) lacking a sibling or unrelated donor. However the impact of reduced intensity (RIC) *vs* myeloablative (MAC) conditioning regimens is not conclusive as no randomized study addressing this question is yet available.

Aims: In the present study we compared the outcome of RIC and MAC in pts with AML older than 45 years (yrs) undergoing haploSCT. The aim of the study was to confirm the efficacy and feasibility of RIC among a population for which the choice of conditioning intensity is more related to center strategy than pts comorbidities or disease status.

Methods: We retrospectively compared the outcomes of 614 pts with de novo or secondary AML transplanted between 2007 and 2015 from an haplo-identical donor using either RIC (n=365) or MAC (n=249) regimens. Age was categorized in three subgroups (45-55 yrs, 55-60 yrs, >60 yrs). Patients receiving a previous allogeneic transplantation were excluded. RIC was defined according to EBMT definitions.

Results: The median follow up for MAC and RIC was 24 and 20 months, respectively and the median year of transplant was 2013 for both. Pts receiving a RIC were older (55 yrs in MAC *vs* 61 yrs in RIC, p=<10^{-4}). Secondary AML was more frequent in RIC *vs* MAC (31% *vs* 22%) while 77% of MAC and 68% of RIC were transplant for de novo AML, p=0.01. No differences were found on disease status and Karnofsky performance status (KPS) at transplant: pts were in CR1 (MAC: 44%, RIC: 40.5%), CR2/3 (MAC: 17%; RIC: 17%) or had active disease (MAC: 40%; RIC: 43%), p=0.68; 12% of pts in both groups had KPS<80, p=0.95. The most frequently used MAC regimen was TBF (56%), while in RIC it was miniTBF (27%) and low dose TBI+Fludarabine (24%). RIC regimens were more frequently associated with the use of peripheral blood as stem cell source (MAC 42% *vs* RIC 55%, p=0.002). Post-transplant cyclophosphamide was used in 69% of both RIC and MAC, p=0.39. Main outcomes were not different according to conditioning regimen: at 2 years RI was 26% *vs* 32% (p=0.29), NRM 31% *vs* 34% (p=0.62), aGVHD II-IV 24% *vs* 31% (p=0.05), and cGVHD 27% *vs* 26% (p=0.94), LFS 42% *vs* 39% (p=0.17), OS 46% *vs* 39% (p=0.15), GRFS 36% *vs* 28% (p=0.10) for MAC *vs* RIC, respectively. The results according to RIC and MAC were not different in any of the three age subgroups. 338 patients died; main causes of death were infections and GVHD to be followed by disease recurrence. In multivariate analysis, the type of conditioning regimen was not associated with risk of relapse or treatment failure: RI (HR: 1.22, p=0.28), NRM (HR: 0.92, p=0.63), acute GVHD grade II-IV (HR: 1.14, p=0.48), chronic GVHD (HR: 1.26, p=0.30), LFS (HR: 1.03, p=0.77), GRFS (HR: 1.07, p=0.55), OS (HR: 1.05, p=0.68). Disease status was associated with outcomes (active disease *vs* CR): RI (HR: 2.44, p=<10^{-4}), LFS (HR: 1.75, p=<10^{-4}), GRFS (HR: 1.72, p=<10^{-4}), OS (HR: 1.71, p=<10^{-4}) as well as KPS>90: NRM (HR: 0.53, p=0.0002), LFS (HR: 0.67, p=0.001), GRFS (HR: 0.74, p=0.014), OS (HR: 0.62, p=0.0002).

Summary/Conclusions: In our study no differences were found between RIC and MAC regimens for haplo-SCT in adults with AML including the age stratified populations. Disease status and performance status were the major predictors of transplantation outcome, while conditioning intensity had no effect. These results may serve as the background for a well design randomized study comparing RIC *vs* MAC for haplo-SCT in adult pts with AML.

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ROLE OF UPFRONT ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH AGGRESSIVE ADULT T-CELL LEUKEMIA-LYMPHOMA: A DECISION ANALYSIS

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Background: Patients with aggressive adult T cell leukemia-lymphoma (ATL) have a dismal outcome even with an intensive chemotherapy. There is still controversy regarding the indication of up-front allogeneic hematopoietic stem cell transplantation (allo-HSCT) as no prospective randomized controlled trial (RCT) has been conducted due to a rarity of patients with ATL even in Japan.

Decision analysis is a computerized modeling analysis which can simulate the clinical outcomes of different therapeutic strategies and identify an appropriate therapeutic strategy.

Aims: The aim of this study is to compare the life expectancy (LE) of chemotherapy followed by up-front allo-HSCT to that of chemotherapy alone using decision analysis in patients with aggressive ATL using database constructed by a nationwide survey.

Methods: We constructed a Markov decision analysis model to compare the outcomes in 2 therapeutic strategies: chemotherapy followed by upfront allo-HSCT *vs* chemotherapy alone. The transition probabilities between each health states were calculated from the database of 1,792 patients and patients were stratified into low-, intermediate- and high-risk groups according to the risk stratification system which we developed previously (Fuji S *et al.* 18th International Conference on Human Retrovirology). The model simulated the LE, quality-adjusted LE (QALE) and survival curve after diagnosis of aggressive ATL. Since QoL data for patients with aggressive ATL are lacking, estimates from a similar decision analysis study of patients with acute myeloid leukemia were used. In terms of the timing of up-front allo-HSCT, it was set as all patients receive up-front allo-HSCT from 2 to 6 months if ATL did not progress before allo-HSCT. We used the TreeAge Pro 2016 software package for decision analysis (TreeAge Software Inc., Williamstown, MA).

Results: In all patients, up-front allo-HSCT was associated with higher LE in comparison to chemotherapy alone (2.26 years *vs* 1.75 years). Stratified into 3 groups according to the prognostic scoring system, LE of up-front allo-HSCT was higher compared to that of chemotherapy alone in the intermediate- (2.27 years *vs* 1.66 years) and high-risk groups (1.50 years *vs* 0.91 years). The estimated survival curve depicted by TreeAge showed the superiority of up-front allo-HSCT as shown in Figure 1A-D. The Monte Carlo simulation showed that the probability of superiority of up-front allo-HSCT was 100% in all patients, 97.1% in the low-risk group, 100% in the intermediate-risk group, and 100% in the high-risk group in terms of LE, and was 99.8% in all patients, 75.2% in the low-risk group, 100% in the intermediate-risk group, and 100% in the high-risk group in terms of QALE.

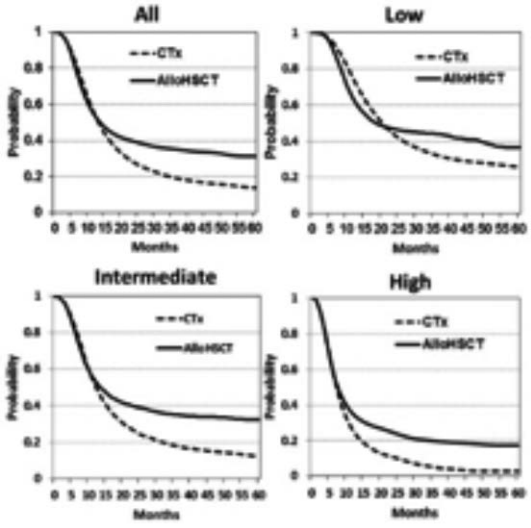

Figure 1.

Summary/Conclusions: Based on decision analysis, up-front allo-HSCT was associated with higher LE and QALE in the intermediate- and high-risk groups in comparison to chemotherapy alone in patients with aggressive ATL. In the absence of prospective randomized controlled trials, our results suggest that up-front allo-HSCT for aggressive ATL is the favored treatment strategy in the intermediate- and high-risk groups.

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OUTCOMES OF THIOTEPA BASED REDUCED-INTENSITY CONDITIONING VERSUS STANDARD REDUCED-INTENSITY CONDITIONING IN ADULT PATIENTS UNDERGOING DOUBLE-UNIT CORD-BLOOD HEMATOPOIETIC STEM CELL TRANSPLANT

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Background: Cord blood transplantation (CBT) is an established alternative source for hematopoietic stem-cells in patients without matched donor. However, the most commonly used high-dose total-body-irradiation (TBI) myeloablative conditioning (MAC) results in high treatment related mortality (TRM). Non-myeloablative and reduced-intensity conditioning (RIC) have been studied to decrease TRM and provide curative chance to the elderly and those with comorbidities. However, these strategies are associated with higher relapse-rate and graft rejection. A novel-RIC using addition of thiotepa and higher dose of TBI to standard RIC has shown to result in sustained donor engraftment. Our study compares transplant-related-outcomes in patients who underwent first double-unit CBT with standard-RIC regimen of fludarabine (Flu, 200mg/m^2), cyclophosphamide (Cy, 50mg/kg), and TBI (200cGy or 300cGy) *versus* this standard-RIC regimen with addition of thiotepa (10mg/kg) and increased dose of TBI (400cGy).

Aims: 1. To compare transplant related outcomes in CBT recipients who received *standard-RIC* (FluCyTBI) to those who received *novel-RIC* (FluCy with addition of thiotepa and increased dose of TBI). 2. To identify optimal conditioning regimen in patients undergoing UCT.

Methods: After IRB approval, consecutive patients undergoing CBT from 08/2009 to 08/2016 were evaluated and data retrospectively abstracted. Patient selection, graft-*versus*-host disease prophylaxis and transfusions were per institutional standards and conditioning regimens were compared as described.

Results: Of the 99 patients who underwent allogeneic double-CBT, 52 received standard-RIC and 47 received novel-RIC. Median age at transplant was 67 years (range, 24-74) and 54 years (range, 25-67) in standard-RIC and novel-RIC cohort respectively. Acute myeloid leukemia was the major indication for transplant in both cohorts. Median hematopoietic stem-cell transplant comorbidity-index (HSCT-CI) was 3 (range, 0-6) and 1 (range, 0-6) in standard-RIC and novel-RIC groups respectively. Four patients suffered engraftment failure (2 in each cohort). Median neutrophil engraftment was 13 days (range, 6-42) and 21 days (range, 12-43) while median platelet engraftment was 37 days (range, 26- 70) and 38 days (range, 24-74) in standard-RIC and novel-RIC groups respectively. Fifty-three suffered acute-GVHD which occurred in 21 (40%) patients (grade 2-4: n=15, 29%; grade 3-4: n=2, 4%) in standard-RIC group and in 32 (68%) patients (grade 2-4: n=29, 62%; grade 3-4: n=5, 11%) in novel-RIC group. Chronic-GVHD (cGVHD) occurred in 18 patients (n=7, 14% in standard-RIC; n=11, 23% in novel-RIC group). The one-year cumulative incidence of relapse was 36% (n=15) in standard-RIC while it was 15% (n=5) in novel-RIC cohort. Median relapse free survival (RFS) was significantly improved in novel-RIC cohort compared to standard-RIC (HR, 0.32, CI:0.11- 0.76, p=0.01). Median RFS was 29 months in standard-RIC cohort while median RFS was not reached in novel-RIC cohort. The one-year cumulative incidence of transplant related mortality (TRM) was 22% (n=10) in those who received standard-RIC while it was 16% (n=7) in those who received novel-RIC. TRM was not significantly different between the standard-RIC and novel-RIC cohorts. Median follow-up in standard-RCI cohort was 9.3 months (range, 0.16- 79) and 13 months (range, 1.4- 36) in novel-RCI cohort. The overall survival (OS) was significantly better in novel-RCI cohort compared to standard-RCI (HR 0.49, CI: 0.25- 0.94, p= 0.03). Median OS was 17 months in standard-RIC cohort while median OS was not reached in novel-RIC group (Figure 1).

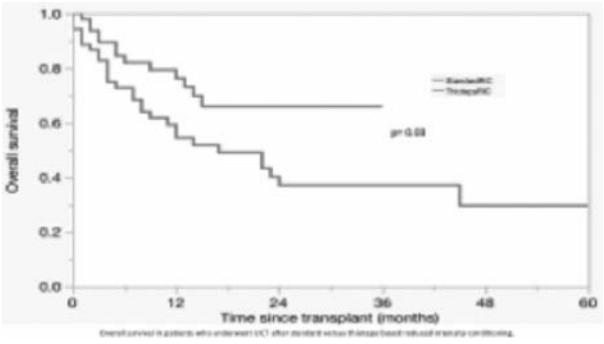

Figure 1.

Summary/Conclusions: In our study, RIC consisting of FluCy with addition of thiotepa and increased dose of TBI in patients undergoing double-cord UCT was associated with improved OS and improved RFS without increase in TRM as compared to standard RIC. While older and more comorbid patients might experience increased TRM with the thiotepa based regimen, these data suggest that consideration of this regimen may be appropriate in fit, older patients.

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INTERFERON-A IS EFFECTIVE FOR TREATMENT OF MINIMAL RESIDUAL DISEASE IN PATIENTS WITH ACUTE LEUKEMIA AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Post-transplant relapse is a major cause of transplant failure. Because impending relapse can be indicated by minimal residual disease (MRD) after allogeneic hematopoietic stem cell transplantation (allo-HSCT), MRD-directed intervention may be a reasonable option for relapse prophylaxis.

Aims: We investigated the efficacy of MRD-directed interferon-α (IFN-α) treatment in acute leukemia patients who were positive for MRD after allo-HSCT.

Methods: A total of 107 patients who were MRD-positive after allo-HSCT were enrolled. MRD-positive status was defined as positivity for leukemia-associated aberrant immune phenotypes or positivity for Wilms' tumor gene 1 in a single bone marrow sample. Recombinant human IFN-α-2b injections were administered subcutaneously 2–3 times per week for 6 months.
Results: The 2-year cumulative incidence of severe acute and chronic graft-*versus*-host disease after IFN-α treatment was 5.7% and 6.6%, respectively. Eighty-one (75.7%) patients turned MRD-negative after IFN-α treatment, including 42 (39.3%), 6 (5.6%), 7 (6.5%), and 26 (24.3%) who turned MRD-negative 1, 2, 3, and >3 months after MRD-directed IFN-α treatment, respectively. Twelve patients showed relapse after IFN-α treatment, and 4 patients died of non-relapse mortality (NRM). The 2-year cumulative incidence of relapse and NRM after IFN-α treatment was 11.5% and 4.3%, respectively. The 2-year probabilities of event-free survival and disease-free survival after IFN-α treatment were 66.5% and 82.4%, respectively. Persistent MRD after IFN-α treatment was significantly associated with higher relapse risk and poorer survival.
Summary/Conclusions: These data confirmed that MRD-directed IFN-α treatment is effective for patients who were MRD-positive after allo-HSCT.

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COMPARABLE LONG-TERM OUTCOME AFTER ALLOGENEIC STEM-CELL TRANSPLANTATION FOR OLDER PATIENTS (AGE ≥50 YEARS) WITH AML FROM SIBLING AND MATCHED UNRELATED DONORS. A REPORT ON BEHALF OF THE ALWP OF EBMT

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Background: Allogeneic stem-cell transplantation (SCT) is curative therapy in AML. Most deaths after SCT occur within the first 2 years. Prior large cohort studies showed that patients (pts) surviving leukemia free 2 years after SCT have high probability of survival at 10 years. Most of these studies were done in younger pts following myeloablative conditioning (MAC). Marked improvement has been achieved in SCT from unrelated donors (UD) in recent years due to improvement in tissue typing, donor selection and supportive care. However, there is relatively limited data on the comparative long-term outcomes (beyond 10 years) of SCT in AML pts (age ≥50 years) from sibling and UD in this setting.
Aims: To compare the long-term outcomes after SCT from sibling and UD using different conditioning intensities in AML pts age ≥50 years.
Methods:
We analyzed long-term outcomes in a relatively large cohort of pts with de-novo AML (n=1134), age ≥50 years, who were alive and leukemia-free 2 years after SCT from matched siblings (n=848) or UD (n=286), in the years 2000-2007, median follow up 8.6 years (2-16.4).
Results: The median patient age was 56 years (50-75) and 58 years (50-74) after SCT from siblings and UD, respectively (P=0.005). 77%, 12% and 11% in the sibling group were in CR1, CR2 and active leukemia at SCT compared to 50%, 25% and 25% in the UD group, respectively (P<0.001). 37% and 38% had reduced-intensity conditioning according to EBMT definitions (P=0.78), while 27% and 70% had in-vivo T-cell depletion (TCD), respectively (P<0.001). Chronic GVHD prior to the 2 year time-point occurred in 61% and 53%, respectively (P=0.02). The 10-year leukemia-free survival (LFS) of pts surviving leukemia-free 2 year after SCT was 72% (68-75) and 62% (55-70) after SCT from sibling and UD, respectively (P=0.30). Multivariate analysis (MVA) identified active leukemia at SCT (HR 1.8, P<0.001) or CR2 (HR 1.5, P=0.02) compared to CR1 and female recipient (HR 0.7, P=0.005) as independent factors predicting LFS. The donor type, conditioning regimen, age, cytogenetics and prior acute or chronic GVHD were not significant. The 10-yearr overall survival was 74% (70-77) and 66% (59-74), respectively (P=0.42). Relapse occurred in 15% (13-18%) and 17% (12-22%, P=0.97), respectively. MVA identified SCT in active disease (HR 2.2, P<0.001) or CR2 (HR 1.9, P=0.006), poor cytogenetics (HR 5.8, P=0.02), in-vivo TCD (HR 5.5, P=0.03) and female gender (HR 0.6, P=0.03) as risk factors for late relapse. Non-relapse mortality (NRM) occurred in 13% (11-16%) and 21% (15-28%, P=0.15), respectively. Advanced age was the only risk factor for late NRM (HR, 1.6, P=0.03). Donor and conditioning type were not predictive of late relapse or NRM. A center effect was documented for NRM (P<0.001) and LFS (P=0.05). There were 209 late deaths after sibling and 72 after UD SCT. Relapse was the cause of death in 53% and 37% of late deaths, respectively (P=0.06). GVHD was the cause of death in 16% and 22% and infection in 9% and 19%, respectively (P=0.05). Second malignancy was the cause of death in 13% and 12% of late deaths, respectively.
Summary/Conclusions: Long term outcome is similar after SCT form sibling or UD in pts with AML, age ≥50 years. Pts who are leukemia free 2 years after SCT can expect good and similar subsequent outcome with both donor types. Disease status was the major predictor of subsequent LFS while conditioning intensity had no effect. While relapse is the major cause of late death after both donor types, NRM and in particular GVHD and infections are more common causes of late death after SCT from UD.

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IMPACT OF AZACITIDINE PRETREATMENT ON OUTCOMES OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH HIGH-RISK MYELODYSPLASTIC SYNDROME

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Background: Myelodysplastic syndrome (MDS) is a heterogeneous myeloid stem cell disorder with ineffective hematopoiesis, dysplastic cell morphology, and a propensity for progression to acute myeloid leukemia. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only curative therapy for MDS. In recent years, azacitidine (AZA) has been increasingly used as pre-transplant induction therapy in high-risk MDS patients. However, the benefits of pretransplant therapy in these patients are unclear, and the optimal therapy regimen remains unknown.
Aims: We conducted a retrospective analysis to elucidate the clinical impact of prior treatment with AZA on outcomes after allo-HSCT in high-risk MDS patients.
Methods: Clinical data were collected from the registry database of the Japan Society for Hematopoietic Cell Transplantation. We selected patients with high-risk MDS at diagnosis (IPSS intermediate 2 or high), aged 16 years or older, who underwent their first transplantation between January 2009 and December 2014 and received AZA or best supportive care (BSC) before allo-HSCT. Patients who received conventional chemotherapy or immunosuppressive therapy prior to allo-HSCT were excluded. We compared overall survival (OS), relapse, non-relapse mortality (NRM), and hematopoietic recovery after allo-HSCT patients were pretreated with AZA or BSC.
OS was estimated by the Kaplan–Meier method, and a log-rank test was used for comparisons. Relapse and NRM were considered competing risk events and were compared using Gray's test. The cumulative neutrophil and platelet recoveries were also compared by Gray's test, considering death without these events as a competing risk. In a multivariate analysis, the Cox proportional hazard model and Fine-Gray methods were used for OS and cumulative incidence of relapse and NRM and hematopoietic recovery, respectively, using the following variables: age, gender, performance status at transplantation, marrow blast at diagnosis, cytogenetic risk, donor source, donor-recipient gender mismatch, ABO mismatch, and conditioning regimen.
Results: Of the 485 patients, 161 patients (33.2%) received AZA and 324 patients (66.8%) received BSC before allo-HSCT. The median age was 60 (18–70) and 56 (18–74) years, respectively (P=0.002). A higher proportion of BSC patients received cord blood transplantation (P=0.005). Bone marrow blast at diagnosis was significantly higher in AZA patients (P <0.001) and cytogenetic risk was better in AZA patients (P=0.02) than those in BSC patients. No differences were observed in other factors. The 2-year OS rate (46.7% and 50.8%, P=0.66), relapse (31.5% and 28.6%, P=0.59), NRM (26.5% and 26.1%, P=0.99), 30-day neutrophil engraftment (88.2% and 83.6%, P=0.18), and 60-day platelet engraftment (72.0% and 69.4%, P=0.36) were not significantly different between the AZA and BSC groups. In multivariate analysis, AZA and BSC showed comparable OS (HR, 1.16; P=0.31), relapse (HR, 1.13; P=0.50), NRM (HR, 0.92; P=0.64), neutrophil engraftment (HR, 1.01; P=0.89), and platelet engraftment (HR, 1.07; P=0.59).
Summary/Conclusions: Our study showed that pretransplant AZA and BSC provide similar outcomes of allo-HSCT in high-risk MDS patients. Further analysis is needed to clarify the role of pretransplant therapy in high-risk MDS and to identify the subset of patients who may benefit from pretransplant AZA.

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LOW-DOSE DECITABINE IMPROVES PLATELET RECOVERY IN PATIENTS WITH ISOLATED THROMBOCYTOPENIA AFTER HSCT

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Background: Isolated thrombocytopenia is a common complication of hematopoietic stem-cell transplantation (HSCT), which was defined as consistent low platelet counts with recovery of the other two cell lines after transplantation. This status leads to an increased risk of life-threatening hemorrhage, frequent requirements of platelet transfusion and extended hospital stays, representing a challenging clinical problem. Previous studies have demonstrated that decitabine, a hypomethylating agent, may increase platelet counts by promoting megakaryocyte maturation and platelet release in mouse model.
Aims: In order to investigate the role of decitabine in patients after HSCT suffering from isolated thrombocytopenia, we conduct a clinical trial to validate this effect in post-HSCT setting.
Methods: We performed a prospective open-label study to evaluate the treatment of low-dose decitabine in patients with hematological malignancies who received allogeneic HSCT and suffered from isolated thrombocytopenia. The inclusion criteria were: (1) Platelet count ≤30 × 109/L persistently at day 60 post-HSCT or later; (2) Recovered neutrophil and hemoglobin; (3) Full donor chimerism; and (4) No response to conventional treatments for a duration of at least 4 weeks. Patients with malignancy relapse, active infections, uncontrolled graft-*versus*-host disease, severe organ damage or transplant-related thrombosis were excluded. From July 2013 to July 2016, 38 patients were randomly assigned into either the control group to receive conventional treatment only, or the test group to receive additional decitabine (15mg/m^2, intravenously daily for 3 consecutive days).
Results: Major response was observed in 16 out of 19 patients (84.2%) in decitabine group, with a median time of 22 days to achieve platelet transfusion-independence. Two patients (10.5%) showed a minor response and 1 patient (5.3%) failed. In contrast, 3 out of 19 patients in the control group (15.8%) showed a major response, 2 patients (10.5%) showed a minor response, 14 patients (73.7%) did not show any improvement, of which 1 patient died of severe hemorrhage in week 5. For bone marrw morpholocial analysis, all 38 patients showed low levels of megakaryocytes at week 0. However, the megakaryocyte counts in decitabine group were significantly increased at week 4, while no significant difference was recorded in control group. After decitabine treatment, we did not observe a change in anti-platelet antibodies levels and T cell subsets ratios. However, reactive oxygen species (ROS) and megakaryocyte counts increased in the test group. No considerable myelosuppression, febrile neutropenia, and nonhematologic toxicities associated with the treatment were observed.
Summary/Conclusions: Our data showed an encouraging efficacy of decitabine in patients after HSCT suffering from isolated thrombocytopenia owing to remarkably increased megakaryocyte counts. Decitabine may improve isolated thrombocytopenia via regulating ROS and megakaryocyte reconstitution.

Thalassemia

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QUANTITATIVE PROTEOMICS OF PLASMA EXTRACELLULAR VESICLES TO IDENTIFY NOVEL BIOMARKERS OF CLINICAL SEVERITY FOR HBE/B-THALASSEMIC PATIENTS

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Background: Hemoglobin (Hb) E/β-thalassemia has a wide spectrum of clinical manifestations that cannot be explained purely by its genetic background. Extracellular vesicles (EV) are one factor that may indicate and/or contribute to disease severity because there is an observed increase in EV release due to the enhanced oxidative stress in thalassemic erythrocytes.
Aims: This study aims to explore the differences in protein composition and abundance between circulating EV from HbE/β-thalassemic patients and normal individuals.
Methods: 15 HbE/β-thalassemia patients and 15 matched-controls from Thailand were fully consented and recruited for this study. Pooled EVs isolated from five thalassemic samples were compared to pooled EVs from five matched controls using a Duplex-Tandem Mass Tag (TMT) mass spectrometry (TMT-MS) analysis. This experiment was repeated three times in total, using different patient and control samples to identify consistent alterations of protein expression in EVs. Finally, protein differences were also confirmed using Western blotting.
Results: The total proteins identified across the three experimental TMT-MS datasets ranged from 1,764 to 2,534 proteins. When restricted to proteins that contained more than one unique peptide, the range of proteins was reduced to 685 to 1,127 proteins. Many proteins were previously reported EV constituents. 19 proteins were consistently increased in patient samples compared to controls across all data sets. The majority of these proteins were chaperone proteins and antioxidant enzymes. Alpha Hemoglobin Stabilizing Protein (AHSP) had the highest increase of between 31 to 47-fold. Other proteins that exhibited increased abundance in thalassemic circulating EV included catalase, superoxide dismutase, T-complex proteins, heat shock protein 70 and ferritin light chain. Importantly, the heme scavenger and plasma proteins – haptoglobin and hemopexin were observed to be consistently decreased in patients' EV across all data sets. Immunoblotting results corroborated the TMT-MS findings.
Summary/Conclusions: We have successfully identified consistent alterations in protein expression levels between EV generated by HbE/β-thalassemic patients and normal individuals. These findings may potentially lead to the development of a prognostic marker, and therefore may improve the therapeutic outcome for the patients suffering from thalassemia.

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A SELECTIVE ORAL GLYT1 INHIBITOR IMPROVES ANEMIA IN A MOUSE MODEL OF BETA-THALASSEMIA

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Background: The anemia of β-thalassemia is due to a combination of reduced red cell survival in the peripheral circulation and ineffective erythropoiesis, the latter due to unbalanced hemoglobin chain synthesis, and hemichrome-induced oxidative damage. Here we used a specific and selective inhibitor of the plasma membrane expressed glycine transporter GlyT1 (RO4917838) in a mouse model for β-thalassemia ($Hbb3^{th/+}$). A previous study in Wistar rats has shown that RO4917838 induces a dose-dependent decrease in MCH, Hb, soluble transferrin receptor, and increase in absolute reticulocytes and RBC counts (Winter *et al*. Exp Hematol, DOI: 10.1016/j.exphem.2016.07.003). This has been linked to the ability of RO4917838 to reduce glycine bioavailability in erythroblasts and decreased heme biosynthesis.
Aims: To evaluate the impact of the glycine transporter GlyT1 selective inhibitor RO4917838 on anemia of a mouse model for β-thalassemia.
Methods: Wild-type control (WT) C57B6/2J, and $Hbb^{th3/+}$ mice (β-Thal) aged between 3 to 4 months were treated daily with either vehicle or RO4917838 at dosages of 3, 10, 30 mg/kg/d for 4-6 weeks by gavage. Hematological parameters, analysis of erythropoiesis, molecular studies of sorted erythroid precursors, indices of hemolysis, hepcidin liver expression and Pearls staining were carried out.
Results: RO4917838 administration was associated with an improvement of β-Thal hematologic phenotype, as supported by (i) the amelioration of red cell morphology; (ii) the increase in Hb levels; (iii) the reduction in reticulocyte count

and in the percentage of circulating erythroblasts; (iv) the increase in β Thal red cell survival. RO4917838 induced a significant reduction in extramedullary erythropoiesis as well as in the amount of insoluble alpha chain aggregates in circulating red cells. It is of note that in β-Thal sorted erythroblasts we found a reduction in HRI and in phospho-eIF2a, indicting a reduction in free heme, which shall resulted in the activation of HRI, in RO4917838 treated β -Thal mice (10 mg/Kg/d, 6 weeks). Finally, in β-Thal mice treated with RO4917838 (4 weeks at 30 mg/kg/d) a reduction in liver and spleen iron-overload was identified, which was associated with increased hepcidin liver expression.
Summary/Conclusions: Our data suggest that RO4917838 ameliorates anemia and ineffective erythropoiesis by reduction of heme biosynthesis in a mouse model for β-thalassemia. RO4917838 is a potential, novel therapeutic approach for the treatment of anemia in patients affected by beta-thalassemia.

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MAY MUTATIONS IN THE KLF1 GENE HAVE WORSENING EFFECTS ON THE BETA THALASSEMIA PHENOTYPE?

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Background: Kruppel-like factor 1 (KLF1) is a pleiotropic erythroid transcriptional factor that plays a key role in erythropoiesis (Siatecka M, Blood 2011; 118: 2044-521). Accordingly, KLF1 mutations have been found to be responsible for a variety of hematological disorders. KLF1 also contributes directly or indirectly to regulate the expression of genes in the beta-globin gene cluster and the fetal-to-adult globin gene switching (Waye JS et al Int. J. Lab. Hem. 2015; 37: 78-84). It has been reported that mutations leading to KLF1 haploinsufficiency are linked to increased fetal hemoglobin (HbF) levels with ameliorative effects on the severity of beta-thalassemia (Liu D. *et al.* Blood 2014; 124: 803-811; Perkins A. *et al.* Blood 2016; 127: 1856-1862).
Aims: This study was aimed at providing a functional characterization of known and novel mutations in the KLF1 gene associated with atypical beta-thalassemia phenotypes.
Methods: Hematological parameters were measured using an automated hematology analyzer (Beckman Coulter) and high performance liquid chromatography (Variant II, Bio-Rad Laboratories). Screening of KLF1 mutations was performed by Sanger sequencing on an Applied Biosystems 3730 DNA analyzer. Functional studies were performed by gene reporter assays and expression vectors for KLF1 mutants in the human K562 erythroleukemia cell line. This study was performed on 19 adult subjects, including 11 beta-thalassemia heterozygotes with an unexpected phenotype of intermediate thalassemia (moderate or severe anemia, elevated HbA_2 and/or HbF levels) and 8 subjects with normal erythrocyte indices and borderline HbA_2 and/or HbF levels without mutations in alpha- and beta-globin gene clusters.

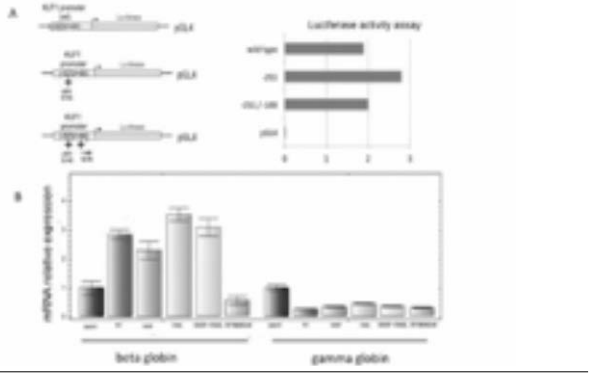

Figure 1.

Results: Of the 19 patients who were tested, 15 were found to be positive for mutations in the KLF1 gene. More in detail, we found 7 mutations, comprising a nucleotide variation (c.-251 C>G) already reported as a single nucleotide polymorphism and a known mutation (c.-148 G>A) in the proximal promoter region, 3 known mutations associated with increased HbA_2 and/or HbF levels (S102P, F182L and M39L) (Radmilovic M. *et al.* Ann. Hematol 2013; 92: 53-58) and two novel mutations (C94X and P173Pfs*236), all of them in the proline-rich domain in exon 2. Functional studies were performed in K562 cells in order to clarify the pathogenic significance of these mutations and to better define the role of KLF1 in atypical thalassemia phenotypes. Interestingly, the c.-251 C>G polymorphism was found to be associated with an increased transcriptional activity of the KLF1 promoter (Figure 1A), thus allowing us to exclude for this nucleotide variation the condition of a neutral polymorphism. Furthermore, unexpectedly, the novel P173Pfs*236 mutation was found to be associated with a dramatic reduction of the beta-globin gene expression levels (Figure 1B).
Summary/Conclusions: Our study confirmed the ameliorative effect of some KLF1 mutations on the thalassemia phenotype that were found to be associated with increased fetal- and/or beta-globin gene expression. In other cases we demonstrated that KLF1 mutations may contribute to worsen the beta thalassemia phenotype or result in a silent beta thalassemia trait. This study provides further insights into the multiple roles of KLF1 in erythropoiesis and highlights an intriguing effect of a subset of KLF1 mutations that may contribute to the severity of the thalassemia phenotype, thus reinforcing the relevant implications of KLF1 screening for genetic counseling and for effectiveness of prevention screening programs for hemoglobinopathies.

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SECONDARY SOLID TUMORS FOLLOWING HEMATOPOIETIC CELL TRANSPLANTATION FOR THALASSEMIA MAJOR

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Background: Secondary solid tumors (SST) have been described after HCT, in particular for patients affected by hematologic malignancies. There is limited information about the incidence of SST following HCT for thalassemia major (TM).
Aims: The aim of this study was to determine the incidence of SST in 134 patients with TM who received HCT in our Center between 1983 and 2013
Methods: 117 patients survived more than 3 years after HCT and were enrolled in the study. Of them, 57 were males and 60 females. Their median age at time of HCT was 10 years (1-29). As conditioning regimen, they received Busulfan (14 mg/Kg) and Cyclophosphamide (200 mg/Kg). The GvHD prophylaxis included Cyclosporine and Methotrexate. All patients received bone marrow cells from an HLA identical donor.
Results: At time of this report, 112 patients were cured, whereas 5 patients rejected their graft and are now under regular transfusion treatment.
Overall, the median follow-up after HCT was 24 years (3-34). Seven patients developed a malignancy 3.2 to 28 years (median 16,4 years) after HCT including 2 carcinomas of the tongue, 1 oral squamous cell carcinoma, 1 colorectal cancer, 1 thyroid carcinoma, 1 carcinoma of the uterine cervix, and 1 parotid carcinoma. The 30-yr cumulative incidence (CI) of developing SST was 10±0.17%. All patients underwent surgical resection of the tumor and in addition 4 of them received chemotherapy and/or radiotherapy. Of relevance, the 3 patients with cancer of the oral cavity were affected by severe chronic GvHD with buccal cavity involvement. Two patients (1 with parotid and 1 with tongue carcinoma) died of tumor progression and 5 are living. We compared these results with 2 case control populations. First of all, we investigated the occurrence of solid tumors in the 117 individuals (64 males, median age 10 years at time of marrow donation), who served as stem cell donors for HCT. One donor developed breast cancer 29 years after marrow donation at age of 38. The 30-yr CI of developing solid tumor for donors was 4.5±0.21% with a statistically significant difference (p=0.03) as compared to that of transplanted patients. The second case control population consisted of 117 patients affected by TM treated with transfusions and iron chelation. The matching technique applied was based on the variables age and sex. One control per case (transplanted patient) was randomly selected from the MIOT (Myocardial Iron Overload in Thalassemia) registry and matched by sex and age with the transplanted patient population. Two patients developed an hepatocellular carcinoma (HCC) at age of 39 and 44 years, respectively. One patient died and one is living. Using the event rate measure, we observed an event rate of 0.102 at 30 years for the transplant group and 0.041 for the nontransplant group (p=0.106).
Summary/Conclusions: This study shows that the magnitude of increased risk of SST is twofold to threefold for patients treated with HCT as compared with an age- and sex matched nontransplant TM patients or with stem cell donors. Notably, among the transplanted patients we didn't observe any case of HCC, which is one of the most frequent solid tumor in nontransplant TM patients, whereas we observed 4 cases of head/neck cancers. In our series, cGvHD seems to be a strong risk factor in the development of new solid tumors. Patients with cGvHD, especially those with involvement of the oral cavity, must receive a very long careful monitoring and surveillance in order to prevent the development of secondary cancers.

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VALIDATING A NOVEL CAPILLARY ELECTROPHORESIS: THE MOST SUITABLE PLATFORM FOR THE NATIONAL NEWBORN SCREENING PROGRAM IN A REGION WITH HIGH PREVALENCE OF THALASSEMIA AND HEMOGLOBINOPATHIES

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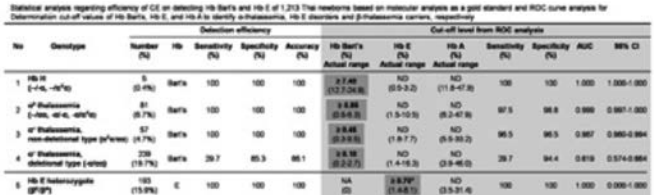
Background: Newborn screening program for thalassemia (thal) and hemoglobinopathies (NBS-Hbs) is crucial for early detecting patients with serious hemoglobinopathies (Hb variants) *e.g.* Sickle cell anemia (Hb SS). NBS-Hbs has been incorporated into a routine neonatal service in several developed countries. However its role on early detection other forms of globin disorders remains unclear. Moreover, NBS-Hbs can detect several types of thalassemia and Hb variants carriers. This application could be useful for the national prevention and control program for severe thalassemia syndromes in many developing countries including Thailand where these conditions are highly prevalent especially β-thal major, Hb E/β-thal and Hb Bart's hydrops fetalis (caused by α^0-thalassemia). Recently a new capillary electrophoresis (CE) has been developed specifically for NBS-Hbs. However there is a limited data on validation of this technology on detecting several types of thalassemia and Hb variants found in Southeast Asia.
Aims: To evaluate and validate a new CE system to screen globin disorders in newborn to initiate the national NBS-Hbs for Thailand
Methods: After informed consent, 1,213 blood samples of 2-day old newborns were collected by heel prick puncture into 5-dried blood spots. After elution, dried blood samples were analyzed by Capillarys 2 NEONAT FAST® (SEBIA, Évry, France). All samples were also extracted for DNA and genotyped by our extensive PCR based panel to detect >98% of abnormal globin alleles found in Thailand using α-thal GAP-PCR, α-thal ARMS-PCR, β-thal ARMS-PCR, and PCR-RFLP for Hb E. We compared CE data with each globin genotypes and use a ROC curve to set up new diagnostic criteria using% Hbs from CE for future cases.
Results: Identification of Hb Bart's provided 100% of sensitivity, specificity and accuracy in most individuals with α-thal. Using ROC analysis, we proposed different cut-off values of Hb Bart's to differentiate Hb H disease, α^0-thal and non-deletional α^+-thal traits; ≥7.40%, ≥0.85%, ≥0.45%, respectively with excellent accuracy (Table 1). Interaction of Hb E with these α-thal genotypes has no effect on these cut-off values (Table 1). However, there was a limitation to identify deletional α^+-thal trait using CE by the level of Hb Bart's ≥0.10% (detectable level). A cut-off level to distinguish Hb EE from Hb E trait was suggested at ≥4.95% *vs* ≥0.7%. Two patients with Hb E/β-thalassemia were identified through this study with different CE pattern from Hb EE. 11 β-thalassemia traits was identified and they had a lower level of Hb A as compared to their gestational age (GA)-sex matched controls with normal β globin genotypes (n=148). We recommend Hb A level ≤10.35%; a cut-off to primarily consider for β-thalassemia carrier followed by molecular analysis.

Table 1.



Summary/Conclusions: This newborn CE platform showed a high efficiency for detecting several types of thalassemia and Hb variants in particular α-thal, β-thal and Hb E using cut-off levels of each Hb species described herein. Besides early detecting of Hb S, we can now apply this NBS into a routine service in order to early detect Hb H disease, Hb E/β thalassemia and the majority of common thalassemia carriers. This NBS-Hbs approach can reinforce and leverage our current program on prevention and control for severe thalassemia syndromes in our region. Moreover, due to population migration from The East to the West, our new diagnostic guideline by CE could be useful and applicable for existing NBS programs currently available in several European countries.

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TRANSIENT ELASTOGRAPHY IN NON TRANSFUSION DEPENDENT THALASSEMIA: A SUCCESSFUL TOOL TO ASSESS AND MONITORING LIVER FIBROSIS

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Background: Non Transfusion Dependent Thalassemia (NTDT) patients are at risk for several complications due to chronic anemia, hypoxia and iron overload. Over the recent years hepatic complications are more frequently observed in these patients probably due to the aging and poor care: monitoring liver fibrosis is becoming part of the follow up. Liver stiffness measurement (LSM) by transient elastography (TE), a widely-used non-invasive tool, in our centre has been included in the regular follow-up of patients with NTDT
Aims: To evaluate by TE liver fibrosis in NTDT patients, its correlation with biochemical, hematological and clinical parameters at baseline and after 5 years
Methods: Hepatic fibrosis and siderosis were evaluated in 101 NTDT patients using respectively TE, and liver iron concentration (LIC) derived from T2* Magnetic Resonance Imaging (MRI) at baseline and, in a subset of patients, after 5 years. The following TE thresholds were taken into account: <5.0 kPa no fibrosis (F0), 5.1-7.9 kPa mild fibrosis (F1), >7.9 kPa moderate fibrosis (F2), >10.3 advanced fibrosis (F3), >11.9 kPa cirrhosis (F4). Biochemical and hematological blood test were collected too. Patients were also tested for HCV antibodies and HCV RNA. Data were analyzed retrospectively
Results: Patient's mean age was 46±11 years, 37/101 (36.6%) were splenectomized, 51/101 (50.5%) had never been transfused, 46/101 (45.5%) were occasionally transfused and 4/101 (3.9%) had been regularly transfused for 10+5yrs. At baseline (T0), the overall mean LSM was 5.9 ±2.6 kPa, mean LIC 6.68±5.37 mg/g dw, ferritin 700±596 ng/ml, Hb 9.3±1.3 g/dl, AST, ALT GGT and ALP were normal. LSM correlate with GGT (p <0.001) and AST values (p=0.02). Seven/101 (6.9%) patients were HCV RNA positive, one of them had never been transfused. At baseline 37/101 (36.6%) patients were on iron chelation (ICT) (29 deferoxamine, 7 deferasirox, 1 deferiprone). At T0 patients with fibrosis (any grade) didn't show differences compared with patients without fibrosis (F0) regarding age, splenectomy, transfusions, ICT and all biochemical tests except for GGT (mean GGT 16.1 ± 10.9 U/L in F0 group, GGT 27.1 ± 24.8 U/L in patients with any grade of fibrosis, p <0.05). For 60 patients data at 5±1 years (T1) were available. LSM remained stable in 35/60 (58.3%): 24 patients did not show fibrosis in both T0 and T1 evaluations, 10 patients showed a F1 fibrosis in both evaluations and one remained in the F4 class. Among these patients 13/35 (37.1%) were on ICT. A reduction in LSM was found in 21/60 (35%) patients (T0=7.09±1.63 kPa, T1=5.07±1.61 kPa, p<0.001), with a reduction trend in LIC (T0 =6.79±4.09 mg/g dw, T1=5.18±3.04 mg/g dw; p=0.09 ns) and a statistical significant reduction in ferritin levels (T0=709±458 ng/ml, T1 436±280 ng/ml, p=0.005); 14/21 (66.6%) were on ICT. Comparing patients who remained stable with patients who ameliorate the grade of fibrosis, a significant difference was found regarding the number of patients on ICT (37.1% *vs* 66.6% respectively, p <0.05). A worsening in LSM was observed in 4/60 (6.6%) patients: among them LSM changed from F0 to F1 in 2 patients, and from F2 to F4 in the other 2 patients. None of these patients presented HCV RNA positivity
Summary/Conclusions: NTDT patients could benefit from regular non-invasive assessment of liver fibrosis. In our study subject who received ICT had best chance to reduce the grade of fibrosis through the reduction in iron overload. In these patients, that usually are HCV RNA negatives, monitoring and treating iron overload is a crucial point in the prevention of hepatic fibrosis being the hepatic siderosis the primary cause of hepatic tissue damage, cirrhosis and hepatocellular carcinoma.

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INCREASING INCIDENCE OF MALIGNACIES IN AGING THALASSEMIC PATIENTS: A SINGLE INSTITUTION'S LONGITUDINAL EXPERIENCE

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Background: The introduction of close monitoring, regular blood transfusions and systematic iron chelation in the management of thalassemia have significantly changed the clinical phenotype of the patients and improved their survival. The patients, who have benefited of the current therapeutic regimen, are now reaching middle age, and they have started facing problems more commonly seen in older people. Recent observations suggest of an increased incidence of malignancies in the aging group of thalassemic patients.
Aims: The purpose of this study is to determine the longitudinal changes in the incidence of malignancies, along with possible correlations to different aspects of the disease.
Methods: A retrospective study in the largest Thalassemia Unit in Greece has been conducted spanning an observation period from 2001-2016. The occurrence and type of cancer, as well as history on transfusion dependence, liver failure, HCV infection and chelation therapy were recorded and analyzed. Statistical analysis was performed with SPSS software package, v. 20. A p-value<0.05 was considered statistically significant.
Results: Records from 591 patients (338 with thalassemia major and 253 with thalassemia intermedia) were evaluated. 27 patients (11 males and 16 females)

with malignancies were identified (incidence: 4.6%). The mean age of the diagnosis of the malignancy was 41.6 years (36.6 years for thyroid gland cancer, 45.8 years for liver, 38 years for hematologic malignancies and 46 for renal cancer). 24 patients were transfusion dependent (TD) (7% of the patients) and 3 non transfusion dependent (1.18%). Liver cancer had the highest incidence 29.6%, following by thyroid gland cancer 25.9%, hematologic malignancies 11.1% and renal cancer 14.8%. HCV infection was found in 56.7% of the patients and a statistical significant relationship between HCV infection and cancer (p=0.001) was detected. No correlation between liver failure and cancer was detected. In the TD group, the age specific ratio of cancer increased with age with the patients >50 years having the highest ratio of 42.3, compared to 36.9 and 17.1 observed in the 46-50 years and 41-45 years age groups, respectively. In regards to chelation therapy, at the time of diagnosis 40.9% of the patients were receiving deferasirox (DFX), 22.7% deferiprone (DFP), 22.7% deferoxamine (DFO), 9.1% no chelation therapy and 4.5% DFO/DFP. No statistical significant difference was observed between the different chelation therapies (p=0.119). As the utilization of different types of chelation changed throughout the years, according to the availability of the chelating agents, we analyzed separately, the patients that developed malignancies in the period after 2010 when longitudinal exposure to all three chelators can be assumed. Even though the results showed a difference (p=0.027) between the different groups with 47.1% of those patients receiving DFX at the time of the diagnosis compared to 27.1% receiving DFP and to 11.8% receiving DFO, this distribution reflects the overall distribution of chelator usage during that period. Apart from the incidence, there was no statistical significant difference between TD and NTD patients with cancer regarding the gender, age and year of diagnosis. The mean cancer mortality rate was 48%, but varied significantly with the type of cancer with liver cancer and hematological malignancies having a mortality of 66% .Overall only 2% of the deaths occurring in our group of patients were attributed to cancer.

Summary/Conclusions: This retrospective study has confirmed the increased incidence of malignancies in thalassemia patients in Greece, which is, at least, partially related to the aging of this population. Based on these observations, adaptation of monitoring guidelines is essential for optimal management of thalassemic patients. Periodic screening for malignancies, especially hepatic, thyroid and hematologic, will allow early detection and timely, and thus, more efficacious treatment of the neoplasia.

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SAFETY AND EFFICACY OF EARLY START WITH SUBOPTIMAL DOSE OF DEFERIPRONE IN MINIMALLY TRANSFUSED INFANTS WITH TRANSFUSION DEPENDENT THALASSEMIA: A RANDOMIZED TRIAL

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Background: Early exposure to Iron toxicity is the main risk factor for morbidity and mortality in patients with transfusion-dependent thalassemia. Current practice is to start chelation therapy only after 10-20 transfusions, or when the serum ferritin (SF) level rises above 1,000 µg/L.

Aims: To evaluate the safety and efficacy of the early use of low-dose deferiprone in minimally transfused pediatric thalassemia patients and to evaluate if it can postpone iron overload in these group of patients.

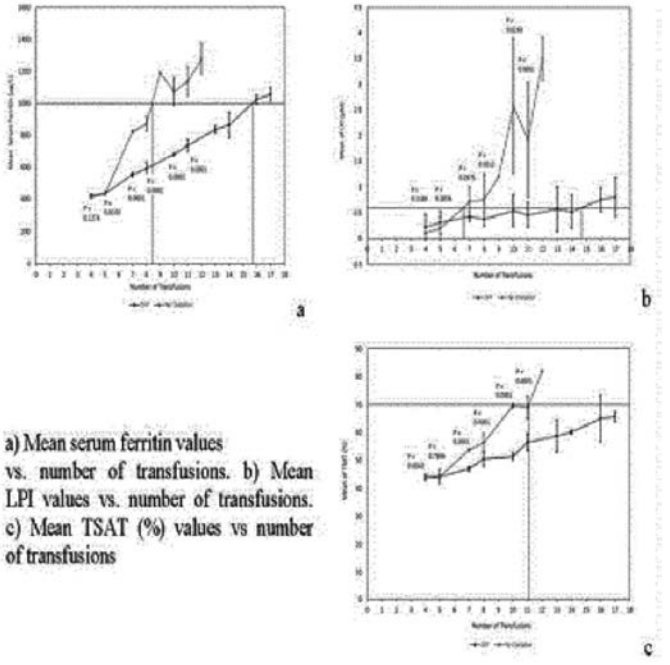
Methods: In the current trial (ClinicalTrials.gov Identifier: NCT02173951), sixty-four children recently diagnosed with thalassemia major who had begun receiving blood transfusions in first year of life to keep pre-transfusion Hb above 10 gm/dl, had not yet started iron chelation therapy and had SF ≥400 µg/L or transferrin saturation (TSAT) ≥70% or labile plasma iron (LPI) ≥0.2 µM were randomized to start deferiprone (DFP) at a sub-therapeutic dose (50 mg/kg/day) or no chelation (NC). Median age at 1st transfusion was 8 months for both DFP-treated and for NC children. The percentage of patients with LPI ≥0.6 µM, SF ≥1000 µg/L or TSAT ≥70% in each study arm was assessed at 6, 9 and 12 months (patients confirmed SF ≥1000 ng/mL were withdrawn from the study and placed on a standard chelation regimen). Complete blood count was done weekly in DFP treated and every 3-4 weeks in NC.

Results: Table 1. Summary of the efficacy results of SF, TSAT, and LPI.

Table 1.

	Baseline		3 months		6 month		9 months		12 months	
	ES-DFP	DC	ES-DFP	DC	ES-DFP	DC	ES-DFP	DC	ES-DFP	DC
SF (µg/L)	431±14 N=32	430±15 N=32	579±36 N=30	862±44 N=31	708±33 N=25	1110±77 N=31	861±68 N=30	1274±89 N=29	1042±40 N=27	
% patients with SF ≥ 1000 µg/L	0%	0%	0%	3%	0%	100%	10%	100%	100%	
TSAT (%)	44.0±0.9 N=31	44.4±2.4 N=31	49.2±2.7 N=27	55.9±3.2 N=28	54.0 ± 2.9 N=25	69.1±3.6 N=26	59.8±3.8 N=25	82.0 N=1	65.4±5.8 N=23	
% patients with TSAT ≥ 70%	0%	0%	0%	0%	0%	54%	0%	100%	26%	
LPI (µM)	0.28±0.2 3 N=31	0.18±0.2 0 N=31	0.40±0.1 3 N=30	0.75±0.4 8 N=31	0.50±0.2 7 N=30	2.00±1.1 4 N=31	0.54±0.3 6 N=28	3.50±0.4 2 N=2	0.79±0.3 2 N=24	

All NC patients were removed from the trial prior to completing 7 months of follow-up (9-11 transfusions) due to confirmed SF ≥1000 µg/L. Mean ± SD time of follow up was 10.4± 4.9 and 5.9 ± 2.5 months for DFP and NC respectively. Most common adverse events in patients on DFP *versus* NC were diarrhoea (19% *vs* 13%, p= 0.73), vomiting (13% *vs* 13%, p=1.00), abdominal colic (13% *vs* 13%), elevated liver enzymes (6% *vs* 3%, p=1.00) and neutropenia (6% *vs* 6%). All adverse events were mild in severity and did not require interruption of DFP use. There were no cases of agranulocytosis or moderate neutropenia, no arthralgia and no serious infections in DFP-treated patients. DFP therapy was associated with a significant reduction in the rate of iron accumulation as measured by SF (P<0.0001), LPI (P<0.001)and TSAT (P<0.001) (Figure 1a, b, c). LPI ≥0.6 µM appeared as early as after 5 transfusions in NC children and was delayed to at least 10 transfusions with DFP therapy. TSAT ≥70% appeared after 10 transfusions in NC children and was delayed to at least 17 transfusions with DFP therapy.The results of this study show that LPI and TSAT may reach values ≥0.6 µM and ≥70%, respectively, after 5 -10 transfusions in children with TM and all NC children had *SF ≥1000* µg/L after 8-9 transfusions.



a) Mean serum ferritin values vs. number of transfusions. b) Mean LPI values vs. number of transfusions. c) Mean TSAT (%) values vs number of transfusions

Figure 1.

Summary/Conclusions: A sub-therapeutic dose of deferiprone for a mean of 12 months in children with TM and low iron overload was not associated with safety concerns and able to significantly reduce the rate of iron accumulation as measured by SF , LPI and TSAT.

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LONGITUDINAL PROSPECTIVE MRI STUDY IN PEDIATRIC PATIENTS WITH THALASSEMIA MAJOR

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Background: No studies are available in literature evaluating, on repeated magnetic resonance (MRI) imaging assessments, changes in myocardial and hepatic iron overload, biventricular function, and development of macroscopic myocardial fibrosis in pediatric patients with thalassemia major (TM)

Aims: This is the first longitudinal prospective MRI study in pediatric TM patients.

Methods: We considered 68 TM patients enrolled in the MIOT (Myocardial Iron Overload in Thalassemia) project with less than 18 years at the first MRI scan and who performed a follow-up (FU) study at 18±3 months.
Myocardial and hepatic iron burdens were quantified by the T2* technique. Atrial dimensions and biventricular function were quantified by cine images. Late gadolinium enhancement (LGE) images were acquired to detect myocardial fibrosis.

Results: At the baseline MRI, 16 (23.5%) patients showed myocardial iron overload (MIO; global heart T2*<20 ms) and 54 patients liver iron overload

(79%). Figure 1 shows the changes in iron levels. Twenty-five patients changed the chelation regimen after the baseline MRI. Globally, a worsening in cardiac iron was found in the 3% of the patients while a worsening in hepatic iron in the 21% of the patients (P=0.003). The LV end-diastolic volume index and all RV volumes as well as the LV mass index were significantly lower at the FU MRI. No significant improvement in left or right global systolic function was found. For 40 patients the presence of myocardial fibrosis was investigated at both baseline and FU scans. Six patients (15.0%) had myocardial fibrosis at the baseline MRI and myocardial fibrosis was detected for all of them also at the FU. The extent of myocardial fibrosis was comparable between the two scans (0.77±0.42% *vs* 0.79±0.51%; P=0.686). At the FU 4 new occurrences of myocardial fibrosis were detected. In patients with baseline MIO no significant correlation was found between the percentage change in cardiac iron and the changes in hepatic iron or the baseline hepatic iron.

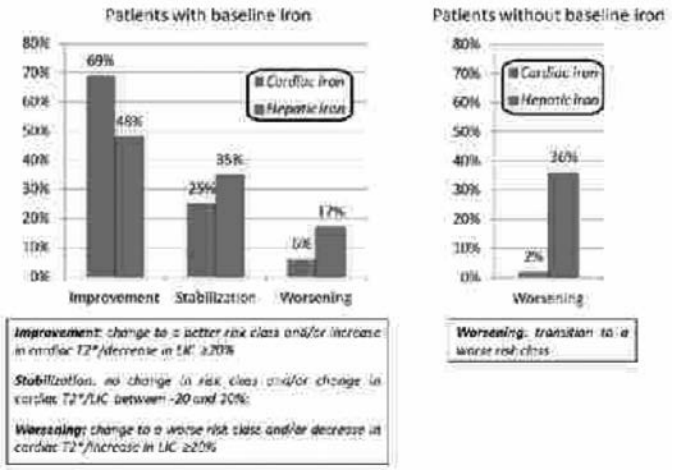

Figure 1.

Summary/Conclusions: Magnetic resonance monitoring in children with TM demonstrated a good control of cardiac iron overload in terms of prevention and treatment but the need for further improvement of liver iron overload. Myocardial fibrosis appears mainly multifocal, non progressive and not reversible over a 18- month period. A prompt and aggressive approach to iron overload and a chelation regimen consistent with the high iron intake and the high rate of severe liver iron overload is recommended in children.

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LONG TERM FOLLOW-UP OF A COHORT OF WELL TREATED B-THALASSEMIA MAJOR PATIENTS BY MULTI-ORGAN R2* MAGNETIC RESONANCE IMAGING

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Background: The introduction of non-invasive multi-organ evaluation of iron overload by R2* Magnetic Resonance Imaging (MRI) in β-thalassemia major (TM) patients has improved the patient care allowing a more careful tailoring of iron chelation therapy.
Aims: We report a cross-sectional and longitudinal experience with the use of MRI-R2* heart, liver and pancreas in a cohort of well treated TM patients.
Methods: TM patients underwent contemporaneous assessment of pancreatic, cardiac and hepatic MRI-R2* (1.5 T GE HDx scanner) in the period Jan08-Dec16.
Results: 69 TM patients: 43% male, age 38±9yrs, median number of observations/patient 6 (IQR:5-7), median number of yrs of the follow-up (f.u.) 8 (IQR:7-8). Iron chelation regimens included deferiprone (basal 30%>f.u.32%), deferasirox (basal 45%>f.u.52%), daily alternating deferasirox+deferiprone (basal 3% -f.u.6%), deferoxamine (basal 9%>f.u.6%) deferoxamine+ deferiprone (basal 13%>f.u.4%). The observation at the baseline showed a positive strong correlation between R2* values of pancreas and both of liver (R_p=0.68, p<0.001) and heart (R_p=0.75, p<0.001), in accordance with literature. Moreover, the ROC analysis confirms the value of 100 Hz for the pancreatic-R2* as the predictor of cardiac R2*>50Hz (*Noetzli et al. Blood 2009*). We observed a significative correlation of ferritin values with R2* of liver (R_p=0.53,p<0.001), heart (R_p=0.24, p=0.04) and pancreas (R_p=0.35,p=0.003). At f.u. the number of cases of moderate/severe iron overload decreases in the liver (from 11 to 0), in the heart (from 14 to 2) and in the pancreas (from 27 to 23) and the correlation of R2* of pancreas with R2* of heart (R_p=0.25, p=0.034) and liver (R_p=0.42, p<0.001) is weaker in respect to the baseline. We observed a significative decrease of R2* values both in the liver (p=0.0012), heart (p=0.017) and pancreas (p=0.018). Once again we found a correlation between the values of ferritin and R2* of liver (R_p=0.37,p=0.0015), heart (R_p=0.26, p=0.028) and pancreas (R_p=0.23,p=0.05). Moreover the variations of ferritin correlate with the variations of R2* of the liver (R_p=0.6,p<0.001), heart (R_p=0.25, p=0.04) and pancreas (R_p=0.41,p= p<0.001). Finally, assuming the cutoff value of 100 Hz for the pancreatic-R2* as the predictor of a cardiac R2*>50Hz, we calculated the numbers of false/true positive/negative according to the rule above. At the baseline we can observe that the number of false positive is the 14/27 (52%). The percentage increases to 91% (21/23) after f.u.: the pancreas-R2*>100Hz in 23 patients but only 2 has iron overload in the heart; the total number of patients with pancreatic-R2*>100Hz is quite the same before and after f.u. (27 compared to 23). We found no correlation between the false positive predicted and particular conditions such as impaired glucose tolerance, diabetes or adipose involution (Table 1).

Table 1.

(A) Multi-organ MRI-R2*evaluations at baseline and at f.u.. (B) Ability of pancreatic-R2* to predict cardiac iron (R2*>50Hz): counts of False/True positive and False/True negative.

(A)	*MRI-R2* and iron overload at baseline and f.u.*									
	Heart		*Liver*					*Pancreas*		
BASELINE	R2* (Hz)	> 50 Hz	R2* (Hz)	Severe	Mod.	Mild	None	R2* (Hz)	> 50 Hz	> 100 Hz
	27 (IQR: 24-41)	14	119 (IQR: 73-253)	3	8	18	40	86 (IQR:63-122)	56	27
F.U.	26 (IQR: 23-30)	2	99 (IQR:46-159)	0	0	18	51	76 (IQR: 48-119)	51	23

(B)	*Ability of pancreatic-R2* to predict cardiac iron (R2*>50Hz)*			
	False Positive	*True Positive*	*True Negative*	*False Negative*
BASELINE	14	13	41	1
F.U.	21	2	46	0

Summary/Conclusions: In this experience we observed that the regular multi-organ assessment of iron overload by R2* is concomitant with a reduction of the iron burden in this cohort of well treated patients confirming that is a careful method to tailoring the iron chelation therapy. However pancreatic-R2* remains above the cut-off for the prediction of cardiac iron overload, so this parameter should be considered with caution in the tuning of the chelation therapy, in order to avoid over-chelation risk. Ferritin values trend agree with R2* values confirming the reliability of this parameter. These results were obtained with a prevalent use of oral chelation regiment (90% of patients).

Transfusion medicine

P401

DEVELOPMENT OF HTLV-1 HYPERIMMUNE GLOBULINS AGAINST HTLV-1 INFECTION

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Background: Adult T-cell leukemia (ATL) is a malignant disease caused by infection with human T-lymphotropic virus (HTLV-1). The prevention of HTLV-1 infection is the most effective strategy to eradicate ATL. However, there is no effective vaccine or anti-viral agent for HTLV-1.
Aims: The aim of this study was to develop an effective HTLV-1 hyperimmune globulin (HTLV-IG) isolated from HTLV-1 positive carriers screened at the Japanese Red Cross.
Methods: We developed two *in vitro* and *in vivo* screening methods to evaluate and characterize the anti-viral effect of HTLV-1 positive plasma and HTLV-IG.
Results: HTLV-1 positive plasma isolated from an HTLV-1 carrier with a proviral load (PVL) >4 inhibited both HTLV-1 infection and syncytia formation. We purified HTLV-IG from the HTLV-1 positive plasma (PVL >4) and evaluated its effect in a humanized mouse model. NOG (NOD.Cg-Prkdcscid Il2rgtm1Sug/Jic) mice were treated with HTLV-IG for 5 days before HTLV-1 infection. During the monitoring period up to 40 days after post-infection, HTLV-1 infection was observed in untreated infected mice, but not in HTLV-IG-treated mice. The inhibitory effect of HTLV-IG was observed at the early stage of HTLV-1 Infection. Treatment with HTLV-IG at 20 days after HTLV-1 infection had a partial inhibitory effect. HTLV-1 gp46 expression in HTLV-1 infected cells was slightly reduced and the localization of these cells was changed in each tissue after the first line of treatment. These data suggest HTLV-IG is effective at the early phase of HTLV-1 infection. We also assessed the viral safety of HTLV-1 during the HTLV-IG manufacturing process. High log reduction values of HTLV-1 were observed during the Cohn fractionation process. Virus safety was assessed by PCR based assay and *in vitro* and *in vivo* infection assays. We next assess the viral safety of HTLV-1 during the HTLV-IG manufacturing process. High log reduction values of HTLV-1 can be seen during the Cohn fractionation process. Virus safety was assessed with PCR based assay and *in vitro* and vivo infection assay.
Summary/Conclusions: These data suggest HTLV-IG is effective and safe for the prevention of HTLV-1 infection.

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THE CONTAMINATION OF TUMOR CELLS IN THE APHERESIS MATERIAL DOES NOT PREDICT THE RESPONSE OF MULTIPLE MYELOMA PATIENTS TO AUTOLOGOUS TRANSPLANTATION

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Background: The use of high dose of chemotherapy followed by autologous stem cell transplantation (ASCT) has improved the prognosis of patients with multiple myeloma (MM) and plasma cell dyscrasia. However, there is controversy over the effect of infusion of atypical plasma cells (PC) on the apheresis product.
Aims: To analyze whether in MM malignant plasma cell reinfusion could negatively affect responses to ASCT.
Methods: Patients (n=114) undergoing ASCT (n=120) for MM between June 2003 and February 2016 were enrolled in a retrospective study to analyze the prognostic value of aberrant (CD38++CD138+CD19-CD45weak) to normal phenotype (CD38++CD138+CD19+CD45+) plasma cells (A:T PC ratio) in the autograft by flow cytometry. The Durie-Salmon stage at diagnosis, response of disease to induction treatment, biological parameters, pre-ASCT percentage of PC in bone marrow and at day +100, and the mobilization scheme were determined. Response was assessed at day +100 after ASCT using the 2006 International Myeloma Working Group uniform response criteria. Data were analyzed with SPSS v20.
Results: Patient characteristics are shown in Table 1. Patients with a better pre-ASCT response to induction therapy (complete response [CR] or very good partial response [VGPR]) had a non-significant different median A:T PC ratio content in the apheresis material, than those with a poorer response (partial response [PR], stable [SD] or progressive disease [PD]), (0.4 *vs* 1, p=0.28). Similarly, a non-significant difference (p=0.251) was observed in the number of atypical PC contained in the autograft of patients with a better *vs* poorer pre-ASCT response (0.06 *vs* 0.08 ×10^6/kg). There was no difference between the type of mobilization (G-CSF *vs* chemotherapy+G-CSF) and the degree of apheresis contamination (median A:T PC ratio 0.5 *vs* 0.8; P=0.86). There was a statistical trend between the degree of infiltration of PC in the bone marrow before ASCT and the detection of atypical PC in the graft (p=0.06). At day +100, 94% of patients with CR or VGPR to induction therapy maintained the response, and 49% of patients in PR, SD or PD achieved post-ASCT CR or VGPR (p=1.24^-7). There was no association between the content of atypical PC in the graft and the response to day +100. However, the percentage of pre-ASCT PC in the bone marrow was significantly related to the response at day +100 (CR or VGPR *vs* PR, SD or PD), p=0.003, as well as the pre-ASCT monoclonal component (p=4.03^-7).

Table 1.

Median age, yr (range)	60 (30-70)
Male (%)	55 (45.8)
Plasma cell dyscrasia, n (%)	
- Multiple myeloma, IgG	66 (55.0)
- Multiple myeloma, IgA	28 (23.3)
- Bence-Jones multiple myeloma	15 (12.5)
- Plasma cell leukemia	5 (4.2)
- Other: Non secretory o plasmocytoma	6 (5.0)
Salmon-Durie staging, n (%)	
- Stage I	10 (8.3)
- Stage II	36 (30.0)
- Stage III	64 (53.3)
- Other (plasma cell leukemia or plasmacytoma)	10 (8.3)
Response to induction treatment before ASCT, n (%)	
- Complete response	36 (30.0)
- Very good partial response	19 (15.8)
- Partial response	55 (45.8)
- Stable disease	9 (7.5)
- Progressive disease	1 (0.8)
Mobilization regimen, n (%)	
- G-CSF	87 (72.5)
- Chemotherapy and G-CSF	33 (27.5)

Summary/Conclusions: Infusion of PC with atypical phenotype does not appear to affect the response at day+100 following ASCT, in patients with MM or plasma cell dyscrasia. Conversely, the quality of response to induction therapy was significantly associated to 100-day outcome after transplantation. These data support that *in vivo* persistent residual cells, but not those being infused with the graft, are the main source of relapse in MM.

P403

EVALUATION OF THERAPEUTIC PLASMA EXCHANGE AT A TERTIARY LONDON HOSPITAL

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Background: Therapeutic plasma exchange (TPE) is used to treat a number of haematological, renal and neurological conditions. Pathogenic antibodies or other plasma molecules are removed, and plasma volume is replaced with fluid. Human albumin solution (HAS) is usually preferred, except in cases of Thrombotic Thrombocytopenic Purpura (TTP) and related conditions. TPE may result in dilutional coagulopathy, and reactions such as hypersensitivity can occur. The British Society for Haematology (BSH) published a 2015 guideline to assist the use of TPE in UK clinical practice, providing evidence-based indications and recommended schedules.
Aims: To evaluate the use of elective TPE at a large tertiary London hospital, compare clinical practice against BSH guideline recommendations, and explore the effect of TPE on coagulation test results.
Methods: Data was collected prospectively over a 2 month period, using patient notes and electronic transfusion records. A data collection form recorded the indication, treatment schedule, replacement fluid, complications, the presence of a written treatment plan, and frequency and results of coagulation testing.
Results: 24 plasma exchanges took place over the period of data collection; there were no cases of TTP. Adherence to BSH guidelines was variable; although most cases (88%) had an evidence-based clinical indication for TPE, just 4% had a full written treatment plan, and only 17% of courses followed recommended scheduling. 75% of patients had received at least one prior course, some outside guideline indications for repeat courses. Most patients (83%) initially received appropriate replacement fluid (HAS), however 87% received FFP at some point during TPE, with 42% receiving Solvent Detergent FFP. In 17% of patients this fluid change was due to a reaction, but for the remainder it was due to dilutional coagulopathy. The guidelines recommend fibrinogen monitoring, and although most patients had baseline measurement (75%), subsequent testing showed wide variation. Despite this, 71% had a fibrinogen of ≤1 g/l measured during TPE. Fibrinogen levels showed some correction by the next day but usually still abnormal. A prolonged APTT and PT was also seen in most patients immediately following TPE, which almost always corrected by the next day.

Summary/Conclusions: TPE use was generally compliant with BSH guidelines regarding clinical indication and initial replacement fluid. However many patients were changed from HAS to FFP due to measured or predicted coagulopathy. This is a recognised complication of TPE, and the guidelines suggest that if possible, TPE can take place on alternate days to ameliorate this. Fluid change to FFP is recommended only for those at increased haemorrhagic risk. Almost all the TPE courses in our study took place over 3 to 5 subsequent days, reflected in the high frequency of hypofibrinogenemia. The optimum frequency of fibrinogen monitoring, and the level that should prompt change to the TPE schedule, require further exploration. The following are planned to enhance adherence to BSH guidelines and improve patient care: 1. Documented treatment plans with clinical indication, proposed treatment schedule, replacement fluid. 2. Local trust guidelines to include recommended TPE schedules, agreed parameters to monitor response, frequency of fibrinogen monitoring, common complications and their management. Where possible, TPE should take place on alternate days to reduce dilutional coagulopathy. 3. Education of staff involved with service provision, and strengthening of the role of apheresis nurse as lead.

P404

A COMPREHENSIVE PROTEOMICS STUDY ON PLATELET CONCENTRATES: PLATELET PROTEOME, STORAGE TIME AND MIRASOL PATHOGEN REDUCTION TECHNOLOGY

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Background: Platelet concentrates (PCs) represent a blood transfusion product with a major concern for safety as their storage temperature (20-24ºC) allows bacterial growth, and their maximum storage time period (less than a week) precludes complete microbiological testing. Pathogen reduction technologies (PRTs) provide an additional layer of safety to the blood transfusion products from known and unknown pathogens (such as bacteria, viruses and parasites). In this context, PRTs (such as Mirasol technology) have been developed and are implemented in many countries. However, several studies have shown *in vitro* that Mirasol PRT induces a certain level of platelet shape change, hyperactivation, basal degranulation and increased oxidative damage during storage. It has been suggested that Mirasol PRT might accelerate what has been described as the platelet storage lesion (PSL), but supportive molecular signatures have not been obtained.
Aims: We aimed at dissecting the influence of both variables, *i.e.* Mirasol PRT and storage time, at the proteome level.
Methods: We present comprehensive proteomics data analysis of control PCs and PCs treated with Mirasol PRT at storage day 2, 6 and 8. Our workflow was set to perform proteomics analysis using a gel-free and label-free quantification (LFQ) approach. Semi-quantification was based on LFQ signal intensities of identified proteins using MaxQuant/Perseus software platform.
Results: We identified marginal differences between Mirasol PRT and untreated PCs during storage. However, those significant changes at the proteome level were specifically related to the functional aspects previously described to affect platelets upon Mirasol PRT, and in addition, the effect of Mirasol PRT on the platelet proteome appeared not to be exclusively related to proteomic changes due to PSL.
Summary/Conclusions: In summary, semi-quantitative proteomics allows to discern between proteome changes due to Mirasol PRT or PSL, and proves to be a methodology suitable to phenotype platelets in an unbiased manner, in various physiological contexts.

P405

USE OF A SURVEY TO ASSESS AND IMPROVE ADHERENCE TO UK BLOOD TRANSFUSION GUIDELINES IN A HOSPITAL SETTING

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Background: UK guidelines to provide evidence-based support for decisions to transfuse packed red cells were published in 2015 by NICE (National Institute for Health and Care excellence). The guidelines specified Hemoglobin (Hb) targets for transfusion, use of single unit transfusion to avoid over-transfusion, information provision to patients for informed consent, and avoidance of pre-operative transfusion by timely identification of iron deficiency for referral through an anemia pathway. A local baseline audit of NICE compliance at our London tertiary referral hospital showed low overall compliance with these recommendations.
Aims: To determine knowledge amongst the prescriber group of transfusion recommendations for stable patients, to gain insight into current patterns of decision-making for transfusion and to impart knowledge of the key NICE guidance to prescribers.
Methods: An online survey, designed to both evaluate and inform participants, was targeted at doctors of different training grades and specialties during a two week period. The outcomes of this are being used to guide further training.
Results: Of 141 participants who took part in the survey, 31% (43) had been qualified for less than two years and 47% (65) were consultants. Specialties included Surgery, Anesthetics, Internal Medicine, Hemato-Oncology and Intensive Care. 60% (84) had prescribed blood within the last month. Despite only 51% (72) awareness of the NICE guidelines, a significant majority (73%, 103) selected the correct Hb threshold of ≤70g/L for transfusion in patients without acute coronary syndrome (ACS). For patients with ACS, the correct Hb threshold of ≤80g/L was selected by 42% (58), but there was a wide spread of answers. 65% (90) of participants were aware that, in a stable patient Hb is checked after each unit of red cell transfusion, but surprisingly a few (4%, 5) did not check post transfusion Hb at all. Ferritin measurement was inconsistent with only 47% (66) routinely measuring this prior to transfusion, and only 31% (44) aware that a ferritin result over 30 days old should be rechecked. This highlighted potentially inadequate identification of iron deficiency anemia. In addition only 40% (57) were aware of the existence of a hospital anaemia clinic for referral. When reflecting on consent methods, 96% (135) of participants explained the indication to patients, and 90% (127) gave an opportunity to ask questions and ensured the patient was content to proceed. Provision of written information was poor (26%, 37) and only 55% (78) recorded the discussion in patients' notes. Exploring barriers to consent, 24% (32) expressed difficulty in obtaining a patient information leaflet, and issues relating to lack of time and confidence were 16% (22) and 9% (12) respectively.
Summary/Conclusions: Although the majority of participants expressed awareness of the NICE guidance, knowledge was not reflected in subsequent questions. The survey allowed simultaneous assessment of knowledge and provision of key information as a factsheet. Almost all participants felt that completion of the survey had been helpful, and as a tool to reach a highly mobile group, the survey is a constructive and supportive method to facilitate implementation of national guidance by medical staff. We were also able to identify areas that need further development including the clinical referral pathway for the anemia clinic and improving the availability of patient information leaflets on hospital wards. At present we are updating the hospital transfusion policy, which will be disseminated to all hospital staff, and carrying out structured case based discussion sessions with junior doctors to enhance knowledge and confidence.

P406

SCREENING OF TRANSFUSION PRODUCTS FOR PRION DISEASES USING APTAMERS AND TUNABLE RESISTIVE PULSE SENSING

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Background: Prion diseases are a group of fatal transmissible neurological conditions whose disease etiology is characterised by the change in conformation of the normal intrinsic cellular prion protein (PrP^{c}) in to the highly ordered insoluble amyloid state conformer (PrP^{sc}). The significant event fundamental to the progression of these diseases is the self-catalytic, and perpetuating, nature of the conversion of PrP^{c} in the presence of PrP^{sc} aggregates. The emergence of Variant Creutzfeldt-Jakob disease (vCJD), the most predominant prion disease in humans in the United Kingdom during the 1990s, is considered to be an effect of dietary exposure to the bovine spongiform encephalopathy (BSE) agent through contaminated meat products. To date, the widely accepted estimate for the prevalence of vCJD in the UK puts the number of potential carriers at 1 in 2000. Since the disease is known to be infectious and transmissible, the iatrogenic ability of this disease is a significant risk to public health through transfusion products and surgical procedures.
Aims: We aim to develop a reliable and robust assay that can be used as a screening tool to detect the infectious PrP^{sc} protein at low levels in human blood with high selectivity and high sensitivity.
Methods: Here we use a technique based on the Coulter Counter principle that uses tunable elastomeric nanopores termed Tunable Resistive Pulse Sensing (TRPS) to detect the prion protein without an amplification step. The first stage optimises the grafting of an ssDNA aptamer onto nanoparticles. In proof of concept work, the functionalized nanoparticles were then used to detect the cellular prion protein in phosphate buffered saline by monitoring the relative change in velocity through the nanopore, which is then converted to zeta potential. The method was then applied to protein rich samples and serum.
Results: By varying the concentration of aptamer relative to the binding capacity of the nanoparticles, a significant change ($p=0.05$) in zeta distributions was observed. Here mean zeta values were -1.94 mV for 0%; -4.43 mV for 33%; and -7.30 mV for 100%. The assay was further developed by monitoring the functionalized particle's translocation velocity as a function of prion protein concentration. Increasing the concentration of the protein caused shielding of the polyanionic DNA by the positive protein at pH 7.4, therefore the velocity of the protein/particle conjugate decreased. The lowest concentration to have a significant change ($p=0.05$) in velocity distribution was 1 nM, with a 2.5% decrease relative to 0 nM. The higher concentration of 50 nM had a bigger effect of 24% decrease.
Summary/Conclusions: TRPS technology presented here offers the ability to observe the interaction of an aptamer and the prion protein using a particle by particle assay design. Any relative change to the functionalized particle's signal could be observed, demonstrating its capability and suitability to detect biological targets.

SIMULTANEOUS SESSIONS II

Front-line combinations in multiple myeloma and amyloidosis

S407

QUADRUPLET *VS* SEQUENTIAL TRIPLET INDUCTION THERAPY FOR MYELOMA PATIENTS: RESULTS OF THE MYELOMA XI STUDY

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Background: Combining anti-myeloma induction therapies limits the impact of clonal heterogeneity on resistance to therapy, maximising response and associated clinical outcomes. Triplet combinations induce deeper, longer remissions than doublets and those containing an immunomodulatory agent, a proteasome inhibitor (PI) or both are the current standard of care in Europe/US. Potential approaches to further improve outcomes include response-adapted induction, treating suboptimal responders with sequential treatment using an agent with a different mechanism of action, or intensifying induction for all patients by the use of quadruplet combinations upfront.
Aims: The UK NCRI Myeloma XI trial is a large, phase III study comparing, in transplant eligible (TE) patients, the induction quadruplet carfilzomib, cyclophosphamide, lenalidomide and dexamethasone (KCRD) to the sequential strategy of triplet immunomodulatory combinations (with thalidomide or lenalidomide) followed by additional pre-transplant consolidation with PI triplet therapy for those with a suboptimal response.
Methods: In 2013, the TE pathway of the Myeloma XI study was amended to include KCRD given in 28 day cycles (carfilzomib 36mg/m^2 IV d1-2,8-9,15-16 (20mg/m^2 #1d1-2), cyclophosphamide (cyclo) 500mg PO d1,8, lenalidomide (len) 25mg PO d1-21, dexamethasone (dex) 40mg PO d1-4,8-9,15-16). Patients were randomised to this up-front quadruplet or the sequential strategy of CRD (cyclo 500mg PO d1,8, len 25mg PO d1-21 PO daily, dex 40mg PO d1-4, 12-15) or CTD (cyclo 500mg PO d1,8,15 thalidomide 100-200mg PO daily, dex 40mg PO d1-4,12-15) given to max. response. Patients with VGPR/CR proceeded straight to ASCT, those with PR/MR were randomised to sequential CVD (cyclo 500mg d1,8,15, bortezomib 1.3mg/m^2 IV/SC d1,4,8,11, dex 20mg PO d1,2,4,5,8,9,11,12) or nothing and those with SD/PD all received sequential CVD. At day 100 post ASCT there was a maintenance randomisation between lenalidomide and observation. The trial has now closed to recruitment and all patients have completed induction therapy. This analysis compares responses and toxicity of the different regimens.

Table 1.

Response at end of first induction therapy	CTD (n=1021)	CRD (n=1021)	KCRD (n=526)
CR	6.1%	8.4%	18.1%
VGPR	46.8%	52.2%	60.6%
PR	29.7%	25.6%	7.6%
Response at day 100 after ASCT (number completing ASCT to date)	(n=647)	(n=672)	(n=332)
CR	20.9%	23.7%	34.9%
VGPR	56.1%	58.5%	57.5%
PR	16.1%	14.4%	4.8%

Results: 2568 TE patients underwent induction randomisation (CTD 1021, CRD 1021, KCRD 526). Patients were comparable with respect to age (median 59 years), sex and other key laboratory parameters. Patients were mandated to receive a minimum of 4 cycles of initial induction with therapy continued to maximum response. The median number of cycles delivered was CTD: 5, CRD: 5, KCRD: 4. Grade ≥3 haematological toxicities differed between the groups. (Neutropenia CTD: 12%, CRD: 22%, KCRD: 16%; Thrombocytopenia CTD: 3.4%, CRD: 4.5%, KCRD: 8.1%; Anaemia CTD: 6.7%, CRD: 9.6%, KCRD 10%). Grade ≥2 neurological toxicity was greater with the thalidomide-containing regimen (Sensory neuropathy CTD: 9.5%, CRD: 3.4%, KCRD: 2.3%). There was no statistically significant difference in rates of investigator reported, all-grade, thromboembolic events between regimens (CTD: 11.8%, CRD 11.1%, KCRD 14.7%). Response to initial induction and following ASCT is shown in **Table 1** indicating deeper responses with the quadruplet compared to triplets both at the end of first induction regimen (p<0.0001) and, importantly, post-ASCT (p<0.0001). These differences were observed despite the use of randomised pre-transplant consolidation for suboptimal responders to triplet immunomodulatory therapy.
Summary/Conclusions: Induction therapy with KCRD, an outpatient delivered quadruplet regimen, was associated with deeper responses than immunomodulatory triplet therapy (CRD/CTD) and was well tolerated. Deeper responses persisted after ASCT, with an impressive response rate ≥VGPR of 92% with KCRD.

S408

DEEP AND DURABLE RESPONSES WITH WEEKLY IXAZOMIB, LENALIDOMIDE AND DEXAMETHASONE IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA: LONG-TERM FOLLOW-UP OF PATIENTS WHO DID NOT UNDERGO SCT

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Background: Triplet combinations that include a proteasome inhibitor (PI) have been proven superior to doublets in newly diagnosed multiple myeloma (NDMM) (San Miguel et al, N Engl J Med 2008, Durie et al, Lancet 2017). The all-oral combination of the novel PI ixazomib plus lenalidomide-dexamethasone (IRd) was evaluated as an induction regimen in NDMM patients, followed by single-agent ixazomib maintenance.
Aims: Here we report updated efficacy and long-term safety data for patients who did not withdraw from the study in order to receive stem cell transplantation (SCT).

Table 1.

Table. Treatment exposure and safety data.

	All pts who did not withdraw to receive SCT (N=42)	Maintenance subset (N=25); AEs with onset during induction (cycles 1–12)	Maintenance subset (N=25); AEs with onset during maintenance (cycles ≥13)
Treatment exposure			
Median number of treatment cycles, (range)	17 (1–73)	41 (15–73)	
Safety summary			
Grade ≥3 treatment-related AEs, n (%)	31 (74)	14 (56)	6 (24)
Treatment-related serious AEs, n (%)	11 (26)	5 (20)	1 (4)
Discontinued due to AEs, n (%)	9 (21)	0	0
AE leading to dose reduction of any drug in the regimen, n (%)	27 (64)	19 (76)	2 (8)
On-study deaths, n (%)	1 (2)	0	0
Most common (≥10% pts) treatment-related grade ≥3 AEs, n (%)			
Neutropenia#	7 (17)	4 (16)	0
Thrombocytopenia*	7 (17)	2 (8)	1 (4)
Fatigue	6 (14)	5 (20)	0
Hypokalemia	4 (10)	2 (8)	1 (4)
Treatment-related AEs of interest, n (%)			
Rash, eruptions, exanthems NEC*	3 (7)	2 (8)	0
Diarrhea	3 (7)	0	0
Nausea	2 (5)	0	0
Peripheral neuropathy NEC*	2 (5)	0	0

*High-level MedDRA terms; *pooled terms

Methods: In this phase 1/2 study (NCT01217957), patients with NDMM received weekly oral ixazomib (1.68-3.95mg/m^2; days 1, 8, and 15) plus lenalidomide (25mg, days 1-21) and dexamethasone (40mg, days 1, 8, 15, and 22) for up to twelve 28-day induction cycles, followed by maintenance therapy with weekly single-agent ixazomib, at the last tolerated dose given during induction, until disease progression or toxicity.
Results: Of the 65 enrolled patients, 42 continued on study treatment without early withdrawal for SCT; the long-term follow-up of these 42 patients is reported here. Baseline patient characteristics included: median age, 68 years (range 34-86); ISS stage I/II/III in 40%/43%/17%. As of October 18, 2016, with median follow-up of 56 months, the confirmed overall response rate (ORR; ≥ partial response [PR]) was 80%, complete plus very good partial response (CR+VGPR) rate was 63%, and CR rate was 32%. Median time to first response was rapid (0.95 months), while median time to CR was 5.6 months. Median progression-free survival (PFS) in these patients not receiving SCT was 25.3 months. Median overall survival (OS) has not been reached at a median follow-up of 56 months; 3-year OS estimate was 87%. Safety findings are summarized in the Table; 74% of patients had grade ≥3 treatment-related adverse events (AEs), and 26% of the patients had treatment-related serious AEs. Among treatment-related AEs of interest, grade ≥3 rash and peripheral neuropathy were infrequent. There was one treatment-related death due to respiratory syncytial viral pneumonia. After completing 12 cycles of induction therapy with IRd, 25 patients went on to receive maintenance single-agent ixazomib. In these 25 patients, at the end of the induction period ORR was 100%, including 44% VGPR and 32% CR. Responses deepened during maintenance; at data cut-off, the response rates in this maintenance therapy population were ORR 100%, VGPR 32%, and CR 44%. Median PFS for patients who received maintenance therapy was 24 months. The occurrence of the most common treatment-related grade ≥3 AEs (neutropenia, thrombocytopenia, and fatigue) was confined almost exclusively to the induction period. During the maintenance period no patients reported onset of grade ≥3 peripheral neuropathy or rash.
Summary/Conclusions: In patients with NDMM, weekly ixazomib plus Rd, followed by single-agent ixazomib maintenance, was highly active, resulting in deep and durable responses, long PFS, and a high 3-year OS estimate. IRd followed by single-agent ixazomib maintenance also showed an acceptable safety profile, with less toxicity reported during the maintenance (single-agent ixazomib) *vs* induction (IRd) periods, with no evidence of cumulative toxicities.

S409

DEPTH OF RESPONSE AS SURROGATE MARKER FOR PROGRESSION-FREE SURVIVAL AND OVERALL SURVIVAL IN ELDERLY NEWLY DIAGNOSED MYELOMA PATIENTS TREATED WITH VMP AND RD: GEM2010MAS65

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Background: Bortezomib plus melphalan and prednisone (VMP) and lenalidomide plus low-dose dexamethasone (Rd) are two standards of care for elderly untreated MM patients. In order to improve its outcome, we decided to use both VMP and Rd for 18 cycles in a sequential or alternating scheme. After a median f/u of 27 months, both regimens (sequential and alternating) showed similar efficacy with an acceptable toxicity profile.
Aims: To consolidate data, we have updated the outcome with long f/u (51 months), evaluating the role of Complete Response and Minimal Residual Disease (MRD) by multiparametric flow cytometry.
Methods: 242 pts were randomized to receive 9 cycles of VMP followed by 9 cycles of Rd or the same regimens in an alternating approach (one cycle of VMP alternating with one Rd, up to 18 cycles. VMP included the iv administration of weekly bortezomib (except in the first cycle that was given twice weekly) at 1.3mg/m^2 in combination with oral melphalan 9mg/m^2 and prednisone 60mg/m^2 once daily on days 1-4. Rd treatment consisted on len 25mg daily on days 1-21 plus dex 40mg weekly. MRD was evaluated by second generation flow (sensitivity level of 10^{-5}).
Results: 233 pts were evaluable for efficacy. Baseline characteristics of the pts were well balanced in both arms. The median progression-free survival (PFS) was of 30m in the sequential and 32m in the alternating arm (p>0,5). The median overall survival (OS) has been reached in the sequential arm (64m) whilst has not been reached yet in the alternating arm (63% at 5y) (p>0,5). 95 patients (41%) achieved >CR (49 and 46 patients in the sequential and alternating arms, respectively). Pts who achieved >CR had a significantly longer PFS (median of 45m) as compared with pts who didn't (median of 22,3 m) (HR: 0,32; p<0.0001). This translated into a benefit in OS: 73% of pts that achieved >CR remain alive at 5 years whilst the median OS was of 49m for patients that didn't achieve CR (HR: 0,34; p<0,0001). No differences were observed between the sequential and alternating arms. Minimal residual disease MRD was evaluated in 83 out of the 95 pts who achieved >CR. In 46 of them (55%) cells were undetectable with a sensitivity threshold of 10^{-5}, and were considered as MRD-ve patients. These pts displayed a significantly longer PFS (median not reached) as compared to MRD+ve pts (median PFS of 40m) (HR: 0.32; p=0,006) as well as significantly longer OS (HR: 0.26; p=0.012). FISH was available in 174 patients: 32 (18%) were considered high-risk (t(4;14), t(14;16) or del 17p). Outcomes were inferior but not significantly different between the high- and standard-risk groups in terms of PFS (26m vs. 33 months (p=0.11)). The achievement of >CR completely overcome the adverse prognosis of the presence of high-risk cytogenetic abnormalities with a median PFS of 47m and 50m in the high and standard-risk subgroup, respectively (p>0.5). This effect was also evident when MRD negativity was achieved. In terms of OS, the outcome for both high and standard risk subgroup of pts was superimposable during the first 20 months but the curves separated since this point, resulting in a median OS of 40m and 63m (p=0,001), respectively. This effect was maintained for pts that achieved >CR including MRD negativity.
Summary/Conclusions: The present therapeutic approach, based on VMP and Rd for newly diagnosed elderly MM pts represents an optimal therapeutic option for fit elderly patients. Pts who achieved >CR and MRD-flow had significantly longer PFS and OS. The achievement of >CR and MRD negativity is able to overcome the poor prognosis of the presence of high risk cytogenetic abnormalities in terms of PFS but continuous therapy is probably required for high risk patients in order to maintain the benefit in OS.

S410

CARFILZOMIB-LENALIDOMIDE-DEXAMETHASONE *VS* CARFILZOMIB-CYCLOPHOSPHAMIDE-DEXAMETHASONE INDUCTION: PLANNED INTERIM ANALYSIS OF THE RANDOMIZED FORTE TRIAL IN NEWLY DIAGNOSED MULTIPLE MYELOMA

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Background: Previous phase I-II studies showed that Carfilzomib-Lenalidomide-Dexamethasone (KRd) and Carfilzomib-Cyclophosphamide-Dexamethasone (KCd) combinations are safe and effective in patients with newly diagnosed multiple myeloma (NDMM) (Jakubowiak Blood 2012, Bringhen Blood 2014).
Aims: The FORTE trial compared KCd *vs* KRd in transplant-eligible patients. Here we report results of the first planned safety interim analysis on induction and mobilization, and preliminary efficacy data.
Methods: NDMM patients younger than 65 years of age were included. Patients were randomized (1:1:1; stratification ISS and age) to: 4 28-day KCd cycles (carfilzomib:20/36mg/m^2 IV d 1, 2, 8, 9, 15, 16; cyclophosphamide 300mg/m^2 d 1, 8, 15; dexamethasone: 20mg d 1, 2, 8, 9, 15, 16) followed by high-dose melphalan and autologous stem cell transplantation (MEL200-ASCT) and consolidation with 4 KCd cycles; or 4 28-day KRd cycles (carfilzomib and dexamethasone as above; lenalidomide:25mg d 1-21) followed by MEL200-ASCT and 4 KRd cycles; or 12 KRd cycles. After the 4th induction cycle, all patients received Cyclophosphamide 2 g/m^2, followed by peripheral blood stem cell collection. For the present interim analysis, we pooled together data of the two KRd groups, because patients in the two groups in fact received the same treatment until mobilization. Data cut-off was October 30, 2016.
Results: A total of 281 patients were evaluated (94 assigned to KCd treatment and 187 to KRd treatment). The most frequent grade 3-4 adverse events (AEs) and serious AEs (SAEs) in both arms were hematological (mainly neutropenia) and infections (mainly pneumonia/fever); increased AST/ALT/GGT (mainly reversible) and dermatological (rash) AEs were more frequent among KRd patients; cardiac AEs were 2% with KRd (including atrial fibrillation [1%] and ischemic heart disease [1%]) *vs* 1% with KCd (atrial fibrillation). Death occurred in 1 patient in the KCd group (infection not treatment-related) *vs* 3 patients in the KRd group (2 cardiac arrest [1 not treatment-related], 1 infection not treatment-related). In the KCd *vs* KRd arms, 99% *vs* 95% (P=0.44) of pts mobilized stem cells (median number of PBSC collected: 9 *vs* 6x10^6CD34/Kg with KCd *vs* KRd). Plerixafor was required in 10% *vs* 24% (P=0.01), respectively. At least a very good partial response (VGPR) was reported in 61% of patients receiving KCd *vs* 74% receiving KRd (P=0.05).

Table 1.

Grade 3-4 AEs/SAEs	KCd	KRd
Hematological	13%	9%
Cardiac	1%	2%
Hypertension	0%	2%
Thromboembolism	0%	1%
Gastrointestinal	0%	3%
AST/ALT/GGT increase	1%*	7%*
Dermatological	0%*	7%*
Infections	6%	9%
Acute Kidney Injury	0%	2%

*p value <0.05

Summary/Conclusions: Safety profile was acceptable; more patients required plerixafor in the KRd arm. Rate of VGPR was higher with KRd. Updated data on a higher number of patients will be presented at the meeting. The trial is registered at Clinicaltrials.gov: NCT02203643

S411

HOVON 104; FINAL RESULTS FROM A MULTICENTER, PROSPECTIVE PHASE II STUDY OF BORTEZOMIB BASED INDUCTION TREATMENT FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH DE NOVO AL AMYLOIDOSIS

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Background: Bortezomib (B) has been reported to be very effective in AL amyloidosis with overall response rates (ORR) varying between 50-80%. However, there are no prospective data from multicenter studies on B treatment in *de novo* patients. We investigated the efficacy and safety of B-Dexamethasone (BD) induction treatment followed by HDM+SCT in *de novo* AL amyloidosis patients.

Aims: The primary aim was to improve the hematological CR rate at 6 months after SCT on intention to treat analysis from 30 to 50%. Secondary aims were OS, PFS, hematologial response rate after BD treatment, organ responses, safety and prognostic factors for survival.

Methods: Patients with biopsy proven AL amyloidosis, aged between 18-70 years, with detectable M-protein and/or level of involved FLC >50mg/L, WHO performance status 0-2, NYHA stage 1-2 and ejection fraction >45% were included. Major exclusion criteria were symptomatic orthostatic hypotension, NT proBNP level >5000 pg/ml, Troponin T> 0.06 ug/l, Bilirubin >2x ULN, eGFR<30 ml/min, CTCAE grade peripheral sensory neuropathy > grade 2 or > grade 1 with pain. Inclusion and exclusion criteria were installed both at entry and before stem cell mobilization (SCM). B was given subcutaneously 1.3mg/m^2 twice a week for 2 weeks in a 21-day cycle, D 20mg orally on each B day and the following day. HDM dosage was 200mg/m^2. Hematological responses were defined according to consensus criteria with the addition of very good partial response (VGPR), defined as the difference between involved and uninvolved FLC<40mg/L. Cardiac, renal and liver response and progression criteria were defined according to consensus criteria with addition of NT proBNP.

Results: Median age was 59 years (range 26-70) and 60% were male. NYHA stage was 1 in 56% and 2 in 42% of patients. Mayo cardiac risk score was I (30%), II (36%), III (34%). Organ involvement was 82% renal, 66% heart, 28% liver, 14% neurological, 8% gastrointestinal and 38% of patients had 3 or more organs involved. Bone marrow plasmacells were >10% in 28% of patients. The median FU for patients alive is 24 (10-55) months. Twelve of 50 (24%) patients could not proceed to SCM. Four patients due to B related toxicity, 3 patients did not fulfill criteria to proceed, 2 patients died (both amyloidosis related) and 3 miscellaneous. Of these 38 patients, 3 went subsequently off protocol because of ineligibility for HDM. Thirty-five out of 50 patients (70%) received HDM + SCT, one patient died of a cardiac arrest after the SCT procedure. The ORR after induction was 80%, ≥VGPR in 54% and CR in 6% of patients. The ORR in the 35 patients at 6 months after SCT was 80%, ≥VGPR in 51% and CR in 43% of patients. On intention to treat analysis the CR rate at 6 months after SCT was 30%. Organ responses at 6 months after SCT were 16/29 renal, 2/8 liver and 13/23 heart. No baseline characterics were identified to be predictive for OS or PFS. BD doses were reduced and delayed after 2 cycles in almost half of patients, mostly because of neurotoxicity, Sensory neuropathy grade 2 or higher was seen in 36% of patients and autonomic neuropathy, mostly dizziness and collapse, in 22%.

Summary/Conclusions: This final analysis demonstrates that the primary aim of improving CR rate at 6 months after SCT from 30 to 50% was not met. This was mainly caused by the high dropout rate before SCT. This may be due to patient selection, but we also demonstrate that BD, given twice weekly sc, despite good efficacy, cannot prevent early amyloidosis related toxicity and can induce grade 2 or higher neurotoxicity.

Trial registration www.trialregister.nl (NTR 3220), EudraCT 2010-021445-42, supported by the Dutch Cancer Society (UU 2010-4884) and by an unrestricted grant from Janssen-Cilag.

Hodgkin and indolent lymphoma - Clinical

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NIVOLUMAB FOR RELAPSED/REFRACTORY CLASSICAL HODGKIN LYMPHOMA AFTER AUTOLOGOUS TRANSPLANT: FULL RESULTS AFTER EXTENDED FOLLOW-UP OF THE MULTICOHORT MULTICENTER PHASE 2 CHECKMATE 205 TRIAL

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Background: Nivolumab, a fully human IgG4 monoclonal antibody targeting programmed death-1, is an immune checkpoint inhibitor that augments T-cell activation and antitumor responses. Nivolumab is indicated for pts with relapsed/refractory (RR) classical Hodgkin lymphoma (cHL) following autologous stem cell transplantation (ASCT) and brentuximab vedotin (BV) treatment. The multicohort phase 2 CheckMate 205 trial (NCT02181738) enrolled pts with RR cHL after ASCT. Initial analyses revealed high objective response rates (ORR), encouraging duration of response (DOR) and an acceptable safety profile (Younes A et al, *Lancet Oncol* 2016). Durable responses to therapy are valuable in pts with progressive disease after failure of ASCT due to their limited treatment options.
Aims: To report extended follow-up data for all pts with RR cHL after failure of ASCT in CheckMate 205.
Methods: This single-arm multicenter trial enrolled pts (age ≥18 y) with RR cHL after ASCT into 1 of 3 independent cohorts (Cohort A: BV-naïve; Cohort B: BV only after ASCT; Cohort C: BV before and/or after ASCT). All pts received nivolumab 3mg/kg every 2 wk until disease progression or unacceptable toxicity. Pts in Cohort C with a persistent complete response (CR) for 1 y were to discontinue nivolumab and could resume at relapse. Primary endpoint was ORR per Independent Radiology Review Committee. Secondary endpoints included DOR; progression-free survival (PFS), overall survival (OS), and safety were exploratory endpoints. All pts provided written informed consent.

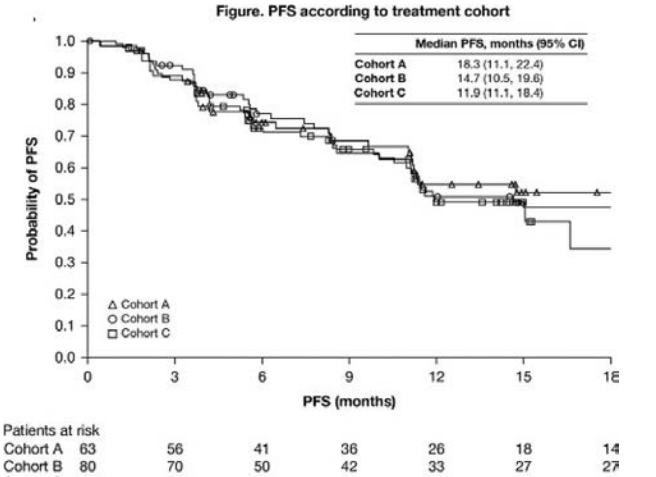

Figure 1.

Results: In total, 243 pts were treated: 63 in Cohort A (BV-naïve), 80 in Cohort B (BV after ASCT), and 100 in Cohort C (BV before [n=33], after [n=58], or before and after [n=9] ASCT). Median (range) age was 34 (18-72) y and 77% of pts had advanced (stage III+) disease at study entry. BV-naïve pts had fewer prior lines of therapy (median of 2 *vs* 4 with prior BV). At Dec 2016 database lock, median (min, max) follow-up was 19 (1, 25), 23 (2, 27) and 16 (1, 20) mo in Cohorts A, B, and C, respectively. Overall, 40% of pts were still on treatment; the most common reason for discontinuation was disease progression (26%). ORR was 65% in Cohort A, 68% in Cohort B, and 73% in Cohort C, with 29%, 13%, and 12% CR, respectively. Median (95% CI) DOR was 20 (13, 20), 16 (8, 20), and 15 (9, 17) mo in Cohorts A, B, and C, respectively. DOR for patients with CR was 20 months for BV-naïve patients (Cohort A) and ≥15 mo for BV-treated patients (Cohorts B and C); DOR for patients with partial response (PR) was 17 and ≥11 months, respectively. PFS by cohort is shown (**Figure 1**). Prolonged median PFS was seen for patients with CR (≥17 mo in each cohort), PR (≥15 mo in each cohort), and stable disease (≥9 mo in each cohort). Median OS was not reached in any cohort. The most common drug-related AEs were fatigue (23%), diarrhea (15%), infusion reactions (IRs; 14%), and rash (12%); grade 3-4 drug-related AEs in ≥3% of pts were lipase increases (5%), alanine aminotransferase increases (3%), and neutropenia (3%). The most common drug-related serious AEs were IRs (2%) and pneumonitis (1%). To facilitate translation to practice, efficacy results by sequencing of prior BV treatment will be presented.
Summary/Conclusion: With extended follow-up, high and durable rates of CR and PR to nivolumab therapy were observed in pts with RR cHL after ASCT, irrespective of BV treatment history.

Study funding: BMS; medical writing support: M Thomas (Caudex), funded by BMS.

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EARLY CHEMOTHERAPY INTENSIFICATION WITH ESCALATED BEACOPP IN ADVANCED-STAGE HODGKIN LYMPHOMA WITH A POSITIVE INTERIM PET-CT AFTER 2 ABVD CYCLES: LONG-TERM RESULTS OF THE GITIL/FIL HD 0607 TRIAL

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Background: Interim 2-[18F]fluoro-2-deoxy-D-glucose Positron Emission Tomography (FDG-PET) performed after 2 chemotherapy cycles (PET2) is the most powerful predictor of treatment outcome in ABVD-treated, advanced-stage classical Hodgkin Lymphoma (cHL). Preliminary reports showed that adapting treatment to PET2 result could increase the efficacy of standard ABVD.
Aims: To confirm in a prospective setting the favorable prognosis of advanced stage PET2 negative patients treated with ABVD, as well as the safety and efficacy of escalated BEACOPP given to PET2 positive patients.
Methods: We conducted a prospective clinical trial (HD0607 ClinicalTRial.gov identifier 00795613), in which advanced-stage (IIB-IVB) cHL patients were treated with 2 ABVD courses, and PET2 performed afterwards. The latter was blindly and independently reviewed by a panel of nuclear medicine experts, using the Deauville 5-point scale (5-PS). PET2+ patients (5-PS 4-5) were randomized to either BEACOPP escalated (Be) plus BEACOPP baseline (Bb) (4+4 courses) or Be+Bb (4+4) and Rituximab (R). PET2- (5-PS 1 to 3) patients continued ABVD treatment with 4 more cycles and, upon CR achievement, randomized to either consolidation radiotherapy (Rxt) on the sites of initial large nodal mass (LNM: diameter >5cm) or no further treatment (NFT).
Results: Starting from June 2008 till June 2014, 782 cHL patients were consecutively enrolled in 24 Italian and 1 Israeli centers. The median age was 31 years (14-60); 35% had stage IIB, 32% stage III and 32% stage IV. The International Prognostic Score (IPS) was 0-1 in 36.6%, 2-3 in 51%, >3 in 12.5%.

Overall, 150 (19.2%) proved PET2+ (97 score 4, 53 score 5) and 630 (80.5%) PET2-. PET2+ patients were more frequently male (56.7% *vs* 47.1%, p=.03), had higher IPS score (P=.0002) and bulky disease (28.0% *vs* 17.9%; p=.0002). Out of 149 PET2+ patients randomized to Be+Bb (76) or Be+Bb+R (73), 136 were evaluable for response: 93 obtained CR and 43 had a treatment failure. Of the remaining 13 patients, 3 died, 7 withdrew their consent and 3 stopped treatment for toxicity. As per study protocol, 627 out of 630 PET2- patients continued with 4 ABVD cycles and 3 withdrew their consent. Out of 296 with LNM, 148 were randomized to RxT and 148 to NFT. Among 627 patients, 574 (91.5%) achieved CR, 50 (8.0%) had a treatment failure and 3 (0.4%) withdrew their consent. Overall, 30 patients (3.8%) died, due to early death (n=2), resistant disease (n=18; 12 with a positive and 6 with a negative PET2), transplant related toxicity (n=5), infections (n=4) and pulmonary fibrosis (n=1). After a median follow-up of 1303 days (2-2857), the 4-Y PFS and OS for all 782 patients was 83% (95% CI 80%>86%) and 96% (95% CI 94%>97%), respectively. For PET2+ and PET2- patients, the 4-Y PFS was 69% (95% CI 60%>76%) and 87% (95% CI 84%>89%), while the 4-Y OS was 89% (95% CI 82%>93%) and 97% (95% CI 95%>98%) (Figure 1, Panel A and B). No outcome difference was observed for Be+Bb *vs* Be+Bb+R patients, with a 4-Y PFS of 69% (95% CI 57%>79%) and 68% (95% CI 55%>78%), respectively (p=.9731). Consolidation RxT in PET2- patients in CR after 6 ABVD and LNM did not translate in to a significant benefit, with a 4-Y PFS of 96% (95% CI 91%>98%) for RxT and 93% (95% CI 87%>96%) for NFT (p=.2882).

Figure 1. Progression Free Survival and Overall Survival by PET2 -/+

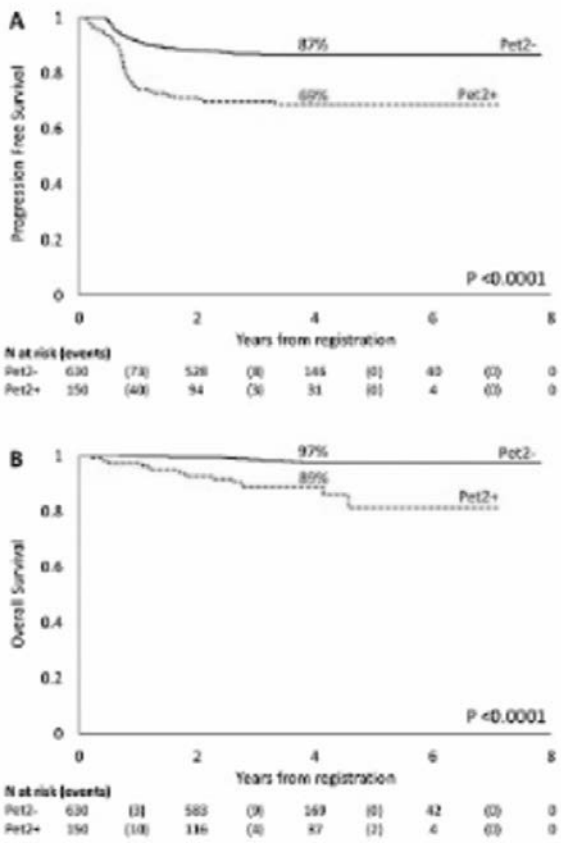

Figure 1.

Summary/Conclusions: These data suggest that 1) an early switch from ABVD to escalated BEACOPP can be safely done in PET2+ advanced-stage cHL; 2) the long-term outcome for the entire patient cohort is superior to standard ABVD; 3) no clinical benefit is associated with post ABVD RxT in PET2- patients presenting with large nodal mass; 4) the addition of Rituximab does not increase the effectiveness of Be+Bb in PET2+ patients.

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DISEASE CHARACTERISTICS AND SURVIVAL AFTER 3RD RECURRENCE OF CLASSICAL HODGKIN LYMPHOMA: AN ANALYSIS OF THE GERMAN HODGKIN STUDY GROUP

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Background: Data on disease presentation, therapeutic options and survival after 3rd or higher relapse of classical Hodgkin lymphoma (cHL) are sparse. Therefore the additional benefit of new agents, which are usually initially investigated after several relapses of cHL, is difficult to estimate.

Aims: The aim of this study was to define and describe a historical control group in European patients from the German Hodgkin Study Group (GHSG) data for comparison of safety and efficacy of novel therapeutic agents.

Methods: Cases with at least three consecutive tumor-related events, either progressive refractory or relapsed disease, were identified in the GHSG database. Detailed information was added from case report forms and physician's letters. Overall survival (OS) was the main and progression free survival (PFS), response to therapy, adverse events, disease and treatment characteristics as secondary endpoints.

Results: Among 12,584 HL patients in the GHSG first-line trials HD7 to HD15 and 449 HL patients in the trials HDR1 and HDR2 a total of 69 cHL patients with ≥3 tumor events were identified. The dates of occurrence of 3rd relapse ranged between 15th of January 1993 and 21st of June 2013. The sample consisted of 51 male (74%) and 18 female (26%) patients. At time of 3rd relapse the age of the patients ranged from 20 to 79 years (mean 39.2 years, standard deviation (SD) 14.0 years) and the majority of patients presented with stage III or IV disease (67%). Time from end of 3rd-line treatment to 3rd relapse was ≤3 months (i.e. GHSG definition of refractory disease) in 15 cases (22%), ≤12 months (early relapse) in 19 cases (28%) and >12 months (late relapse) in 35 cases (51%). All 69 patients were pretreated with chemotherapy, 35 (50.7%) with BEACOPP, 30 (43.5%) with ABVD and no BEACOPP, and 32 (46.6%) with another type of chemotherapy. The number of prior chemotherapies ranged from one to three (median 3). Pretreatment with radiotherapy was observed in 57 (82.6%) patients, with salvage chemotherapy aimed to induce a remission prior to a stem-cell transplantation (SCT) in 58 (84.1%), and with high dose chemotherapy followed by autologous SCT in 50 (72.5%) patients. Four patients (5.8%) had received allogeneic SCT as 3rd-line treatment. None of the patients had received brentuximab vedotin or anti-PD1 antibodies before 3rd relapse. With a median observation time of 63.3 months for OS after 3rd relapse, 45 patients (65.2%) had died and 60 (87.0%) had another PFS event. Twelve months after the 3rd relapse OS was 73.2% (95%>CI 62.6% to 83.8%) and PFS 50.8% (95%>CI 38.9% to 62.8%, Table 1).

Table 1.

	Progression Free Survival (PFS)				Overall Survival (OS)			
		%	95% confidence-interval			%	95% confidence-interval	
	events		Lower limit	Upper limit	events		Lower limit	Upper limit
6 months	16	76.3 %	66.1%	86.4%	7	89.6 %	82.4%	96.9%
12 months	33	50.8 %	38.9%	62.8%	18	73.2 %	62.6%	83.8%
18 months	47	29.5 %	18.5%	40.5%	23	65.6 %	54.2%	77.0%

Summary/Conclusions: Patients with a 3rd relapse or progression of cHL have a dismal, mostly palliative prognosis due to frequent tumor progression. Within one year half of the patients have a PFS event and one fourth die.

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A REVISED STAGING SYSTEM FOR WALDENSTRÖM'S MACROGLOBULINEMIA

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Background: Waldenström's macroglobulinemia (WM) is a rare low-grade B-cell lymphoma characterized by the lymphoplasmacytic bone marrow infiltration and the presence of monoclonal IgM immunoglobulin in the serum. WM is an indolent lymphoma that has heterogeneous clinical manifestations and patients with this disease may have a prolonged disease course; however, there are groups of patients with poor outcomes after a relatively short disease course. In order to develop a robust staging system a collaborative effort resulted in the formulation of the International Prognostic Scoring System for WM (IPSS-WM) which was developed in 2009 based on data of patients that were treated primarily without rituximab and mainly with alkylators and nucleoside analogues. IPSSWM is based on five covariates (age, hemoglobin, platelet counts, IgM levels and b2 microglobulin) and stratifies WM patients into 3 broad risk groups. IPSSWM does not take into account non-WM related mortality, which is common and quite different among patients over the age of 75 year and does not include LDH, which is a well identified prognostic factor both in lymphomas and multiple myeloma.

Aims: The aim of the current study was to revise the current IPSSWM by using a large dataset of symptomatic WM patients treated with different types of primary therapy that included rituximab and other new agents.
Methods: The analysis included 492 patients from the prospectively maintained database of the Greek Myeloma Study Group with a median follow up of 10 years. All patients fulfilled criteria for diagnosis and for treatment initiation according to Consensus Recommendations.

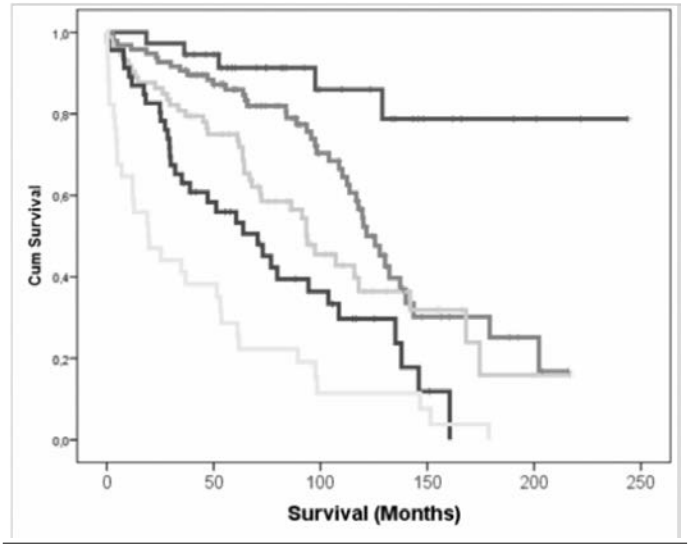

Figure 1.

Results: In univariate analysis factors such as age, beta-2 microglobulin, serum albumin and LDH were all associated with poor outcome. The IPSSWM includes age and b2 microglobulin but not serum albumin, or LDH, while the presence of very high IgM (>7gr/dl) was quite rare and of limited prognostic value. The presence of anemia <11.5gr/dl was common across all subgroups while low platelet counts <100x109/L was found in relatively few patients and had no prognostic significance. Based on ROC analysis for early death (within 3 years), serum albumin <3.5gr/dL and b2microglobulin >4mg/L were the two most important prognostic factors of early WM-related death. Age >65 years was associated with increased risk of death, however, age >75 years conferred additional risk (double hazard of death compared to those 65-75 years and fourfold compared to patients <65 years). Thus, we formulated a score in which high b2 microglobulin, elevated LDH and low serum albumin are scored with 1 point each, age 66-75 years is scored with 1 point but age >75 years is scored with 2. As a result, patients with scores 0, 1, 2, 3 or 4-5 had 3-year WM-related death rate of 3%, 7%, 14%, 19% and 48% (chi-square: 80.7, p<0.001). Regarding overall survival, 10-year survival rate was 85%, 59%, 39%, 28% and 12% (p<0.001) (Figure 1). Because age is a major determinant of disposition we also evaluated this staging system in patients >65 years and retained it prognostic significance. Compared to IPSSWM, this new staging system outperformed ISSWM: c-statistics, a measure of performance of a prognostic tool, was 0.652 for IPSSWM (95%CI 0.627-0.677) *vs* 0.711 (95% CI 0.659-0.763) for the new staging system.
Summary/Conclusions: A revised staging system, based on b2 microglobulin, elevated LDH, low serum albumin and age identifies groups with very different outcomes among patients with symptomatic WM treated with contemporary regimens and may outperform IPSSWM.

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SPLENIC MARGINAL ZONE LYMPHOMA (SMZL) TREATED WITH RITUXIMAB (R) MONOTHERAPY: A LONG TERM FOLLOW-UP STUDY ON 104 PATIENTS

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Background: Rituximab monotherapy has been used successfully in the treatment of SMZL and it can replace splenectomy, at least in 1st line.

Aims: To present our data on the outcome of R monotherapy treated pts after a long term follow-up.
Methods: The diagnosis of SMZL was based on the WHO criteria. Criteria for treatment initiation included: bulky/symptomatic splenomegaly, cytopenias or presence of B-symptoms. All pts received 6 weekly cycles of R as 1st line therapy at a dose of 375mg/m^2 (induction phase). None of the pts had been splenectomised before R treatment. Maintenance with R at a dose of 375mg/m^2 every 2 months for 1-2 years was given according to physician's discretion. Response assessment was based on the SLSG consensus criteria. Survival curves were estimated using the Kaplan Meier method and compared by log-rank test.
Results: 104 pts with SMZL were included. 45% were males with a median age of 66 y (41-91). At diagnosis all pts had bone marrow infiltration with a median % of infiltration of 40 (10-85). Anemia and thrombocytopenia were present in 30% and 19%, respectively. 40% had absolute lymphocytosis. LDH was elevated in 43%. According to the SLSG prognostic system, 39% were classified in group A, 56% in group B and 5% in group C. The median time from diagnosis to treatment initiation was 2 months (0-203). 71 pts received R maintenance. The overall response rate 2 months after the end of induction treatment was 93% (CR, CRu and PR in 42%, 21% and 30%, respectively). Maintenance therapy improved the quality of response in 19 of them, 52 pts maintained their initial response and one relapsed during maintenance phase. The 5- and 10-year PFS, OS and CSS were 70% and 64%, 93% and 88%, 99% and 93%, respectively. Maintenance therapy was associated with better PFS (p=0.008). 22 pts relapsed (6 of them with histologic transformation to DLBCL). 11/22 were retreated with R and 9/11 responded. 8 deaths were recorded: 3 of them disease related. R therapy was well tolerated. Only one pt could not complete treatment due to intolerance.

Table 1.

Table. Baseline characteristics and outcome of 104 SMZL pts treated with R monotherapy

Parameter	# of cases	%
Male sex	47	45
Median age (range)	66 (41-91)	
B-symptoms	7	7
Elevated LDH	44/102	43
Anemia	31/102	30
Thrombocytopenia	19/102	19
Lymphocytosis	40/100	40
SLSG prognostic system		
Group A	39/100	39
B	56/100	56
C	5/100	5
5-year PFS	64%	
10-year PFS	74%	
5-year OS	93%	
10-year OS	88%	
5-year CSS	99%	
10-year CSS	93%	

Summary/Conclusions: The present study, includes a large number of pts with a long follow-up, confirms that R monotherapy is very effective in SMZL with minimal toxicity and is recommended as the treatment of choice for this disease.

Biology of MPN: JAK2 and beyond

S417

YOU DON'T KNOW JAK: A PROGRAMMED RIBOSOMAL FRAMESHIFTING DEFECT POTENTIATES THE TRANSFORMING ACTIVITY OF THE JAK2-V617F MUTATION

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Background: The JAK-STAT pathway is a critical controller of cellular proliferation, differentiation, survival and apoptosis in response to external stimuli. Promiscuous activation of this pathway is an important driver in the pathogenesis of BCR/ABL-negative chronic myeloproliferative neoplasms. The JAK2-V617F allele is the most common and characterized mutation linked to this class of leukemia. The increased activation of JAK-STAT signaling in JAK2-V617F cells can be partially explained by increased JAK2 autophosphorylation. It is unclear however if these effects are sufficient to fully account for the strong activation of the JAK-STAT pathway induced by JAK2-V617F. We recently described programmed -1 ribosomal frameshifting (-1 PRF) as a novel mechanism regulating the expression of ~10% of human genes, including cytokine receptors (Belew AT et al, *Nature*, 2014). In this process, cis-acting mRNA elements (-1 PRF signals, which consist of a slippery site followed by a pseudoknot) direct translating ribosomes to slip by one base in the 5' direction, establishing a new reading frame. This directs ribosomes towards premature termination codons, resulting in destabilization of the -1 PRF signal-containing mRNA via nonsense-mediated mRNA decay (Figure 1). There is thus an inverse relationship between -1 PRF efficiency and mRNA stability.
Aims: To investigate whether the JAK2-V617F mutation, shown here to be located in the pseudoknot of a -1 PRF signal in the JAK2 mRNA, impacts disease progression through ablation of -1 PRF.
Methods: Computationally predicted -1 PRF signals were validated using dual luciferase reporters and proteomic analysis of a -1 PRF fusion protein. -1 PRF as well as mRNA abundance and decay were assayed in HEK293T and HeLa cells. Transformation assays were performed in hEpoR expressing Ba/F3 cells. *in vivo* experiments were performed in BALB/c mice.

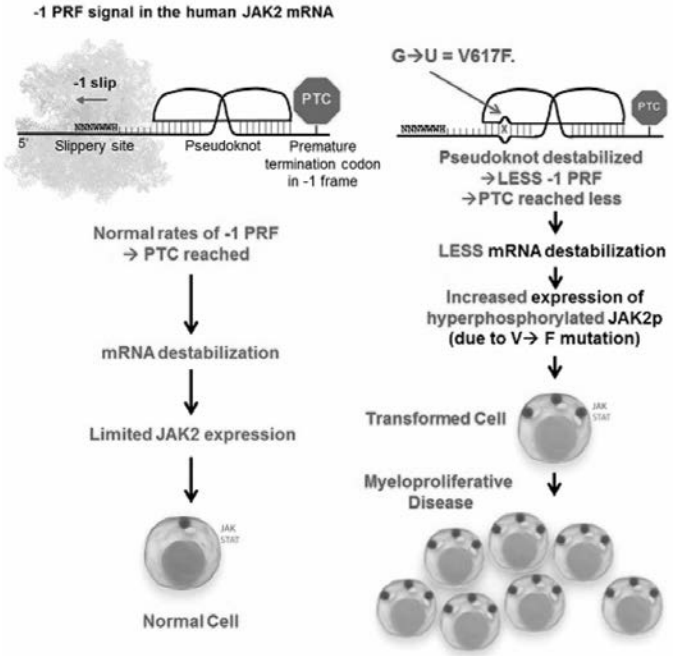

Figure 1.

Results: We demonstrate in human cell lines that the JAK2-V617F mutation structurally disrupts the -1 PRF signal in the JAK2 mRNA, leading to ~2-fold lower rates of -1 PRF and increased abundance of the JAK2 mRNA and protein. The transforming potential of a series of mutants designed to manipulate -1 PRF independent of V617F was assayed in a Ba/F3 cell model. Silent protein coding changes in the pseudoknot of the -1 PRF signal at position V617 (V617m) or the slippery site (SSm), both of which reduced frameshifting, increased JAK2 expression and led to transforming activity, albeit less than V617F. Importantly, the V617F+SSm combination conferred an additive effect on cellular transformation. Ba/F3 cells expressing these JAK2 variants were also introduced into mice. Whereas mice injected with wild type JAK2 remained healthy, both V617m and SSm induced similar leukemia phenotypes as V617F and V617F+SSm, with a ~2-fold longer disease latency of 8-10 weeks. Increased JAK2 mRNA abundance in JAK2-V617F homozygous patients as well as the presence of three additional -1 PRF signals in the JAK2 mRNA further suggest a prominent role for -1 PRF in controlling JAK2 production.
Summary/Conclusions: We demonstrate that the JAK2-V617F mutation diminishes -1 PRF on the JAK2 transcript, stabilizing the mRNA and increasing JAK2 expression, contributing to its transforming activity *in vitro* and disease onset *in vivo*. We suggest that -1 PRF normally provides a layer of control by limiting JAK2 translation. Defective -1 PRF synergizes with the transforming activity of the JAK2-V617F protein by causing its overexpression, explaining why this particular mutation causes such aggressive malignancies. In support of this, the combination of ruxolitinib and an HSP-90 inhibitor, which reduce kinase activity and JAK2 expression respectively, leads to increased therapeutic efficacy in myeloproliferative neoplasms (Bhagwat N et al, *Blood*, 2014).

S418

EFFECTIVENESS OF LSD1 INHIBITION FOR THE TREATMENT OF MPN

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Background: Treatment of MPN with JAK1/2 inhibitors ameliorates symptoms and splenomegaly but does not meaningfully reduce the *JAK2^{V617F}* allele burden. Though curative, stem cell transplantation is associated with extensive morbidity and mortality highlighting the need for novel effective therapies. The histone "lysine-specific demethylase 1A" (LSD1/KDM1A) is being explored as a drug target in AML with several inhibitors already in the clinic. The enzyme is critical for sustaining self-renewal in leukemic initiating cells; inhibiting LSD1 induces monocytic differentiation and reduces engraftment of AML cell *in vivo*. *LSD1* is over-expressed in a number of myeloid diseases including MPN. Preliminary data in a mutant *Mpl* mouse model of MPN showed that a 28-day course of LSD1 inhibition had a beneficial impact on spleen size, cytokines and mutant cell burden. In a mutant *JAK2* mouse model of MPN, we have characterized the disease-modifying activity of the LSD1 inhibitor (IMG-7289), a compound in clinical development for myeloid diseases (NCT02842827).
Aims: We assessed the pharmacodynamic effects of continuous daily treatment with IMG-7289 in a *JAK2^{V617F}* knock-in murine MPN with established disease. Animals were treated for up to 56 days. Outcome measures included complete blood counts (CBC), hematological phenotype, overall survival, spleen size, bone marrow morphology and the *JAK2^{V617F}* allele burden. Moreover, pro-inflammatory cytokine were monitored during the course of treatment as well as chromatin changes by western blotting and ChIPseq.
Methods: *Jak2^{V617F}*-L2 mice were crossed to *MxCRE* mice and displayed a fulminant MPN phenotype without dIdC induction. CBC and BM FACS analysis were conducted as previously described. We designed a qPCR assay to quantitate murine *JAK2^{V617F}* allele burden.
Results: IMG-7289 treatment was exceptionally well tolerated and mice showed drastic decreases in platelet count (208 *vs* 2063*10^3/µl), reticulocytes (800 *vs* 1674*10^3/µl), monocytes and neutrophils 14 days after the start of treatment. HCT and WBC started to decrease after 28 days. While the *JAK2* mutant allele burden increased over time in untreated mice, it decreased in IMG-7289 treated mice and was statistically significantly lower in peripheral blood as well as in spleen. We observed a drastic increase in the pro-inflammatory cytokine CXCL5 in untreated mice during the course of investigation while CXCL5 levels of treated mice decreased to levels of wild-type littermates. Moreover, treated mice showed a highly significantly increased survival over untreated mice, even in a late stage of disease. Lastly, we were able to show that global H3K9me2, which is generally associated with gene expression silencing, was increased in the bone marrow of IMG-7289 treated mice compared to control mice. The remaining pathophysiological data and functional data on epigenetic regulation will be presented.
Summary/Conclusions: The LSD1 inhibitor IMG-7289 normalizes or stabilizes elevated CBCs in a *JAK2^{V617F}* MPN mouse model. It decreases *JAK2* mutant allele burden, pro-inflammatory cytokine levels and confers a clear survival advantage. Our preliminary data show that LSD1 is a potent target with disease-modifying potential in MPN. Clinical studies with IMG-7289 testing this hypothesis have just begun. Owing to its mode of action, altering epigenetics, and the potential reversibility of drug-induced epigenetic remodeling, a long treatment period in MPN patients may be necessary to eliminate disease. Combining IMG-7289 with JAK1/2 inhibitors might accelerate treatment effects.

S419

LOSS OF RAF KINASE INHIBITOR PROTEIN IS INVOLVED IN MYELOMONOCYTIC LINEAGE COMMITMENT AND AGGRAVATES THE DEVELOPMENT OF CHRONIC MYELOMONOCYTIC LEUKEMIA IN A MURINE IN-VIVO MODEL

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Background: Chronic myelomonocytic leukemia (CMML) is characterized by increased proliferation and myelomonocytic lineage commitment of hematopoietic stem cells (HSCs). Mutations in the RAS-pathway occur frequently in CMML patients and lead to a CMML-like myeloproliferative disorder (CMML-MPD) in mice via causing hypersensitivity to GM-CSF. Loss of RAF kinase inhibitor protein (RKIP), a negative regulator of RAS signaling, is frequent in myelomonocytic and monocytic subtypes of acute myeloid leukemia (AML) and is often associated with RAS mutations. Moreover, RKIP loss has recently been shown to increase the proliferation of AML cell lines.
Aims: In this work, we aimed at investigating the role of RKIP in the development of CMML.
Methods: RKIP expression was measured by immunoblot and quantitative real-time PCR in 23 primary CMML patient samples as well as in CD34+ HSCs, B-lymphocytes, granulocytes and monocytes of four healthy donors. Sequence analysis of CMML samples was done with an Ion Torrent Next Generation Sequencing platform using an amplicon panel covering 39 genes recurrently mutated in myeloid neoplasms. Effects of RKIP on GM-CSF-induced myelomonocytic differentiation were studied in human CD34+ HSCs lentivirally transduced with RKIP shRNA, as well as in a genetic mouse model for RKIP deletion ($RKIP^{-/-}$). Effects of RKIP on CMML development were initially studied in the same $RKIP^{-/-}$ model. Additionally, these mice were crossed with animals exhibiting a somatically inducible mutation in NRAS ($RKIP^{-/-}$Mx1-Cre-$NRAS^{G12D}$) and the severity of CMML-MPD onset was studied at an age of six months.
Results: Loss of RKIP protein expression was observed in 6/23 (26%) CMML patient specimens and was associated with decreased mRNA levels as well (P<0.001). Patients with RKIP loss exhibited an increased percentage of myelomonocytic cells in the peripheral blood (86% *vs* 75%; P=0.0226). One or more mutations affecting the RAS signaling pathway were detected in all specimens with RKIP loss. In addition to the previously demonstrated induction of proliferation, we then aimed to delineate a role of RKIP loss in myeloid lineage commitment. When studying healthy donors, we observed that RKIP expression was high in HSCs and lymphoid cells, but significantly decreased in cells belonging to the myeloid lineage (monocytes, P=0.001 and granulocytes, P<0.001). In functional experiments, knockdown of RKIP increased the GM-CSF-induced myelomonocytic lineage commitment of both, human and murine HSCs (P<0.05 and P=0.0295, respectively). These results could be corroborated in-vivo, as intraperitoneal injection of GM-CSF caused a significant increase of myelomonocytic cells in the intraperitoneal cavity (P=0.006), bone marrow (P=0.007) and peripheral blood (P=0.027) in $RKIP^{-/-}$ mice when compared to their wildtype littermates. In a final step, we evaluated the potential of RKIP loss to cause CMML-MPD in mice. While it proved to be insufficient to cause the disease as a single event in $RKIP^{-/-}$ mice, it aggravated the CMML-MPD phenotype in animals carrying an additional mutation in NRAS. In this case, RKIP deletion caused worsening of leucocytosis (P=0.036) and splenomegaly (P=0.035), which was associated with increased levels of myelomonocytic cells in the bone marrow (P=0.028), peripheral blood (P=0.002) and spleen (P=0.025).
Summary/Conclusions: RKIP loss is a frequent event in CMML and is associated with mutations affecting the RAS signaling cascade. Loss of RKIP is functionally involved in myelomonocytic lineage commitment of HSCs and aggravates CMML-MPD development in mice carrying an additional mutation in NRAS.

S420

JAK2 V617F HAEMATOPOIETIC CLONES WITH DIFFERENT EXPANSION KINETICS ARE DETECTABLE SEVERAL YEARS PRIOR TO MPN DIAGNOSIS

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Background: *JAK2* V617F is the most common somatic mutation in the classical myeloproliferative neoplasms (MPNs) and is also frequent amongst healthy individuals with age-related clonal haemopoiesis (ARCH).
Aims: To investigate the pre-clinical clonal evolution of MPNs.
Methods: We identified 12 individuals with *JAK2* V617F mutant MPN from whom blood DNA was available from the time of MPN diagnosis and also from an earlier time point of between 4.5-15.2 years previously (median 10.2 years) when blood was donated for registration to the Cyprus Bone Marrow Donor Registry. We used deep DNA sequencing to interrogate all 24 samples at 15 myeloid mutation hotspots including *JAK2* V617, using an established multiplex PCR/MiSeq sequencing protocol that reliably detects nucleotide substitutions present at a variant allele fraction (VAF) ≥0.008. Additionally, for 12 samples with sufficient DNA available, we performed targeted DNA capture for all exons of 41 genes recurrently mutated in myeloid neoplasms using a custom RNA-bait library followed by sequencing on Illumina HiSeq 2500. Finally, we genotyped archival Registry samples for the rs12343867 single nucleotide polymorphism (SNP) (C/T) linked to the *JAK2* 46/1 haplotype.

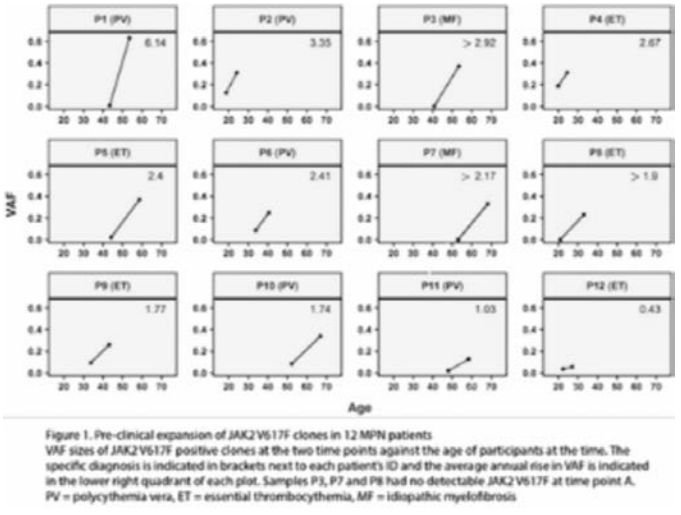

Figure 1. Pre-clinical expansion of JAK2 V617F clones in 12 MPN patients
VAF sizes of JAK2 V617F positive clones at the two time points against the age of participants at the time. The specific diagnosis is indicated in brackets next to each patient's ID and the average annual rise in VAF is indicated in the lower right quadrant of each plot. Samples P3, P7 and P8 had no detectable JAK2 V617F at time point A. PV = polycythemia vera, ET = essential thrombocythemia, MF = idiopathic myelofibrosis

Figure 1.

Results: Amplicon sequencing returned a median coverage of 6641 reads per nucleotide (nt) at the studied hotspots. This confirmed the presence of *JAK2* V617F in all 12 diagnostic and 9 of 12 archival samples. The remaining 3 samples were *JAK2* V617F negative at the sensitivity of our assay (VAF≥0.008). The only other hotspot mutation identified was *SRSF2* P95R in one patient, P3, whom had a diagnosis of myelofibrosis. Pulldown sequencing of all exons of 41 genes from 12 samples with sufficient DNA returned an average coverage of 1978 reads per nt and showed a close correlation in *JAK2* V617F and *SRSF2* P95R VAF quantitations with amplicon sequencing. The *JAK2* V617F VAF at MPN diagnosis differed between patients as expected, however the average rate of clonal growth also varied widely between individuals, ranging from 0.36 to 6.2% per annum (Figure 1). Targeted exon capture from 12 of 24 samples, only identified one co-mutation with a VAF >0.02, the *SRSF2* P95R in patient P3. As this locus was also amplified by amplicon sequencing, we were able to quantify the *SRSF2* P95R VAF in both the diagnostic and the archival DNA sample taken 12.6 years earlier. In the P3 diagnostic sample the VAFs for *JAK2* V617F and *SRSF2* P95R were similar (0.37 and 0.41 respectively) indicating that they co-occurred in most cells of the neoplastic clone. In the archival sample from P3, the *SRSF2* P95R was detectable at a VAF of 0.06, however the *JAK2* V617F was absent/undetectable at the sensitivity of our method (VAF≥0.008) indicating the *SRSF2* P95R was the clone-founding mutation in this neoplasm. The genotyping results for the rs12343867 SNP revealed a tentative association in our small cohort between homozygosity for the risk allele (C) linked to the *JAK2* 46/1 haplotype and the average annual increase in *JAK2* V617F VAF. This will need to be verified in larger studies.
Summary/Conclusions: Our findings reveal that *JAK2* V617F neoplasms develop from clonal haematopoiesis over many years. The rate of clonal expansion of *JAK2* V617F clones in the pre-clinical phase was highly variable and although it was tentatively associated with the 46/1 haplotype, the high variability observed suggests that other factors likely influence clonal progression.

S421

DISRUPTION OF HAEMATOPOIETIC STEM CELL HETEROGENEITY IN A MOUSE MODEL OF MYELOPROLIFERATIVE NEOPLASM

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Background: The hematopoietic stem cell (HSC) compartment in mice encompasses a broad range of heterogeneous cell types including highly lineage-biased HSCs, such as platelet-biased HSCs (PMID:23934107). Myeloproliferative neoplasms (MPNs) are a heterogeneous spectrum of clonal hematopoietic disorders, that includes essential thrombocythemia (ET), a MPN-subtype usually presenting with isolated thrombocytosis. Most ET patients carry a gain-of-function point mutation in JAK2 (JAK2V617F), with several other collaborating hits reported to co-occur with JAK2V617F at lower frequencies, including loss-of-function mutations of the epigenetic regulator EZH2, which are more frequent in advanced MPN.
Aims: Although it is broadly accepted that MPNs are propagated by counterparts of HSCs, the impact of collaborating MPN-associated mutations arising in different HSC subsets remains unclear. We aimed to explore the possibility that platelet-biased HSCs might selectively promote development of an ET phenotype.
Methods: We generated a novel mouse model of MPN that carries a conditional knock-in of heterozygous human JAK2V617F (hJAK2V617F) and the conditional knock-out (KO) of EZH2 together with an inducible Mx1-Cre transgene. To analyse platelet-biased HSC subsets upon onset of the mutation(s), we also crossed in the vwf-eGFP transgene, which is selectively expressed in platelet-biased HSCs.
Results: Compared to wild-type and single mutant mice, EZH2-KO hJAK2V617F mice showed increased platelet counts, including a subset of mice which became acutely unwell with an extreme thrombocytosis. Strikingly, in serial bone marrow (BM) transplantation assays, EZH2-KO fully rescued the previously described hJAK2V617F-associated transplantation defect (PMID:20489053). EZH2-KO hJAK2V617F BM recipients showed long-term serial engraftment that was fully restricted to the platelet and myeloid lineages with a persistent thrombocytosis and absence of lymphoid reconstitution. RNA-sequencing revealed upregulation of several signaling pathways, including Hedgehog, and increased inflammation associated gene expression in EZH2-KO hJAK2V617F HSCs. Unexpectedly in this mouse model of thrombocytosis, phenotypic analysis of the HSC compartment in the BM showed that vwf-eGFP+ve HSCs were selectively lost (fold change[FC]=0.12 p=0.009), while vwf-eGFP–ve HSC numbers remained unaffected (FC=1.06 p=0.88) in Ezh2-KO hJAK2V617F mice. To assess a differential contribution of vwf-eGFP+ve HSCs *vs* vwf-eGFP-ve HSCs in the ability to propagate MPN, we sorted HSCs according to vwf-eGFP expression and transplanted them into recipient mice. Unlike their normal counterparts, which showed lymphoid-biased reconstitution, vwf-eGFP–ve HSCs from Ezh2-KO hJAK2V617F mice primarily gave rise to platelets and myeloid cells. In contrast, vwf-eGFP+ve HSCs from Ezh2-KO hJAK2V617F mice engrafted poorly without recapitulating the disease in recipients.
Summary/Conclusions: In this novel Ezh2-KO hJAK2V617F mouse model, Ezh2 loss collaborates to worsen thrombocytosis and rescue the HSC function defect in hJAK2V617F mice. We also observed a striking disruption of phenotypic and functional HSC heterogeneity in Ezh2-KO hJAK2V617F mice with an unexpected and selective loss of vwf-eGFP+ve HSCs together with subversion of vwf-eGFP–ve HSCs towards platelet-myeloid lineage committment. This previously undescribed disruption of HSC heterogeneity in myeloid malignancy together with the clonal advantage conferred to HSCs by EZH2-KO helps to explain how this collaborating mutation might promote the development of more advanced MPN.

Clinical trials including treatment discontinuation in CML

S422

DASATINIB IN CHILDREN AND ADOLESCENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP) FROM A PHASE 2 TRIAL

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Background: As safe and effective frontline treatment options for children and adolescents with CML are limited, and no approved therapies exist for patients (pts) resistant/intolerant to imatinib (IM), additional treatment options and alternative formulations are greatly needed for this younger population. Dasatinib (DAS) has proven efficacy in adults with newly diagnosed CML-CP, as well as those resistant/intolerant to IM (Cortes JCO 2016, Shah AJH 2016). Results of a phase 1 study confirmed its dosing and safety in pediatric pts (Zwaan JCO 2013); however, a larger prospective study is necessary to further support the use of DAS in pediatric pts with newly diagnosed or IM-resistant/intolerant CML-CP.
Aims: To determine whether DAS is safe and effective in pediatric pts with CML-CP newly diagnosed or resistant/intolerant to IM enrolled in a phase 2, open-label, nonrandomized prospective clinical trial (CA180-226/NCT00777036).

Table 1.

	Treated pts with CML-CP (n=113)		
	IM-resistant/intolerant CML-CP (n=29)	Newly diagnosed CML-CP (n=84)	
		Tablet (n=51)	PFOS (n=33)
Median average daily dose, mg/m² (range)	58 (35-72)	59 (37-78)	65 (38-99)
Median duration of treatment, months (range)	50 (2-90+)	52 (8-75+)	27 (<1-42+)
Pts on treatment, n (%)	14 (48)	61 (73)	
		37 (73)	24 (73)
Reasons for discontinuation, n (%)			
Progressive disease	5 (17)	5 (10)	1 (3)
Study drug toxicity	0	0	1 (3)
Pt withdrawal[a]	3 (10)	2 (4)	1 (3)
Maximum clinical benefit	2 (7)	1 (2)	0
Noncompliance	1 (3)	0	0
Other	4 (14)	6 (12)	6 (18)
MCyR,[b] % (95% CI)			
By 3 months	55 (36, 74)	57 (46, 68)	
		55 (40, 69)	61 (42, 77)
By 12 and 24 months	90 (73, 98)	96 (90, 99)	
		98 (90, 100)	94 (80, 99)
Median time to MCyR,[b] months (95% CI)	3.1 (2.8, 4.1)	3.0 (2.9, 4.3)	
		3.3 (2.9, 5.6)	3.0 (2.8, 5.0)
CCyR,[b] % (95% CI)			
By 6 months	66 (46, 82)	64 (53, 74)	
		61 (46, 74)	70 (51, 84)
By 12 months	76 (57, 90)	92 (84, 97)	
		94 (84, 99)	88 (72, 97)
By 24 months	83 (64, 94)	94 (87, 98)	
		96 (87, 100)	91 (76, 98)
Median time to CCyR,[b] months (95% CI)	3.9 (2.8, 5.6)	5.6 (5.0, 6.0)	
		5.7 (3.7, 6.2)	5.6 (3.1, 6.0)
MMR, % (95% CI)			
By 12 months	41 (24, 61)	52 (41, 63)	
		57 (42, 71)	46 (28, 64)
By 24 months	55 (36, 74)	70 (59, 80)	
		75 (60, 86)	64 (45, 80)
Estimated 48-month PFS, % (95% CI)	78 (57, 89)	93 (83, 97)	
		92 (80, 97)	97 (79, 100)

[a]Refers to either study treatment discontinuation by pt, or withdrawing consent from further follow-up.
[b]Responses based on ≥20 metaphases.
CI=confidence interval; MMR=major molecular response.

Methods: Pts aged <18 years were recruited into 3 separate cohorts: (1) IM-resistant/intolerant CML-CP treated with DAS tablets 60mg/m^2 QD, (2) IM-resistant/intolerant CML-AP/BP or Ph+ ALL (enrollment closed early due to poor response), and (3) newly diagnosed CML-CP treated with DAS tablets 60mg/m^2 or DAS 72mg/m^2 powder for oral suspension (PFOS) QD for ≥1 year. PFOS dose was increased by 20% to match the exposure of the tablet in order to maintain a desired efficacy based on the findings from a bioequivalence study in adults. Primary objectives were major cytogenetic response (MCyR) for CML-CP resistant/intolerant to IM and complete cytogenetic response (CCyR) for newly diagnosed CML-CP (MCyR >30% and CCyR >55% considered of clinical interest). Study cohorts were not designed to be comparative.
Results: From 145 pts enrolled, 130 were treated; 54% were aged ≥12-<18 years. Within the IM resistant/intolerant group, 25 were resistant, 2 were intolerant, and 2 were undetermined. For pts with CML-CP (n=113), 48% of pts with IM-resistant/intolerant CML-CP and 73% with newly diagnosed CML-CP remained on treatment at the time of this analysis (table 1). Cumulative rate of MCyR >30% was reached as early as 3 months for IM-resistant/intolerant CML-CP, and a cumulative rate of CCyR >55% was reached as early as 6 months for newly diagnosed CML-CP (table). Estimated progression-free survival (PFS) by 48 months was 78% for IM-resistant/intolerant CML-CP and 93% for newly diagnosed CML-CP (table). Reasons for progression were loss of MCyR (n=3 IM-resistant/intolerant; n=4 newly diagnosed), loss of complete hematologic response (n=2 each), and development of CML-BP (n=2 IM-resistant/intolerant; n=1 newly diagnosed). One death was reported in the IM-resistant/intolerant CML-CP cohort 1 year after stopping DAS (gastrointestinal bleeding). Adverse events (AEs) were consistent with reports in DAS-treated adults, except no DAS-related pleural/pericardial effusion, pulmonary edema/hypertension, or pulmonary arterial hypertension were reported here. Hypersensitivity in a newly diagnosed pt was the only DAS-related AE that led to discontinuation.
Summary/Conclusions: Results from the largest prospective and registrational trial of pediatric pts with CML-CP demonstrate that DAS is a safe and effective treatment for pediatric CML-CP. Target responses to first- or second-line that DAS were met as early as 3 and 6 months, respectively, and deep responses were observed. Efficacy and safety of DAS in pediatric pts were similar to those observed in adults; however, unlike in adults, no cases of pleural/pericardial effusion were reported.

S423

INITIAL REDUCTION OF THERAPY BEFORE COMPLETE WITHDRAWAL IMPROVES THE CHANCE OF SUCCESSFUL TREATMENT DISCONTINUATION IN CHRONIC MYELOID LEUKAEMIA (CML): YEAR 2 RESULTS IN THE BRITISH DESTINY STUDY

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Background: In CML, there is considerable current interest in whether some patients can safely discontinue tyrosine kinase inhibitor (TKI) therapy. However, all studies so far have examined patients in stable MR4 at entry, *i.e.* BCR-ABL1/ABL1 ratio ≤0.01%. Patients in stable major molecular response (MMR) but not MR4 (<0.1 but >0.01%) have not been formally studied; neither have the effects of stepwise TKI withdrawal.
Aims: The present British De-Escalation and Stopping Therapy with Imatinib, Nilotinib or sprYcel (DESTINY) study examines treatment de-escalation as a prelude to complete cessation, in patients in not only stable MR4 but also those with MMR but not MR4.
Methods: Trial entry required first chronic phase of CML, TKI treatment for ≥3 years, and either the same TKI (imatinib, dasatinib or nilotinib) since diagnosis or only one switch for intolerance. All PCR tests (minimum of 3) in the 12 months before trial entry must have been ≤0.1% (*i.e.* MMR), each with ≥10,000 ABL1 control transcripts; those with all results ≤0.01% were allocated to the 'stable MR4' group; the remainder to the 'MMR but not MR4' group. Entry criteria were thus virtually identical to the EUROSKI study except that patients with MMR but not MR4 were also separately eligible. TKI treatment was reduced to half the standard dose (imatinib 200mg daily, dasatinib 50mg daily or nilotinib 200mg twice daily) for the first 12 months, then stopped completely. Central PCR monitoring was carried out monthly, expressed according to International Scale. Molecular recurrence was timed as the first of 2 consecutive samples with loss of MMR (>0.1%) and mandated resumption of full TKI dose.
Results: 174 patients (male 98; female 76) were recruited after giving informed consent from 20 UK centres. At entry, 148 patients were receiving imatinib, 16 nilotinib and 10 dasatinib, for a median duration of 6.8 years. We reported at ASH 2016 that after 12 months of half-dose therapy, molecular recurrence was lower in patients with stable MR4 at entry (3 of 125 patients; 2.4%) than in those in MMR but not MR4 (9 of 49 patients; 18.4%) (p<0.001). We now show in the Figure below that during the subsequent 12 months of complete treatment cessation in 117 stable MR4 patients, only 26 further recurrences and 4 withdrawals occurred, giving a recurrence free survival (RFS) of 77% (90% CI: 71-83%) for the overall 24 months for this patient group. The recurrence rate on cessation is higher in the MMR but not MR4 group (20 recurrences and 4 withdrawals among 36 patients during cessation; 39% RFS overall (90% CI: 29-52%); p=<0.001). In both the stable MR4 group and the MMR but not MR4 groups, no difference in RFS was seen between patients in MR4.5 at entry and those not. In multivariable Cox proportional hazards modelling, addition of the baseline entry PCR result did not add to the predictive effect on RFS of the prior 12 month PCR pattern, whereas the duration of TKI treatment was an additional predictive factor (p=0.058; HR 0.93), in line with recent data from EUROSKI. The probability of RFS remains unrelated to age, gender, performance status or prior TKI (imatinib *vs* second generation). No progression to advanced phase was seen; one case lost haematological response.

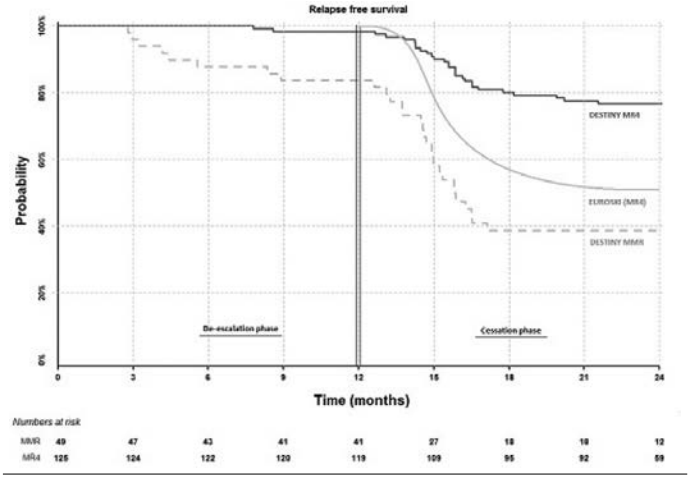

Figure 1.

Summary/Conclusions: The present 24 month RFS of 77% for the overall 24 months in patients in stable MR4 appears better than in any comparable study to date, and implies that the initial 12 months of dose reduction may be responsible, perhaps via improved compliance in the few months prior to stopping or through an as yet undefined mechanism.

S424

ASSESSMENT OF IMATINIB 400MG AS FIRST LINE TREATMENT OF CHRONIC MYELOID LEUKEMIA: 10 -YEAR SURVIVAL RESULTS OF THE RANDOMIZED CML STUDY IV

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Background: The optimum initial treatment of chronic myeloid leukemia (CML) is unknown.
Aims: CML-study IV was designed to confirm the International Randomized Study on Interferon (IFN) and STI571 (IRIS) and to explore whether treatment with imatinib (IM) at 400mg/day could be optimized.
Methods: From July 2002 to March 2012, 1551 newly diagnosed patients in chronic phase (CP) were randomized into a 5-arm study. 1536 patients were evaluable, 400 for IM400mg, 430 for IM + IFN, 420 for IM800mg, 158 for IM + cytarabine and 128 for IM-after-IFN-failure. Recruitment to the latter two arms was stopped after a pilot-phase.
Results: After a median observation time of 9.5 years, 10-year overall survival (OS) of all patients was 82%, 10-year progression free survival 80% and, 10-year relative survival 92%. 10-year OS was 80% with IM400mg, 84% with IM + IFN, 79% with IM800mg, 84% with IM + cytarabine and 79% with IM after IFN (Figure 1). The differences were not significant in spite of faster response with IM800mg. In a multivariate analysis, risk group, comorbidities, major route chromosomal aberrations, smoking and type of treatment center (academic *vs* other) influenced survival, but not gender, transcript type or any form of treatment optimization. Patients reaching the molecular response milestones at 3, 6 and 12 months had a significantly better survival, the faster response of a treatment group (IM800mg) did not translate into a detectable survival advantage.

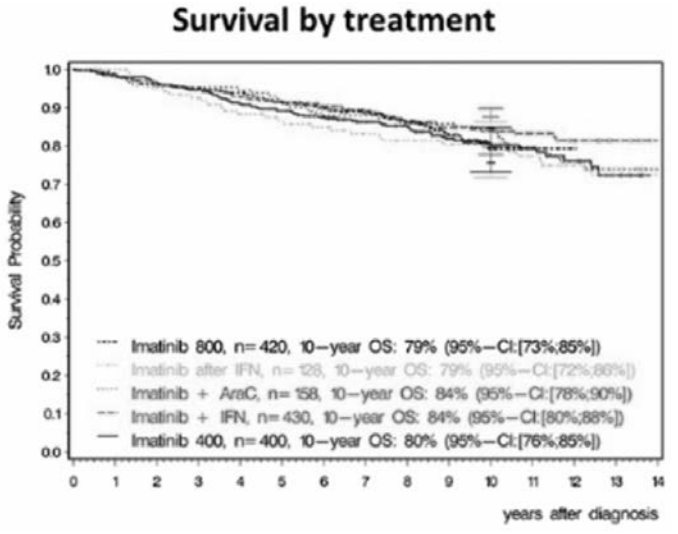

Figure 1.

Summary/Conclusions: Monotherapy with IM400mg provides a close to normal life expectancy. Faster response does not necessarily translate into better survival. Outcome of CML is currently more determined by disease biology and demographics than by treatment optimization.

S425

BOSUTINIB *VS* IMATINIB FOR NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA: INITIAL RESULTS FROM THE BFORE TRIAL

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Background: Bosutinib (BOS) is a potent, dual SRC/ABL tyrosine kinase inhibitor approved for treatment of adults with Philadelphia chromosome-positive (Ph+) chronic myeloid leukemia (CML) resistant or intolerant to prior therapy.
Aims: To assess the efficacy and safety of BOS *versus* imatinib (IM) for first-line treatment of chronic phase (CP) CML in the BFORE trial (NCT02130557).
Methods: In this ongoing, multinational, phase 3, open-label study, 536 patients with newly diagnosed CP CML were randomized 1:1 to BOS 400mg once daily (n=268) or IM 400mg once daily (n=268 [3 not treated]). Informed consent was obtained from all patients. Per protocol, efficacy was assessed in a modified intent-to-treat (mITT) population of 487 Ph+ patients (BOS, n=246; IM, n=241) with e13a2/e14a2 transcripts; Ph– patients and those with unknown Ph status and/or BCR-ABL transcript type were excluded from this population.
Results: After ≥12 months of follow-up, 78.0% of BOS and 73.2% of IM patients remain on treatment with median treatment durations of 14.1 months and 13.8 months, respectively. Major molecular response (MMR) rate at 12 months (primary endpoint) was significantly higher with BOS *versus* IM in the mITT population (47.2% *vs* 36.9%; P=0.02) as well as in the ITT population of all randomized patients (46.6% *vs* 36.2%; P<0.02). In the mITT population, time to MMR was shorter for BOS (hazard ratio=1.34 based on cumulative incidence; P<0.02). Rate of complete cytogenetic response (CCyR) by 12 months was also significantly higher with BOS *versus* IM (77.2% *vs* 66.4%; P<0.008), with time to CCyR shorter for BOS (hazard ratio=1.38; P≤0.001). Rate of BCR-ABL transcripts ≤10% (Intl. Scale) at 3 months was higher with BOS *versus* IM (75.2% *vs* 57.3%; P<0.001); rates of deep molecular response over time were also generally higher with BOS (Table). Results for molecular endpoints were similar in the ITT population. The only baseline characteristic identified as a significant predictor of MMR at 12 months besides treatment arm was Sokal risk group (high *vs* low; P<0.0001 and intermediate *vs* low; P<0.05 [mITT]). On-treatment progression to accelerated or blast phase occurred in 4 patients (1.6%) receiving BOS and 6 patients (2.5%) receiving IM in the mITT population. One BOS-treated and 4 IM-treated patients discontinued treatment due to progression to accelerated or blast phase. Among all treated patients, there were no deaths within 28 days of last dose of BOS and 4 with IM. Safety data for treated patients were consistent with the known safety profiles of BOS and IM. Discontinuation due to drug-related toxicity occurred with 12.7% of BOS patients and 8.7% of IM patients. Grade ≥3 diarrhea (7.8% *vs* 0.8%) and increased alanine (19.0% *vs* 1.5%) and aspartate (9.7% *vs* 1.9%) aminotransferase levels were more common with BOS. Cardiovascular, peripheral vascular, and cerebrovascular events were infrequent in both groups (all grades: 3.0%, 1.5%, and 0% BOS *vs* 0.4%, 1.1%, and 0.4% IM; grade ≥3: 1.5%, 0%, and 0% BOS *vs* 0%, 0%, and 0.4% IM).

Table 1.

Table

Response[a]	BOS (n=246)	IM (n=241)	*P*-value
MMR, n (%)			
3 mo	10 (4.1)	4 (1.7)	0.1156
6 mo	86 (35.0)	44 (18.3)	<0.0001
9 mo	104 (42.3)	71 (29.5)	0.0029
12 mo	116 (47.2)	89 (36.9)	0.0200
MR[4], n (%)			
3 mo	1 (0.4)	0	0.3297
6 mo	24 (9.8)	11 (4.6)	0.0289
9 mo	34 (13.8)	20 (8.3)	0.0593
12 mo	51 (20.7)	29 (12.0)	0.0104
MR[4.5], n (%)			
3 mo	0	0	-
6 mo	5 (2.0)	2 (0.8)	0.2770
9 mo	11 (4.5)	7 (2.9)	0.3758
12 mo	20 (8.1)	8 (3.3)	0.0238

MMR=major molecular response; MR=molecular response.
[a]In the modified ITT population excluding 12 Ph– patients (ie, 0 out of ≥10–99 metaphases at baseline; 6 in each arm), 8 patients with atypical transcripts (3 bosutinib and 5 imatinib), and 31 patients with unknown Ph status (13 bosutinib and 18 imatinib, including 2 imatinib patients also listed as having atypical transcripts).

Summary/Conclusions: Patients on BOS had significantly higher rates of 12-month MMR and CCyR and achieved responses faster than those on IM. Consistent with the known safety profile, higher incidences of gastrointestinal events and transaminase elevations were observed with BOS. Primary results from this study suggest BOS may be an important treatment option for patients with newly diagnosed CP CML.

S426

CHRONIC MYELOID LEUKEMIA PATIENTS WERE NOT DIFFERENT IN MOLECULAR RELAPSE AFTER STOPPING IMATINIB IN MR4 WHETHER RESIDUAL DISEASE WAS DETECTED OR NOT - WHEN ADJUSTING FOR NUMBER OF CONTROL TRANSCRIPTS

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Background: With imatinib (IM), most patients with chronic myeloid leukemia (CML) achieve deep molecular responses. Six months after stopping tyrosine kinase inhibitor in deep response in the EURO-SKI trial, 61% of the patients were in molecular relapse-free survival (RFS) *i.e.* alive and in major molecular remission (3-log reduction in BCR-ABL1 levels) (Mahon ASH 2016). Between patients with and without BCR-ABL1, the difference in RFS at 6 months was not significant when assessing BCR-ABL1 detectability at the MR4.5 level (at least a 4.5-log reduction in BCR-ABL1) (Pfirrmann ASH 2016).
Aims: For 91 of 448 patients of the EURO-SKI learning sample, the sensitivity to claim undetectable disease at the MR4.5 level was not given. Aim was to investigate whether RFS probabilities would be different when comparing detectable and undetectable disease at the MR4 level.
Methods: Detectability of BCR-ABL1 depends on the number of control gene transcripts. To reduce bias when comparing "MR4 detectable disease" (MR4 but still detectable BCR-ABL1 transcripts; *i.e.* 0.01- 0.0033% IS) and "MR4 undetectable disease" (MR4 without detectable BCR-ABL1; based on 10,000-31,999 ABL1 or 24,000-76,999 GUSB copies), two samples with similar sensitivity of identifying BCR-ABL1 were to be identified using propensity score (PS) matching (Rosenbaum, Rubin 1983). Apart from type (ABL1 or GUSB) and number of control gene transcripts, matching variables were interferon alpha pre-treatment, duration of MR4, and the IM treatment time before observation of MR4. Logistic regression was used to compare RFS at 6 months. Significance level was 0.05.
Results: A total of 448 patients had eligible, complete, and sufficient molecular data prior to and within the first 6 months after stopping IM treatment. All molecular results had sensitivity at the MR4 level with yet detectable disease in 196 patients (44%). With small differences in GUSB copy numbers (used in 96 of 448 cases, U test (detectable *vs* undetectable): P>0.5), prior to PS matching, median numbers of ABL1 transcripts were higher with MR4 detectable disease (78,975 *vs* 68,925 with undetectable disease; P=0.0511, not significant (n.s.)). In 196 patients with detectable disease, RFS at 6 months was 52% (95% confidence interval (CI): 45-59%) and in 252 patients with undetectable disease 63% (CI: 58-69%). Relapse was significantly higher in patients with detectable disease (odds ratio: 1.603 (CI: 1.096-2.343). PS matching resulted in 173 patients per group. Median numbers of ABL1 transcripts changed to 82.142 (detectable) and 75.750 (undetectable disease; n. s.). At 6 months, patients with detectable disease again had 52% (95% confidence interval (CI): 45-59%) RFS probability, and patients with undetectable disease 59% (CI: 52-66%). In the logistic model stratified for the matched pairs, for relapse at 6 months, the odds ratio for MR4 with detectable to undetectable disease was 1.308 (CI: 0.862-1.984, n. s.).
Summary/Conclusions: Using the MR4 threshold, after matching on number of control transcripts and other factors, results suggest little or no impact of detectability of BCR-ABL1 on RFS. Time in deep response seems to be more important. In daily routine, many labs produce reliable outcome at the MR4 but not always at the MR4.5 level. Discontinuation at the MR4 level, irrespective of detectability of BCR-ABL1 residual disease, appears safe, with a good chance of success when performed as in EURO-SKI. With PS matching, bias and differences but also power was reduced. To judge whether molecular response on the MR4 level is sufficient, further data is welcome.

AML Biology II: Epigenetic targets

S427

ETO2-GLIS2 RECRUITS ETO2/ERG COMPLEX AT SUPER-ENHANCERS TO CONTROL TRANSCRIPTION AND DRIVE LEUKEMIC PROPERTIES IN PEDIATRIC ACUTE MEGAKARYOBLASTIC LEUKEMIA

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Background: Deregulated expression programs due to genetic alterations, such as gene fusions affecting transcription and/or epigenetic factors is the hallmark of acute myeloid leukemia (AML) and the basis for the associated differentiation block of hematopoietic progenitors. Acute megakaryoblastic leukemia (AMKL) is a subtype of poor prognosis AML affecting primarily young children. Recently, the *ETO2-GLIS2* fusion has been identified in 20-30% of *de novo* AMKL and associated with the worst prognosis in this subtype of AML.
Aims: Our goal was to characterize the mechanisms of cellular transformation induced by ETO2-GLIS2.
Methods: We first defined the consequences of ETO2-GLIS2 expression on hematopoietic progenitors and the contribution of ETO2 and GLIS2 on differentiation and self-renewal by methylcellulose replating assays and phenotype characterization. We then assessed global expression profiling and ETO2-GLIS2 direct binding on DNA by ChIPseq experiments. With immunoprecipitation experiments, we identified some ETO2-GLIS2 complex members. Finally, we tested the effects of a small peptide that could inhibit ETO2-GLIS2 complex stabilization both *in vitro* and *in vivo*.
Results: We showed that the GLIS2 moiety drives the megakaryocytic phenotype whereas both the ETO2 and GLIS2 moieties are required for maintaining self-renewal. Global expression profiling and comparison to patients' signature consistently identified ETO2-GLIS2-mediated deregulation of major transcriptional regulators of hematopoiesis and leukemogenesis. Especially, ETO2-GLIS2 brings on an unbalance in ETS/GATA factors illustrated by an extinction of *GATA1* and an overexpression of the *ERG* oncogene. We identified that ETO2-GLIS2 directly binds DNA via ETO2 complexes and through its GLIS2 moiety. Moreover, the ETO2-GLIS2 fusion localizes at half of H3K27ac-dense enhancers, so called super-enhancers, to control transcription of associated genes, in close association with ERG. Dimerization of ETO2-GLIS2 and interaction with endogenous ETO2 via its NHR2 domains were demonstrated with immunoprecipitation experiments. A NHR2 peptide-interference strategy inhibited the oligomerization, reversed the transcriptional activation at enhancers, promoted megakaryocytic differentiation and abrogated human AMKL cells maintenance *in vivo*. So, the interaction of ETO2-GLIS2 with ETO2 complexes is an essential node for the transcriptional control by the fusion at enhancer elements. Finally, ERG is localized at super-enhancers and is associated with up-regulation of associated genes. ERG knockdown or genetic inactivation downregulates expression of ETO2-GLIS2 targets required for leukemic cells survival. Together, the strong up-regulation of ERG by the fusion and the presence of ERG at super-enhancers suggest a feed forward mechanism to impose gene deregulation.
Summary/Conclusions: In conclusion, we propose that the megakaryocytic differentiation arrest and self-renewal controlled by ETO2-GLIS2 results from imbalanced expression of master transcription factors imposed by aberrant chromatin structures at enhancers that may be disrupted by targeting the NHR2 interface.

S428

NUCLEOSOME BINDING PROTEIN HMGN1 BLOCKS MYELOIDDIFFERENTIATION AND PROMOTES CLONAL DOMINANCE VIA ABERRANT HISTONE ACETYLATION

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Background: Acute myeloid leukemia (AML) is characterized by rapid growth and block in differentiation of myeloid progenitors. The AML blast is defined by having "open" chromatin. We hypothesized that alterations of chromatin compaction may promote AML. Reversing those changes could represent a novel therapeutic approach.
Aims: Gain of chr21q22 is the most common focal amplification in complex karyotype AML. HMGN1 is a chromatin-regulatory protein on 21q22 known to affect lymphoid development, and our preliminary data suggested that HMGN1 could directly mediate a myeloid differentiation block. Since HMGN1 is known to decompact chromatin and alter histone marks, our goal was to *define and therapeutically target the mechanisms by which HMGN1 overexpression disrupts myeloid differentiation and promotes clonal dominance.*
Methods: We immortalized bone marrow progenitors from wild-type (WT) or OE-HMGN1 mice (transgenic overexpressing HMGN1) with an estrogen receptor-HoxB8 fusion protein. Using exogenous estrogen to control nuclear translocation of HoxB8, we analyzed synchronized myeloid differentiation by flow cytometry, RNAseq, and TMT proteomic analysis. We performed MINT-ChIP-seq (MNase Indexed T7-chromatin IP) to measure the histone marks H3K27ac, H3K27me3, H3K4me3 and total Histone H3. We also measured histone marks in hematopoietic stem and progenitor subpopulations *in vivo*. We performed competitive bone marrow transplantation with CD45.1 WT and CD45.2 OE-HMGN1 donors and measured the relative contribution to hematopoiesis over time.
Results: Synchronized differentiation in WT cells progressed over 6 days from myeloid progenitors to mature neutrophils and monocytes, analyzed by cell surface markers, morphology, and gene and protein expression. OE-HMGN1 cells proliferated faster and remained as undifferentiated myeloblasts (84% Cd11b+Gr1+ in WT *vs* 4% in OE-HMGN1, p=0.002; **Fig A**). Gene set enrichment analysis revealed more similarity to undifferentiated hematopoiesis and leukemia signatures in OE-HMGN1 cells. MINT-ChIP indicated higher global and locus-specific levels of H3K27ac in OE-HMGN1 cells (**Fig B**, upper panel), consistent with an increase in gene transcription, confirmed by RNA-seq. We found a specific increase in HoxA cluster expression in OE-HMGN1 cells, highest at HoxA7 and HoxA9, genes known to be important in AML pathogenesis. In agreement with gene expression, among the most differentially measured histone peaks genome-wide were higher H3K27ac at HoxA gene promoters at all differentiation time points analyzed (**Fig B**, lower panel). Competitive transplantation demonstrated an advantage to OE-HMGN1 stem and progenitor cells. The clonal dominance of OE-HMGN1 over WT cells extended to all populations analyzed (long- and short-term HSCs, multipotent progenitors, CMP, GMP and MEP; **Fig C**) and to mature lineages (myeloid, B and T cells). MINT-ChIP in LK and LKS stem and progenitor cells revealed an increase in H3K27ac peaks at cell cycle and leukemia-related genes in the context of OE-HMGN1. H3K27 acetylation is catalyzed by the CBP/p300 histone acetyltransferase (HAT), suggesting that HAT inhibition could target leukemias with HMGN1 overexpression. Indeed, treatment of myeloid progenitors with the CBP/p300 inhibitor C646 rescued the differentiation block in OE-HMGN1 cells (93% CD11b+Gr1+ in WT *vs* 80% in OE-HMGN1, p=NS).

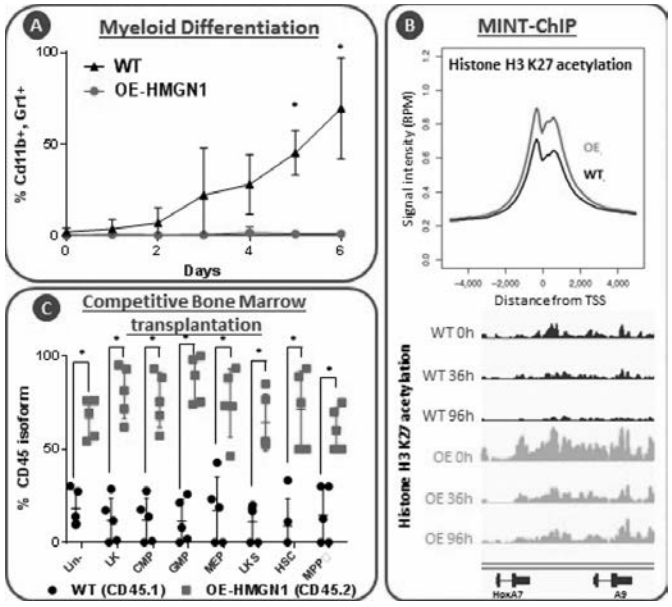

Figure 1.

Summary/Conclusions: Our study suggests that HMGN1 overexpression blocks myeloid differentiation and promotes proliferation in hematopoietic progenitors via increased H3K27 acetylation. Targeting epigenetic changes downstream of HMGN1 or interfering with HMGN1 itself may represent a novel therapeutic strategy in AML.

S429

PIWIL4 ACTS AS A PIRNA BINDING, EPIGENETICALLY ACTIVE AND GROWTH REGULATORY PROTEIN IN HUMAN ACUTE MYELOID LEUKEMIA

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Background: Piwi proteins are critically important for maintaining the self-renewing stem cell population in lower organisms through epigenetic silencing of transposable elements via DNA methylation and H3K9me3 marks, in close interaction with a novel class of non-coding RNA called piwi interacting RNA (piRNA).
Aims: There are neither precise data on the function of Piwi proteins in human acute myeloid leukemia (AML), nor are there reports on expression of piRNAs in this disease. We employed functional techniques and NGS to understand the role of human PIWI-like protein, PIWIL4 and its associated piRNA in AML.
Methods: We assessed the expression of human PIWIL genes in AML and healthy bone marrow cells using qRT-PCR. Murine stem progenitors were transduced with AML specific oncogenes to evaluate the effect on Piwil4 expression. shRNA mediated knockdown (KD) of PIWIL4 was performed on AML cell lines, AML patient bone marrow (BM) cells and healthy cord blood CD34+ stem progenitors and the impact on growth was determined using *in vitro* and *in vivo* assays. Western blot, ChIP-seq for H3K9me3 and RNA-seq were performed to assess the impact of PIWIL4 KD on the epigenetic landscape and transcriptome of the AML cell line THP-1. IP for PIWIL4 followed by LC-MS was performed to determine the binding partners of PIWIL4. PAR-CLIP and microarray were performed to identify piRNAs that physically bind to PIWIL4 and to test the impact of PIWIL4 KD on piRNA expression.
Results: Among the family of human PIWIL genes, PIWIL4 showed the highest expression level and was ubiquitously expressed in healthy hematopoietic stem/progenitors, mature lymphoid and myeloid cells. Importantly, PIWIL4 was aberrantly higher expressed in more than 89% of the AML patients (n=68; p<0.0001) compared to normal CD34+ BM and total BM cells (n=3). Overexpression of AML specific oncogenes in murine stem progenitors, within 96h post-transduction, induced a 6 to 8 fold increase in Piwil4 expression compared to GFP control (n=3, p<0.0001). Knockdown (KD) of PIWIL4 in AML cell lines significantly impaired proliferation and clonogenic growth *in vitro* (n=3; p<0.001) and delayed onset of leukemia in NSG mice (n=8; p<0.0001). PIWIL4 KD in primary AML patient BM cells lead to 5-fold decrease in clonogenicity (n=3, p<0.001), but had no impact on clonogenicity of healthy stem progenitors *in vitro* (n=4). Western blot and ChIP-seq (n=2, MACS1.4, p<0.01, FDR<0.01) in THP-1 cell line revealed a marked global reduction in repressive H3K9me3 marks upon PIWIL4 KD. Over 500 promoter and 600 gene body associated loci exhibited loss of H3K9me3 marks. RNA-seq analyses revealed over 4000 differentially expressed genes upon PIWIL4 depletion. 30% of the loci that lost H3K9me3 marks at promoters and gene body were differentially expressed in RNA-seq (fold>0.05, adj. p<0.01). These genes belonged to pathways associated with RNA metabolism, transcription and cell death. Moreover, these genes were enriched for binding sites of SETDB1, an H3K9me3 establishing histone methyltransferase (ENRICHR, p<0.01, FDR<0.01). Notably, using IP/LC-MS, PIWIL4 was found to associate with SETDB1 in 293T cells. 560 unique piRNAs were found to physically bind to PIWIL4 and 981 unique piRNAs were differentially expressed upon PIWIL4 depletion in THP-1 cells.
Summary/Conclusions: Thus, collectively, we could show for the first time that PIWIL4 expression is deregulated in human AML and acts as a piRNA binding, epigenetically active growth regulatory protein in human AML.

S430

METTL3 CONTROLS TRANSLATION OF TARGET MRNAS BY N6 METHYLATION OF ADENOSINE RESIDUES IN THEIR CODING SEQUENCE AND CONSTITUTES A NOVEL THERAPEUTIC VULNERABILITY OF ACUTE MYELOID LEUKAEMIA

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Background: Acute myeloid leukaemia (AML) patient survival remains below 30% and there have been no major new anti-AML therapies for decades.

Aims: To identify novel therapeutic vulnerabilities of AML.
Methods: A CRISPR-Cas9 drop-out screen was used to identify genetic vulnerabilities of AML. Downstream, we study the function of the RNA methyltransferase METTL3, a novel therapeutic vulnerability of AML. These included in-vitro and in-vivo validation of METTL3 as a therapeutic target using CRISPR/gRNA or shRNA, and investigation of its function using ChIP-seq, RNA-IP-seq, ribosome footprinting (RFP) and bioinformatic analyses.
Results: We performed a genome-wide CRISPR screen on AML cells from *Flt3*$^{ITD/+}$*;Rosa*$^{Cas9/+}$ mice transformed with MLL-AF9 lentivirus and identified >1500 cell-essential genes of which ~250 were AML-specific and included many MLL-AF9 interactors and several putative RNA methyltransferase genes: *METTL1, METTL3, METTL14* and *METTL16* (Fig 1A). Focusing on *METTL3*, we show that its disruption with Cas9/gRNA promoted differentiation of murine and human *MLL-AF9* AML cells and inhibited their growth *in vitro* and *in vivo* (Fig 1B), but did not affect primary murine haematopoietic stem/progenitor cells. To investigate METTL3 function we performed chromatin immunoprecipitation (ChIP) for METTL3 and H3K4me3 and identified 126 METTL3 peaks, localized mainly at promoters with bimodal H3K4me3. METTL3 binding was highest at transcription start sites (TSS) (Fig 1C) and the most enriched transcription factor motif at METTL3 sites was that for the NFY complex. Using available ChIP-seq datasets we found that NFYA, NFYB, H3R2me2s, WDR5 and KLF9 showed strong co-binding with METTL3. Also shRNA knock-down (KD) of WDR5, led to reduced METTL3 binding to target genes SP1 and SP2.
To investigate if/how METTL3 controls expression of target genes we first noted that their mRNA levels were unaffected by METTL3 KD. As METTL3 is an N6-methyladenosine (m6A) methyltranferase, we then performed RNAseq after IP with an m6A-specific antibody (m6A-IP). This identified >4000 METTL3-dependent m6A peaks on poly-A+ RNA. m6A peaks were seen on 72.4% of METTL3-bound gene transcripts and were located in the coding region (CDS) in contrast to STOP codon enrichment in the general transcriptome (Fig 1D). Also, METTL3-bound transcripts were enriched for the [GAG]n motif, which was almost always in the +2 reading frame (Fig1E). As this raised the possibility that m6A may regulate translation of these transcripts, we performed RFP on wild type (WT) and *METTL3* KD MOLM13 cells, to evaluate translational efficiency (TE). Strikingly, upon *METTL3* KD, m6A-marked genes had increased TE, whilst transcripts with METTL3 at their TSS had reduced TE (Fig1F). We then mapped ribosomal pausing sites on mRNAs from METTL3-bound TSSs and found that GAN codons were more occupied (paused) in *METTL3* KD *vs* WT cells (Fig1G). To understand consequences at the protein level, we studied METTL3 targets SP1 and SP2. Remarkably, in contrast to their mRNA levels, SP1 and SP2 proteins were markedly reduced upon *METTL3* KD (Fig1H). SP1 and SP2 targets including c-MYC, were also downregulated upon *METTL3* KD and simultaneous SP1/SP2 gRNA KD markedly reduced proliferation of MLL-AF9/Flt3$^{ITD/+}$ cells.

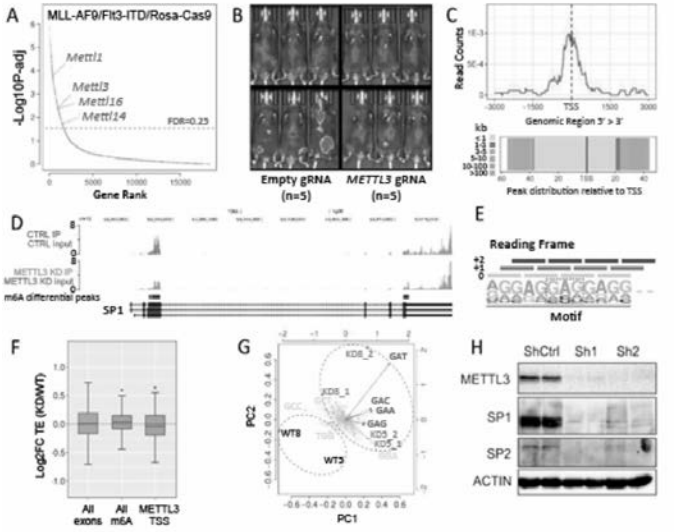

Figure 1: METTL3 controls translational efficiency of mRNAs involved in leukaemogenesis and represents a novel vulnerability in AML
A. Results of genome-wide drop-out screen highlighting METTL genes essential for survival of MLL-AF9-driven AML cells from RosaCas9/+ / Flt3ITD/+ mice. B. Dramatic reduction of luciferase-expressing MOLM13 cell engraftment in immunocompromised (NSG) mice by gRNA against: METTL3. C. Distribution of METTL3 ChIP-seq reads showing that METTL3 binding centres around transcription start sites (TSS) of target genes. D. Visualization of m6A-IP signal on the SP1 gene transcript, showing that the modification is highest in the coding sequence (MOLM13 cells). E. [GAG]n motif enriched in mRNAs from METTL3-bound TSSs, located in the +2 reading frame. F. Reduction in translational efficiency (TE) of *m6A-modified mRNAs* with METTL3 at their TSS, in contrast to other *m6A-modified mRNAs*. G. Principal component analysis of pausing site codon distribution on *mRNAs* from METTL3-bound TSSs identified by ribosome footprinting of METTL3 KD or CTRL MOLM13 cells after doxycycline administration. Note enrichment of "GAN" codons. H. Dramatic reduction in SP1 and SP2 protein levels upon METTL3 KD.

Figure 1.

Summary/Conclusions: Our results show that METTL3 controls translation of specific mRNAs by binding their TSS and introducing m6A at [GAG]n motifs within their CDS, in turn increasing their TE. These mRNAs code for proteins essential for AML cell survival, making METTL3 a novel therapeutic vulnerability of AML.

Acquired and inherited platelet disorders

S431

THE COMBINATION OF ORAL ALL-TRANS RETINOIC ACID AND DANAZOL *VS* DANAZOL AS SECOND-LINE TREATMENT IN ADULT IMMUNE THROMBOCYTOPENIA: A MULTICENTRE, RANDOMIZED, OPEN-LABEL TRIAL

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Background: Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by increased platelet destruction and impaired platelet production. Despite decades of basic and clinical research, the treatment of severe, corticosteroid-resistant or relapsed disease remains a great challenge. Our preliminary study indicated the effectiveness of all-trans retinoid acid (ATRA) for ITP (Wang M, *et al.* ASH 2012, Abstract #3338). This has been coupled with previous discoveries of an immune-modulation effect of ATRA in ITP, including its role to induce changes in Treg cells (Ruan CG 2016), and to correct the imbalance of aberrant macrophage polarization (unpublished data), indicating ATRA as a potential therapeutic regimen. Danazol has been used in the treatment of ITP for more than 30 years. Apart from its haemopoietic stimulatory and immune-modulatory effect, it has recently been shown to reverse abnormal telomere/telomerase function in patients with thrombocytopenia (Townsley DM 2016). The combination of ATRA and danazol may work synergistically based on the mechanism of action targeting both increased platelet destruction and insufficient platelet production.
Aims: To investigate the efficacy and safety of ATRA plus danazol in patients with corticosteroid-resistant and/or relapsed ITP.
Methods: A multicentre prospective study was performed in non-splenectomized corticosteroid resistant/relapsed ITP patients. Participants were at least 18 years of age, had a platelet count of less than 30×10^9/L at enrolment, and did not achieve a sustained response to treatment with full-dose corticosteroids for a minimum duration of 4 weeks or relapsed during steroid-tapering or after its discontinuation. Written informed consents were obtained from all of the participants. The primary endpoint was a sustained response. The secondary endpoints included overall response, time of response, duration of response, incidence of bleeding symptoms and safety.
Results: From 2012 to 2016, 130 consecutive patients were enrolled from 5 different tertiary medical centres in China. Thirty-seven patients were ineligible and excluded, leaving 93 patients randomized to the ATRA+danazol group (n=45) and the danazol group (n=48). At 12 months' follow-up, sustained partial or complete response was achieved in 71.6% of patients in the danazol+ATRA group, significantly higher than 47.2% for danazol monotherapy ($p<0.001$). Additionally, 92.5% and 42.5% of patients receiving ATRA+danazol achieved at least one response (R), while only 58.3% and 11.1% of patients with danazol monotherapy achieved R and CR, respectively. In patients achieving CR or R, the median time to treatment response was 30.5 days with a peak platelet count of 155×10^9/L in the danazol+ATRA group compared with 49 days with a peak PLT of 69×10^9/L in the danazol group. Multivariate analysis revealed that the initial response at day 28 and the median ITP duration were the potential variables associated with a sustained response. There was no treatment-related death due to adverse events. One patient receiving danazol monotherapy died from intracranial haemorrhage 4 weeks after study enrollment.
Summary/Conclusions: Our findings demonstrate that the combination of ATRA and danazol is safe and effective in achieving a rapid and long-lasting response, making it a potential promising therapeutic option for patients with non-splenectomized corticosteroid-resistant/relapsed ITP. This study is registered at www.clinicaltrials.gov as # NCT01667263.

S432

NOVEL PERSPECTIVES IN GENOTYPE-PHENOTYPE CORRELATIONS IN MYH9-RELATED DISEASE: NO LONGER JUST A MATTER OF HEAD OR TAIL

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Background: *MYH9*-related disease (*MYH9*-RD) is an autosomal-dominant disorder caused by mutations in *MYH9*, the gene for non-muscle myosin heavy

chain IIA (NMMHC-IIA), and represents the most frequent inherited thrombocytopenia worldwide. NMMHC-IIA comprises two distinct domains, the N-terminal globular head domain (HD) and the C-terminal tail domain (TD), and causative mutations hit either the HD or the TD. All patients present at birth with macrothrombocytopenia and only some of them develop during life additional manifestations, including nephropathy often leading to end-stage renal disease (ESRD), sensorineural deafness, and/or cataract. Thus, the search for genotype-phenotype correlations in *MYH9*-RD has been an important research topic since the identification of the disorder. In 2008, the analysis of 108 patients allowed to conclude that the mutations affecting the HD were associated with evolution to early-onset ESRD and deafness, whereas the risk of non-hematological manifestations was much lower for patients carrying mutations of the TD. In 2014, raising to 255 the number of patients, we suggested that evolution to juvenile ESRD associated only with the most frequent among HD mutations, *i.e.* substitution of the arginine 702 (R702). Conversely, the other HD mutations, which were almost all localized in a distinct hydrophobic region at the interface between the SH3 subdomain and the motor domain (SH3/MD interface), may be associated with a much less severe evolution.
Aims: To improve prognostic assessment of patients with *MYH9*-RD.
Methods: All the consecutive patients enrolled in the Italian registry for MYH9-RD until June 2016 were included. The association of *MYH9* genotype with phenotype was assessed by a generalized linear regression model (event-free survival analysis).
Results: We enrolled 350 patients belonging to 199 *MYH9*-RD pedigrees. Mutational screening allowed us to identify 6 novel causative mutations in the HD in 6 different pedigrees. Interestingly, all of these variants were localized in the hydrophobic region at the SH3/MD interface. By raising the number of patients with mutations in this region from 14 to 26, and increasing the observation time, we could demonstrate that the mutations in the SH3/MD interface are associated with development of deafness at young-middle age, but low risk of kidney disease and cataract. The other previously identified genotype-phenotype correlations were confirmed. In particular, mutations hitting the R702 in the HD resulted in constant evolution toward juvenile ESRD and severe deafness. Among mutations different from R702 substitutions, the p.D1424H in the TD associated with the highest risk to develop non-congenital manifestations of the disease.
Summary/Conclusions: Mutations in the HD of the NMMHC-IIA are almost all localized in a specific region at the SH3/MD interface, which therefore represents a critical region for *MYH9*-RD pathogenesis. Most importantly, patients with HD mutations are distinguished into two different prognostic groups: subjects with R702 substitutions are expected to early develop a severe syndromic disorder, whereas mutations in the SH3/MD interface are associated with evolution to a milder phenotype, characterized by development of hearing impairment only ("auditory" phenotype). Our study confirmed a genotype-phenotype model for *MYH9*-RD that overcomes the previously reported dualism between HD *vs* TD mutation.

S433

A MONOALLELIC LOSS-OF-FUNCTION MUTATION IN THE THROMBOPOIETIN (THPO) GENE IS RESPONSIBLE FOR A NEW FORM OF INHERITED THROMBOCYTOPENIA (IT)

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Background: The THPO-MPL axis plays a central role in platelet biogenesis: it activates the signaling cascade inducing megakaryocytes (MKs) differentiation from progenitor cells and regulates MKs maturation, proplatelet extension, and nascent platelets release into the bloodstream. Different diseases are known to derive from inherited abnormalities of *MPL* and *THPO*. Gain-of-function mutations in both genes cause congenital thrombocytosis, while loss-of-function mutations in *MPL* result in congenital amegakaryocytic thrombocytopenia: patients affected by this form of inherited thrombocytopenia (IT) present at birth with isolated thrombocytopenia, which always evolves into severe bone marrow aplasia. Similarly, a homozygous loss-of-function variant in the *THPO* gene was found to be responsible for recessive aplastic anemia in a Micronesian family.
Aims: To unravel the molecular basis of ITs and to improve the clinical and laboratory characterization of the new ITs identified.
Methods: Whole exome sequencing (WES) was performed in 86 propositi with an unknown IT. They were part of our case series of 274 consecutive families, 151 of which remained without a definite diagnosis at the end of the diagnostic workup carried out according to the diagnostic algorithm proposed in 2003 by the Italian platelet study group and subsequently updated to include the most recent discovered disorders (Clin Genet 2016;89:141). The investigation was approved by the Institutional Review Board of the IRCCS Policlinico San Matteo Foundation and all patients gave written informed consent.
Results: WES in 86 propositi with unknown IT identified 2 unrelated individuals (family A and B) carrying the heterozygous variant c.91C>T (p. Arg31*), which is expected to result in a mutant protein degradation and THPO haploinsufficiency. In each family the segregation with the disorder was confirmed analyzing one affected relative. Bleeding tendency was absent in all cases. All patients had mild thrombocytopenia; blood film examination did not identify any morphological abnormality of platelets, except for some elements with slightly increased size in patients of family A. *In vitro* platelet aggregation and surface expression of GPIIb/IIIa and GPIb/IX were investigated in the two patients of Family B and gave normal results. The mild severity of thrombocytopenia and the absence of qualitative platelet defects, at least in the two patients of family B, are consistent with the absence of bleeding tendency in affected subjects. THPO serum level was at the lower limit of the normal range in the two subjects of family B, the only available for this assay. This result was in agreement with our hypothesis that *THPO* mutations were expected to result in haploinsufficiency.
Summary/Conclusions: The p.Arg31* mutation in *THPO* causes a new autosomal dominant form of mild, non-syndromic thrombocytopenia. This innocuous disorder is relatively rare (1.3% of families of our case series) but it has to be distinguished from the more severe autosomal dominant ITs with normal platelet size deriving from mutations in *ETV6*, *ANKRD26* and *RUNX1*, since they predispose to the development of hematological malignancies. Because of the similarity of the clinical features and the lack of reliable laboratory markers, we suggest to perform genetic analysis in all subjects with autosomal dominant thrombocytopenia and normal platelet size in order to identify their disorders, define prognosis and organize an appropriate follow-up regimen.

S434

POSITION OF THE GFI1B ZINC FINGER MUTATION DECOUPLES CD34 EXPRESSION FROM ALPHA-GRANULE DEFICIENCY IN GFI1B-RELATED PLATELET DISORDERS

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Background: GFI1B is a transcription factor that plays an important role in haematopoiesis. Families with a mutation of the fifth DNA-binding zinc-finger domain of GFI1B experience bleeding and have a platelet phenotype characterised by macrothrombocytopenia, increased CD34 expression and alpha-granule deficiency.
Aims: To explore the function of other zinc finger domains of GFI1B we have characterised two unrelated families with a GFI1B variant, C168F, predicted to disrupt the first Zn-finger domain and compared the phenotype with a previously described pedigree with the H294fs mutation that disrupts the fifth Zn-finger domain.
Methods: Clinical platelet phenotypes were determined by light and transmission electron microscopy and functional studies performed by light transmission and whole blood impedance aggregometry. Platelet protein expression was measured by flow cytometry and western blotting. DNA-binding of variants was determined by gel mobility shift assays (EMSA) and changes in gene transcription by luciferase assays. Cellular phenotypes were then studied in patient specific iPSC derived megakaryocytes.
Results: Individuals with GFI1B C168F are thrombocytopenic (mean platelet count =107 $x10^9$/L, n=8) but lack the collagen induced aggregation defects and bleeding symptoms observed in individuals with H294fs (ISTH BAT, P=0.015). Alpha granule content observed by microscopy and quantitated by western blotting of granule related proteins, P-selectin and fibrinogen, were similar between C168F and control platelets and this was significantly greater than that observed for the H294fs mutation (P<0.01). EMSA studies indicate that the C168F variant retains the ability to bind DNA whereas the H294fs mutation altering Zn finger 5 abrogates DNA binding. Despite retaining the ability to bind DNA, the C168F variant de-represses gene transcription at *TUBB1*, *MEIS1* and *CD34* target genes (P<0.01). This de-repression is less marked than that observed with the non-DNA-binding H294fs mutation (P<0.01). The transcriptional de-repression observed at the *CD34* promoter with both Zn finger domain 1 and 5 variants was validated by an increase in platelet surface CD34 measured by flow cytometry and total CD34 protein measured by western blotting (P<0.01). This increased CD34 expression appears specific for *GFI1B* mutation as increased CD34 expression was not observed in platelets derived from individuals with *FLI1*, *RUNX1* or *MYH9* mutation. To validate these clinical observations, iPSCs were generated from the different pedigrees and megakaryocyte differentiation performed *in vitro*. Megakaryocyte CD34 expression was increased in cells derived from individuals with both C168F and H294fs variants but alpha granule deficiency was only observed in cells containing the non-DNA-binding H294fs mutation.
Summary/Conclusions: Mutations altering GFI1B Zn finger 1 cause thrombocytopenia with increased CD34 expression but these platelets retain relatively normal alpha-granule content and these individuals lack clinical bleeding symptomatology. This mutation is mechanistically distinct from the Zn finger 5 mutation that abrogates DNA binding with a subsequent phenotype characterised by alpha-granule deficiency and clinical bleeding.

S435

TREATMENT OF PRIMARY ADULT CHRONIC IMMUNE THROMBOCYTOPENIA (CITP) WITH FOSTAMATINIB, AN ORAL SYK INHIBITOR: RESULTS OF TWO RANDOMIZED, PLACEBO-CONTROLLED PHASE 3 STUDIES

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Background: ITP is characterized by autoantibody-directed platelet destruction mediated by activated monocyte Fc receptors which signal via spleen tyrosine kinase (syk). A Phase 2 trial of the oral syk inhibitor Fostamatinib (FOSTA) in 16 patients (pts) with refractory ITP provided preliminary efficacy and safety data (Podolanczuk *et al.*, 2009).
Aims: To evaluate the efficacy and safety of FOSTA in adult cITP in 2 parallel, identical, multi-center, randomized, double-blind phase 3 studies (S047 and S048) of 24 weeks duration, followed by an open label study (S049).
Methods: 150 pts with 3 platelet (plt) counts (ct) <30K/μL were enrolled (76 in S047, 74 in S048) with a 2:1 randomization to FOSTA 100mg or placebo bid, and stratification by prior splenectomy and baseline plt ct<or ≥15K/μL. Sixty-one % of pts were female; median age was 54 y (20-88); 93% were Caucasian; 93% had cITP; median disease duration: 8.5 y; median baseline plt ct: 16K/μL. Prior therapies received by pts included 94% steroids, 47% TPO-RAs, 35% splenectomy, and 32% rituximab. Stable response (SR) was defined as a plt ct ≥50K/μL at 4 of 6 biweekly visits over Weeks 14-24; intermediate response (IR) as at least 2 consecutive bi-weekly plt cts ≥50K/μL, both without rescue treatment.
Results: Across both studies, a SR occurred in 18/101 (18%) FOSTA *vs* 1/49 (2%) placebo pts (p=0.007); 11 additional FOSTA and no placebo patients achieved an IR, making the overall response rate 29% (29/101) for FOSTA *vs* 2% (1/49) for placebo (p<0.0001). The median plt cts were 95K, 49K, 20.5K and 17.5K/μL in SR, IR, non-responders (NR) and placebo pts, respectively. In SR and IR, median time to first plt ct ≥50K/μL was 2 weeks. Age (< or ≥65 y), gender, baseline plt ct <15K/μL, prior TPO-RA or splenectomy did not substantially affect response. In S049, 9/41 (22%) pts newly treated with FOSTA have a SR, consistent with S047 and S048. Fifty-four of 101 (54%) FOSTA pts and 14/49 (29%) placebo pts had a plt increase ≥20K/μL (p=0.005). Three of 18 (17%) SR and 1/11 (9%) IR to FOSTA compared to 26/72 (36%) NR and 22/49 (45%) of the placebo group received ≥1 rescue medication, respectively. In S047-S048, serious bleeding occurred in 5.6% of the NR and 10.2% of placebo pts, but not in the 29 responders. The number of pts with ≥1 adverse event (AE) was similar in FOSTA *vs* placebo (83% *vs* 75%). The majority AEs on FOSTA were mild or moderate; all resolved over time. Most common AEs were: diarrhea (29% *vs* 15%), nausea (19 *vs* 8%), hypertension (20% *vs* 8%), ALT/AST increase (10% *vs* 0%). Serious AEs were reported in 13% FOSTA *vs* 21% placebo pts.
Summary/Conclusions: Fostamatinib substantially improves plt cts in certain pts with heavily pre-treated, severe cITP of long disease duration. AEs are mostly mild or moderate in severity. Given its unique mechanism of action based on inhibition of syk, FOSTA could, if approved, be an important alternative as single agent and be a useful component of combination therapy for pts with difficult cITP.

References

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Acute lymphoblastic leukemia - Biology

S436

THE YING AND YANG OF JAK SIGNALING : LOSS OF USP9X BUFFERS JAK SIGNALING AND ENHANCES SURVIVAL OF CRLF2-JAK-STAT EXPRESSING B CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Children with Down syndrome (DS) are prone to development of high risk B cell precursor (BCP) acute lymphoblastic leukemias (DS-ALL) that differ genetically from most sporadic pediatric ALLs. Chromosomal rearrangements causing increased expression of CRLF2, the receptor for thymic stromal lymphopoietin (TSLP), characterize about half of DS-ALLs.
Aims: Understanding the pathogenesis of relapse of DS-ALL relating to their CRLF2 status.
Methods: Integrative genomic analysis of matched diagnosis remission and relapse DS-ALLs, pharmacological inhibition and genetic CRISPR mediated silencing.
Results: Genomic analysis of 25 matched diagnosis remission and relapse DS-ALLs reveled lesions affecting known driver genes in the samples. In 80% of the patients we detected activating mutations in genes whose protein products are involved in signaling, including receptors (CRLF2, IL7R, FLT3), or downstream effector enzymes (JAK1/2, KRAS and NRAS). In contrast to a previous report, we observed that lesions in CRLF2 are early events during DS-ALL evolution, as is evident by its high allelic frequency, and are maintained at relapse. The genetic make-up differed significantly between these two major subtypes of DS-ALLs. CRLF2neg DSALLs were characterized by enhanced RAS signaling coupled by mutations in chromatin remodeling genes, in particular CREBBP. In contrast CRLF2pos DS-ALLs were characterized by high dynamics of proliferative signaling. At diagnosis CRLF2 rearrangements were almost always combined with secondary activating signaling events in JAK-STAT pathway suggesting that signaling is driving the development of these leukemias. However JAK2 mutations were often lost at relapse and replaced by clones with mutated RAS. Thus the presence of JAK2 activating mutations at the time of diagnosis are associated with sensitivity to upfront chemotherapy. Surprisingly we discovered loss-of-function mutations or deletions of USP9X, a deubiquitinase previously described as an oncogene that positively regulates JAK2 signaling, in 25% of CRLF2pos ALLs, in both our study and published data,. We therefore tested the counterintuitive hypothesis that loss of a positive regulator of JAK2 enhances the fitness of JAK-STAT driven JAK2 mutated leukemic cells. Both pharmacological inhibition and genetic CRISPR mediated silencing of USP9X reduced STAT5 phosphorylation and enhanced the survival of CRLF2-JAK2R683Gs transduced ALL cells. To test directly the effect of JAK inhibition, we treated CRLF2/JAK2R683G transduced cells with increasing doses of ruxolitinib, a JAK inhibitor currently in clinical trials for CRLF2-JAK-STAT ALLs. Strikingly while high doses (>2μM) were cytotoxic, low doses (0.25μM) enhanced the survival of CRLF2-JAK2R683Gs expressing ALL cells.
Summary/Conclusions: These observations suggest that genetic or pharmacological restraining of JAK-STAT signaling may be beneficial to leukemic B cell precursors by enhancing the fitness of JAK-STAT "driven" ALL. This and the reduction of JAK-mutated clones at relapse suggest that the therapeutic effect of JAK2 specific inhibitors may be limited. Rather, combined signaling inhibitors or direct targeting of the TSLP receptor may be a useful therapeutic strategy for DS-ALL.

S437

TNF RECEPTOR 2 IS REQUIRED FOR RIP1-DEPENDENT CELL DEATH IN LEUKEMIA

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Background: Persistence of residual leukemia cells, due to deficiencies in apoptotic programs, is a major driver of relapse. Activation of alternative non-apoptotic cell death pathways such as necroptosis represents an attractive strategy to eliminate residual leukemia cells and prevent relapse. We have previously shown that SMAC-mimetics (SM) potently induce cell death by simultaneous RIP1-dependent apoptosis and necroptosis in a subset of refractory acute lymphoblastic leukemia (B-ALL) patient-derived samples. The molecular signals that drive sensitivity to RIP1-dependent cell death remained elusive so far.
Aims: The aim of this project was to understand the mechanisms that determine the specific vulnerability to necroptosis in ALL.
Methods: To identify molecular determinants of sensitivity to SM, we correlated the gene expression profiles of 17 primary samples with high and low sensitivity to SM with the IC50 in response to two SM compounds, birinapant and LCL161. We confirmed the top scoring genes including TNF receptor 1 (TNFR1) and TNFR2 by quantitative RT-PCR in patient-derived xenografts. We further validated our results by quantifying the expression of the candidate genes in an independent cohort of relapsed primary B-ALL and by screening samples with different expression levels of TNFR1 and 2 for their response to SM *in vitro*.
To assess the mechanistic role of TNFR1 and 2 in the response to SM, we generated patient-derived TNFR1 and TNFR2 knockout cells using the CRISPR/Cas9 gene editing technology, and evaluated their response to SM *in vitro* and *in vivo* using a CRISPR selection model. Additionally, we overexpressed TNFR2 and evaluated the cell death phenotype. To determine the mechanism of TNFR2-mediated sensitization to SM, we investigated the formation of the pro-death RIP1-TNFR1 complex in wild type *versus* TNFR2ko and in SM sensitive and resistant ALL by immunoprecipitation in primary ALL samples.
Results: Comparative gene expression profiling indicated a correlation of the expression of TNFR2 with sensitivity to SM in primary ALL. Using an independent cohort of relapsed ALL samples, we found that high TNFR2 expression predicted sensitivity to SM in an *ex vivo* model of the bone marrow. Deletion of either TNFR1 or TNFR2 using CRISPR/Cas9 in patient-derived ALL conferred resistance to treatment with SM *in vivo* in the xenograft model, indicating that TNFR1 and 2 are both functionally required for cell death. In agreement with an important role for TNFR2 in the response to SM, the overexpression of TNFR2 leads to increased sensitivity to the TNFR1/RIP1 death axis. On the mechanistic level, recruitment of RIP1 to TNFR1 is a key event in the activation of cell death, which is abolished in TNFR2-deficient leukemia and does not occur in SM resistant cases.

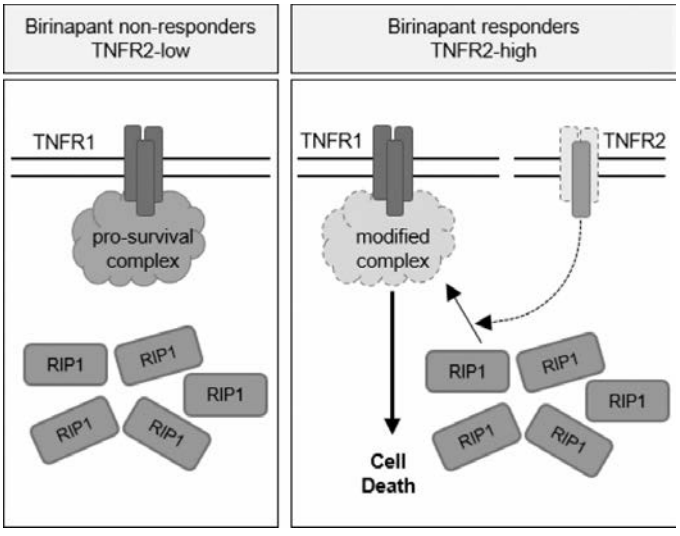

Figure 1.

Summary/Conclusions: Taken together, our data reveal a novel function of TNFR2 in cell death signaling, as TNFR2 predicts sensitivity to SMAC mimetics and plays a key role in activating the TNFR1/RIP1 cell death pathway, which underlies the switch from RIP1-controlled cell survival to cell death and characterizes a distinct vulnerability in ALL.

S438

THERAPEUTIC TARGETING OF ONCOGENIC MYB ACTIVITY IN T-ALL

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Background: T-lineage acute lymphoblastic leukemia (T-ALL) is an aggressive hematologic malignancy that accounts for 10%–15% of pediatric and 25% of adult ALL cases. The prognosis of T-ALL has gradually improved, however, the outcome of T-ALL patients with primary resistant or relapsed leukemia remains poor. Thus, further advances in the treatment of T-ALL require the development of effective and highly specific molecularly targeted antileukemic drugs. The proto-oncogene *MYB* (encodes c-MYB) is aberrantly activated in a subset of T-ALL patients through T-cell receptor driven translocations or genomic duplications of the MYB locus itself. Recently, a new genetic mechanism for the generation of oncogenic super-enhancers in malignant T cells was identified, and suggests a general role for MYB in the regulation of T-cell specific super-enhancer activity.
Aims: We want to identify the role of enhanced MYB activity in super-enhancer driven oncogenic transcription in the context of malignant T-cell development and investigate the *in vivo* role of *cMyb* in the initiation and maintenance of T-ALL.
Methods: To evaluate if *cMyb* could act as a bona fide oncogene in the pathogenesis of T-ALL, we developed a conditional R26-driven cMyb overexpression mouse model. For this, we used a targeting vector that contained a floxed stop cassette followed by the *cMyb* gene and a EGFP/luciferase reporter, which was subsequently targeted in mESCs using recombinase-mediated cassette exchange (RMCE).
Results: Here, we report a novel conditional Myb knockin mouse model (R26-Myb). To study the *in vivo* oncogenic capacity of Myb, we initially crossed this conditional Myb knockin model with VaviCre mice, in order to obtain hematopoeitic specific expression of Myb and the EGFP/luciferase from the ROSA26-promoter. Notably, Vav-iCre$^{tg/+}$ R26-Myb$^{tg/tg}$ mice developed T-cell lymphomas with a median latency of 77 weeks, suggesting that Myb can act as a *bona fide* oncogene in malignant T-cell transformation (Figure 1A). Next, we crossed our Myb transgenic model with Pten conditional knockout mice, to allow comparative analysis of tumors with and without T-cell specific Myb expression. Genetic inactivation of *Pten* is frequently observed in human T-ALL, and T-cell specific deletion of *Pten* (using Lck-Cre) results in T-cell leukemia/lymphoma development with an average of 17 weeks. Using this strategy, we obtained mice that overexpress R26-driven cMyb and lack Pten in developing T-cells and found that cMyb expression synergizes with Pten deletion, resulting in fully penetrant and accelerated T-ALL formation (median survival of 84 days instead of 118; p =0.0003; Figure 1B). Finally, we used this novel murine T-ALL model to identify new therapeutic strategies for MYB dependent T-ALL. Importantly, the tumor cells from the cMyb knockin mice are luciferase-positive and are therefore suitable for *in vivo* drug testing using bioluminescence. Using this model, we evaluated the *in vivo* anti-leukemic efficacy of a variety of small molecules and identified new drugs that impede Myb protein stability or Myb-mediated transactivation in Myb driven tumorigenesis.

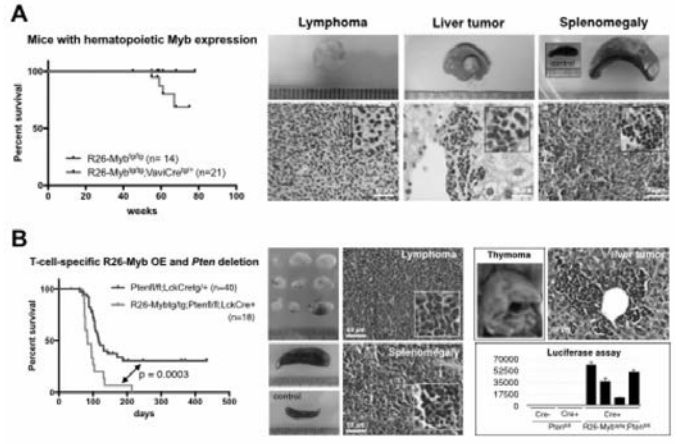

Figure 1.

Summary/Conclusions: We developed a novel Myb-driven T-ALL mouse model and could demonstrate a pathogenic role for *cMYB* in T-cell leukemia. In addition, the Myb-driven preclinical mouse model will open new avenues for therapeutic intervention in T-ALL.

S439

THE T-CELL LEUKEMIA ASSOCIATED RIBOSOMAL RPL10 R98S MUTATION ENHANCES JAK-STAT SIGNALING

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Background: Several somatic ribosome defects have recently been discovered in cancer, yet their underlying oncogenic mechanisms remain poorly understood. Alterations in ribosomal protein genes *RPL5*, *RPL10*, and *RPL22* have been described in ~20% of T-cell acute lymphoblastic leukemia (T-ALL) cases. Whereas *RPL5* and *RPL22* show heterozygous inactivating mutations and deletions, RPL10 contains a clear mutational hotspot at residue arginine 98 (R98), with 8% of pediatric T-ALL patients harboring this RPL10 R98S missense mutation.
Aims: Investigating the pathogenic role of the recurrent R98S mutation in ribosomal protein L10 (RPL10) in T-ALL.
Methods: A label-free quantitative proteomics experiment was performed to screen for differentially expressed proteins in engineered mouse lymphoid Ba/F3 cells expressing RPL10 WT or RPL10 R98S. Differences in protein expression were further validated in hematopoietic cells derived from a transgenic RPL10 R98S knock-in mouse model and in material derived from xenografted T-ALL patient samples.

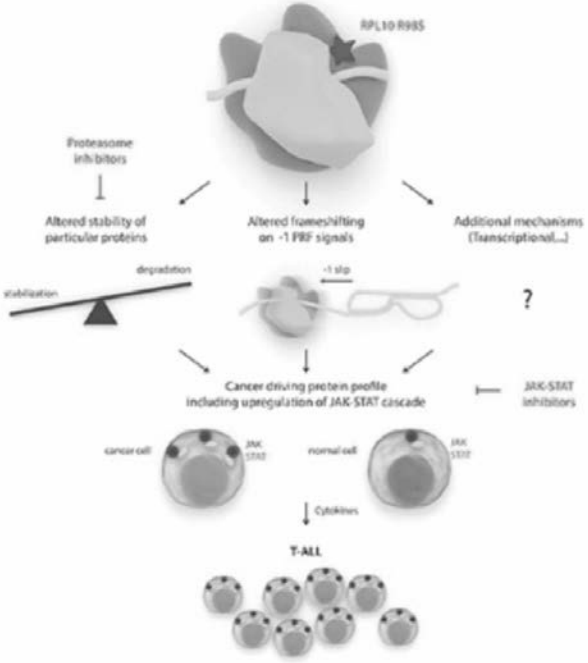

Figure 1.

Results: The differential proteome screen revealed overexpression of several Jak-Stat signaling components (Csf2rb/2, Jak1, Stat1, Stat3, Stat5a/b and Stat6) in engineered RPL10 R98S mouse lymphoid cells, which we confirmed in hematopoietic cells derived from a transgenic RPL10 R98S mouse model. The relevance of this overexpression was illustrated by enhanced Jak-Stat pathway activation upon cytokine stimulation in RPL10 R98S lymphoid cells, as well as increased sensitivity of these cells to clinically used JAK-STAT inhibitors ruxolitinib and pimozide. RPL10 R98S positive leukemia patients likewise showed overexpression of IL7RA, JAK1 and STAT5, increased sensitivity to pimozide, as well as a mutually exclusive mutation pattern between RPL10 R98S and JAK-STAT lesions, suggesting that RPL10-R98S also modulates the cascade in human T-ALL. Programmed -1 ribosomal frameshifting (-1 PRF) recently emerged as a post-transcriptional mechanism regulating expression of cytokine receptors. We identified -1 PRF signals in mouse and human Jak-Stat genes and observed RPL10 R98S associated frameshifting reduction in several of these, which may contribute to their overexpression. Altered levels of -1 PRF can however only partially explain observed JAK-STAT protein expression changes, and transcriptional changes and altered protein stability are also involved. Indeed, our data point to altered proteasome activity and composition in RPL10 R98S cells, with upregulation of immunoproteasome specific catalytic subunits, which may explain the increased stability of particular proteins such as Jak1. Of further medical interest, RPL10 R98S cells showed reduced proteasome activity and enhanced sensitivity to the clinically used proteasome inhibitors bortezomib and carfilzomib.
Summary/Conclusions: We explored the molecular mechanism by which the RPL10 R98S mutation contributes to the pathogenesis of T-ALL. We propose a model in which R98S associated decreases in -1 PRF levels, combined with changes in the degradation of particular proteins and potential other mechanisms such as transcriptional regulation, leads to selective upregulation of the oncogenic JAK-STAT cascade (Figure 1). Besides expanding the relevance of the JAK-STAT cascade in T-ALL and leukemia in general, our results have therapeutic potential since cells harboring the RPL10 R98S mutation are sensitized towards clinically used JAK-STAT and proteasome inhibitors.

S440

NFATC3-PLA2G15 IS A NOVEL INTERGENICALLY SPLICED CHIMERA THAT IS ASSOCIATED WITH AGGRESSIVE T-ACUTE LYMPHOBLASTIC LEUKAEMIA BIOLOGY

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Background: Transcriptional read-through of a single mRNA between contiguous loci, or *cis*-splicing of adjacent genes (*cis*-SAGe), results in transcription of intergenically-spliced chimeric RNAs (ISCs) in the absence of structural genomic changes. Recent advances in high-throughput RNA-sequencing analysis have permitted identification of aberrant ISC expression as a potential cancer driver, but knowledge of leukaemia-related ISCs is lacking.
Aims: To examine whether *cis*-SAGe generates biologically important ISCs in T-acute lymphoblastic leukemia (T-ALL).
Methods: We performed RNA-sequencing of 12 cases of T-ALL and normal thymic RNA, and used targeted analysis pipelines to detect T-ALL-specific fusion chimeras.
Results: We identified 140 T-ALL-specific fusions, of which 55 involved genes located within 30kb of each other, in the same transcriptional orientation. This distance is consistent with that previously observed for *cis*-SAGE, suggesting that ISC expression is common in T-ALL. In total, putative ISCs were detected in 10/12 samples, with a median of 4 (range 0-15) per patient. We performed further analysis on the candidate ISC *NFATC3-PLA2G15*, which includes the Nuclear Factor of Activated T-cells (NFAT) family member NFATC3, a critical regulator of normal thymopoiesis and known modulator of T-ALL biology. We found that primary T-ALLs exhibited a wide range of *NFATC3-PLA2G15* expression, while levels in normal tissue were either very low or undetectable. 5' RACE PCR analysis of leukemic cDNA revealed that fusion transcription was initiated in exon 1 of *NFATC3*. We also performed array competitive genomic hybridization of 115 diagnostic T-ALL samples, and found no evidence of microdeletions that would result in *NFATC3-PLA2G15* expression, providing strong evidence that *NFATC3-PLA2G15* is a true ISC that is generated by *cis*-SAGe. We found that the NFATC3-PLA2G15 fusion had lower activity than wild-type NFATC3 in both luciferase reporter experiments and proliferation and survival complementation assays in NFAT-null ALL cell lines *in vitro*. Gene set enrichment analysis revealed that primary T-ALL blasts with elevated *NFATC3-PLA2G15* levels had reduced transcription of canonical NFAT target genes *in vivo*, suggesting that these cases may have lower activity of normal physiological NFAT pathways. Strikingly, we found that higher *NFATC3-PLA2G15* levels strongly correlated with both shorter time to leukemia development (p=0.01) and survival (p=0.003) in patient-derived T-ALL xenografts in immunodeficient mice. These findings were corroborated by survival analyses of human T-ALL patients treated as part of the Francophone multinational GRAALL-2003 and -2005 studies, as cases with the highest quartile of *NFATC3-PLA2G15* expression had significantly reduced 5 year overall survival (52.6%, 95% CI 33.3% - 68.7%) compared with *NFATC3-PLA2G15* low cases (69.8%, 95% CI 58.8% - 78.3%, p=0.047).
Summary/Conclusions: Our results suggest that ISC expression is common in T-ALL, and that high expression of the *NFATC3-PLA2G15* ISC correlates with reduced canonical NFAT pathway activity and poor patient outcome.

Thrombotic disorders

S441

ASSESSING THE RISK-BENEFIT OF ANTICOAGULANTS IN ELDERLY PATIENTS WITH CANCER-ASSOCIATED VENOUS THROMBOEMBOLISM: A POPULATION BASED STUDY

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Background: Cancer patients have a higher risk of venous thromboembolism (VTE) which conveys a higher subsequent mortality risk; conversely, they also have a higher risk for bleeding due to many factors including abnormal tumor anatomy and the use of chemotherapy agents with the associated risk for thrombocytopenia. However, the consequences of a recurrent VTE or a major bleeding (MB) event might be different in terms of mortality. As a result, the risk of VTE recurrence or a MB event might bear different weights. A previous systematic review has suggested that the case fatality rates of VTE recurrence and MB are similar. However, heterogeneity in study design, outcomes and in particular the types of populations included, limited the interpretation and applicability of the results. Clinical decision making uses estimations of risk and benefit for any given intervention. In the case of VTE, anticoagulants are the cornerstone of treatment having a proven benefit in reducing the risk of recurrent VTE events with an associated increase in the risk of bleeding. Therefore, determining the risk-benefit of anticoagulants might allow for better informed treatment decisions, in particular in a population at high risk for both ends of the spectrum. Therefore, herein we sought to estimate the risk and benefit of anticoagulant therapy in cancer patients developing a VTE using data from administrative databases.
Aims: To estimate case fatality rates of VTE recurrence and MB, as well as the case fatality rate-ratio for MB and VTE recurrence in cancer patients developing a VTE treated with anticoagulants.
Methods: We conducted a retrospective population-based cohort study in Ontario, Canada using de-identified linked administrative healthcare databases housed at the Institute for Clinical Evalutive Sciences (ICES). We included patients over 65 years of age with a diagnosis of cancer defined using provincial, ICD-9 and ICD-10 codes for major malignancies and who developed a VTE event within 6 months of the initial cancer diagnosis. VTE was identified through a previously validated algorithm using a combination of diagnostic codes for deep vein thrombosis (DVT) and pulmonary embolism (PE) and codes identifying diagnostic procedures for VTE (i.e. ultrasound, CT pulmonary angiography, lung scintigraphy) within 7 days of each other. Recurrent VTE and MB events were assessed within 180 days from the index date. MB was identified using a previously validated algorithm and included upper and lower gastrointestinal and intracranial bleeding events. Treatment was classified based on the first available prescription within 7 days of the index VTE. We estimated mortality within 7 days of the VTE recurrence or MB events using an unadjusted Cox proportional hazards model and competing risk analysis. Ratios of the mortality for MB compared to VTE recurrence were calculated and 95% confidence intervals were estimated using non-parametric models.
Results: Between 2004 and 2014 there were 6967 VTE events identified in cancer patients over 65 years of age and treated with an anticoagulant. Mean age was 75 years, and 47.6% patients were women. Of all patients, 59.9% received prescriptions for LMWH alone, 15.3% for LMWH followed by warfarin, 22.1% for warfarin and 2.7% for rivaroxaban. At 180 days after the index VTE event there were 235 (3%) MB events and 1184 (17%) VTE recurrences. Within 7 days of the outcome event there were 26 (11%) deaths after MB and 6 (0.5%) after VTE. The mortality ratio for MB *versus* VTE was 21.8 (95% CI 9-53). In exploratory analyses we did not find differences according to type of anticoagulant prescription.
Summary/Conclusions: In cancer patients 65 years or older treated with anticoagulants for a VTE, the 7 days mortality after a MB event is at least 9 times higher than after a VTE recurrence, although the estimate is imprecise. This data should be taken into consideration when designing studies and interventions involving anticoagulant therapy in this population.

This study was supported by the Institute for Clinical Evaluative Sciences (ICES), which is funded by an annual grant from the Ontario Ministry of Health and Long-Term Care (MOHLTC). The opinions, results and conclusions reported in this paper are those of the authors and are independent from the funding sources. No endorsement by ICES or the Ontario MOHLTC is intended or should be inferred. AL-L was supported in part for this study by a grant from the Canadian Institutes of Health Research (CanVECTOR Network CDT-142654).

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RISK OF THROMBOSIS IN PATIENTS WITH NON-HODGKIN'S LYMPHOMA: A POPULATION-BASED COHORT STUDY

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Background: Lymphoma is a malignancy that originates in lymph nodes and lymphoid tissue. The main category of lymphomas is non-Hodgkin's lymphoma (NHL). NHL comprise about 3% of all cancers in Sweden. Some are aggressive and fast growing, while others are more indolent and do not necessarily require treatment. It is well known that cancer increases the risk of thrombosis, especially venous thromboembolism, but data are scarce on the risk of thrombosis in NHL patients.
Aims: The aim of this study is to evaluate the risk of thrombosis in NHL patients compared to controls and to study time trends in the risk of thromboembolism with recent advances in the treatment of these diseases.
Methods: The study population consisted of individuals diagnosed with NHL in Sweden 1980-2013 (n=40,354), and up to four matched controls (n=115,677). The risk of the first thrombosis was evaluated after the diagnosis of NHL (and corresponding date for controls) and the ones that occurred less than 30 days prior to diagnosis of NHL. Kaplan-Meier survival analysis was used to estimate the risk of thrombosis and a log-rank test performed to assess statistical significance. Cox regression analysis was used to calculate hazard ratios (HRs) and 95% confidence intervals (CI) (adjusting for age, sex, year of diagnosis, and previous history of thrombosis). Risk of deep vein thrombosis, pulmonary embolism and arterial thrombosis was evaluated. Arterial thrombosis was defined as cerebral infarct, transient ischemic attack, angina pectoris, myocardial infarction, and arterial embolism and thrombosis.
Results: NHL patients had a statistically significant increase in risk of any type of thrombosis compared to controls (HR: 1.58, 95% CI: 1.53-1.62). The risk was significantly increased for all three types of thrombosis; deep vein thrombosis (HR: 3.11; 95% CI: 2.93-3.31), pulmonary embolism (HR: 3.16; 95% CI: 2.95-3.39) and arterial thrombosis (HR:1.20; 95% CI: 1.16-1.23). The risk of thrombosis did not change during the study period for the NHL patients. There was an increased risk of thrombosis for NHL patients when compared to controls, independent of previous history of thrombosis (HR: 1.64; 95% CI: 1.59-1.69 if no previous history, HR: 1.43; 95% CI: 1.37-1.50 if previous history of thrombosis). The incidence of thrombosis for NHL patients started to increase about five months before the diagnosis of NHL, and reached its peak a month before diagnosis. The incidence stayed increased for the first year after diagnosis.
Summary/Conclusions: In this large, population-based, study based on more than 40,000 patients with NHL and almost 116,000 controls, we demonstrated that there is an increased risk of thrombosis in patients with NHL when compared to controls. This is true for all types of thrombosis. We therefore conclude that hypercoagulability seems to increase with diagnosis of NHL. Several factors may contribute to this prothrombotic state, including chemotherapy and other treatment related factors as well as the disease itself. Considering that the increase in the incidence of thrombosis was highest before and around the time of diagnosis for NHL patients, that indicates that the tumor itself may have a great impact on the hypercoagulability of these patients.

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COMPARATIVE ANALYSIS OF PREDICTIVE MODELS FOR THROMBOEMBOLIC EVENTS IN LYMPHOMA PATIENTS

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Background: Actual guidelines recommend Padua and Khorana score for thromboembolic (TE) risk estimation for cancer patients in general. These existing models are quite limited for designation of lymphoma patients for TE events, as their development is not based on features specific for hematological patients.
Aims: The aim of this study was to compare diagnostic performance of these suggested predictive models, as well as Thrombosis lymphoma (Throly) score, developed by our group, which is more specific for lymphoma patients.
Methods: The study population included all consecutive patients with a confirmed diagnosis of non-Hodgkin lymphoma (NHL), Hodgkin lymphoma (HL), and chronic lymphocytic leukemia (CLL)/ small lymphocytic lymphoma (SLL), who were treated in the Lymphoma Departments of Clinical Center Serbia and Clinical Center Kragujevac in period from 2006 to 2014. Data for newly diagnosed and relapsed patients who had completed a minimum of one chemotherapy cycle were prospectively collected for all venous and arterial TE events from time of diagnosis to 3 months after the last cycle of therapy. Data for specific demographic, clinical, and laboratory variables known to be associated with TE from Padua and Khoranapredictive scores were also extracted. Moreover, potential disease-related risk factors were gathered. The study population was divided based on a split-sample random method into the model developing

and validation cohorts. The ThroLy model was developed using data solely from a derivation cohort, which included 1236 patients. Variables were evaluated by univariate logistic regression analysis, while the model was developed using a stepwise multivariate logistic regression analysis. Once a final model was defined, patients were divided into *low risk* and *at risk* groups. The final model was assessed in the validation cohort (584 patients). The studied population was also divided, based on Khorana and Padua score, into *low risk* and *at risk* groups.

Results: The study population included 1820 eligible lymphoma patients. The mean patient's age was 53.1 years (range, 15–87 years). Most patients (83%) were newly diagnosed and had advanced stage disease: Ann Arbor stage III, 14.7% and stage IV, 44%. A total of 778 patients (42.7%) had high-grade lymphoma; 351 (19.3%) had low-grade lymphoma; 266 (14.6%) had HL; 156 (8.6%) had other forms; and 269 (14.8%) had CLL/SLL. Of all the patients included in the study, 99 (5.4%) developed at least one TE during the follow-up period. There were 73 patients with venous TE (73.7%), and 25 with arterial TE (25.3%), while 1 patient had both. Patients with aggressive NHL had significantly higher odds of developing TE compared to patients with any other lymphoma type (RR=1.5; 95% CI for RR 1.1–2.4; p=0.027). The incidence of thromboembolism was 81 (5.3%) in the newly diagnosed patients and 18 (6.2%) in relapsed patients. Overall, 35.4% (35/99) of the patients with thromboembolism experienced the event before the start of chemotherapy. The majority of patients (64.6%) had TE events during chemotherapy or within 3 months after chemotherapy. For patients classified *at risk* according to ThroLy score in derivation cohort, the model produced negative predictive value (NPV) of 98.5%, positive predictive value (PPV) of 25.1%, sensitivity of 75.4%, and specificity of 87.5%. In validation cohort PPV for Throly score was 28.9%. Padua and Khorana score had PPV of 15.5% and 14.8% in derivation, and 11.5% and 14.8% in validation cohort, respectively.

Summary/Conclusions: Lymphoma patients are at increased risk of thromboembolic events but thromboprophylaxis in these patients is largely underused. ThroLy score is more specific for lymphoma patients than suggested Padua and Khorana score, but external validation in large prospective cohort studies is required.

Table 1.

Table 1. Clinical features in Group1, Group 2 and both.

	GROUP 1 (n= 782) (March 2014- June 2015)	GROUP 2 (n=469) (July 2015- December 2016)	BOTH (n=1251)	p
Sex (male/female) (%)	58.5/41.5	57.4/42.6	58.0/42	n.s
Mean Age (years)	61.8+/-13.2	61.9 +/-13.6	61.9+/-13.4	n.s
Advanced tumour stage (%)	50.4	60.6	54.0	0.0009
Mean platelets (x10E9/L)	232+/-126	224+/-132	232+/-128	n.s
Mean Haemoglobin (g/dL)	11.3+/-2.1	11.1+/-2.2	11.2+/-2.1	n.s
Mean Leukocytes (x10E9/L)	8.1+/-7.9	8.4+/-6.7	8.2+/-7.5	n.s
Chemotherapy (%)	82.9	84.9	83.6	n.s
Type of tumour (Hematologic/non Hematologic %)	21.6/78.4	19.6/80.4	20.8/79.2	n.s
Mean Hospital stay (days)	4 (1-140)	6 (1-101)	5 (1-140)	
≥4 days	419 (53.6%)	325 (69.4%)	744 (59.4%)	<0.0001
Risk score (Pretemed)	5 (3-13)	5 (3-13)	5 (3-13)	n.s

Summary/Conclusions: The new e-alert system further increases the use of VTE prophylaxis in hospitalised cancer patients, although this was not associated with a reduction in the VTE incidence. A relevant number of VTE events occur despite prophylaxis with standard LMWH. Identificaton of risk factors for thromboprophylaxis failure is needed.

This work has been funded by a biomedical research grant with the Laboratory of Pharmaceutical ROVI and an aid to the research projects of the Instituto de Salud Carlos III and the FEDER (PI13 / 01029).

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IMPACT OF A NEW ELECTRONIC ALERT SYSTEM (V2.0) FOR VENOUS THROMBOEMBOLISM PREVENTION IN HOSPITALISED CANCER PATIENTS

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Background: Onco-hematologic hospitalised patients constitute a group at high risk of venous thromboembolism (VTE). Current clinical practice guidelines recommend prophylaxis with low molecular weight heparin (LMWH) during hospitalisation, unless contraindicated. However, its underuse is a worldwide problem. Electronic alert systems (e-alerts) can improve the use of appropriate thromboprophylaxis and reduce the incidence of VTE.

Aims: To evaluate the impact of a new version (v2.0) of our e-alert system for VTE prevention compared with the initial software version. Secondary endpoints try to identify predictive factors for prophylaxis use and thrombotic events.

Methods: Prospective study including consecutive adult cancer patients admitted at our centre. From April 2014 to June 2015 (first period) the initial e-alert system version remained operative and from July 2015 to December 2016 (second period) the new version was active. The v2.0 displayed a second window that asked physicians about the reason why LMHW was not prescribed. The main outcomes were: VTE (confirmed by objective methods), clinically relevant bleeding, and mortality. All patients were followed-up during hospitalisation and 30 days after discharge. Descriptive statistical analysis and correlation between clinical variables and main outcomes were performed by using the software package SSPS v20.

Results: 1251 patients were included, 782 patients in the first period and 469 in the second one (main clinical features are shown in Table 1). E-alerts v2.0 was associated with an increase of appropriate LMWH prophylaxis during hospitalisation (65.2% *vs* 72.2%; p=0.015). However, this improvement did not result in a reduction of VTE during admission or follow-up (2.3% *vs* 2.3%; p=0.89). Interestingly, almost 80% of VTE events occurred despite LMWH use. No differences in the rate of major bleeding (2.8% *vs* 3.2%; p=0.83), and mortality (10.6% *vs* 14.3%; p=0.07) were observed, either. The main reason for not prescribing LMWH prophylaxis was bleeding risk, but in 17% of cases physicians did not consider that the patient really had a high VTE risk. No significant correlation was found between any of the clinical variables analyzed and the risk of VTE. Prophylaxis use was more frequent among patients with solid cancer (vs hematologic), advanced stage, active chemotherapy treatment and longer hospital stay.

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IDENTIFICATION OF A NEW AND RELATIVELY FREQUENT SERPINC1 GENE DEFECT CAUSING ANTITHROMBIN DEFICIENCY HARDLY DETECTED BY CURRENT MOLECULAR METHODS: DUPLICATION OF EXON 6

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Background: Antithrombin (AT) deficiency was the first thrombophilia described 50 years ago and so far the strongest one. Up to 78% of cases are explained by point mutations or small deletion/insertions in exons or flanking regions of *SERPINC1* that are easily detected by sequencing analysis. A low proportion of cases (2%) is explained by gross gene defects, mainly deletions, which are detected by multiplex ligation-dependent probe amplification (MLPA) analysis. However, the molecular base of AT deficiency is unknown using current methods in 20% of cases.

Aims: To identify new *SERPINC1* defects causing AT deficiency.

Methods: We studied 271 unrelated cases with AT deficiency. Functional and biochemical assays characterized plasma AT. Genetic analyses involved Sanger and Next Generation Sequencing (NGS) (PGM, Ion Torrent), MLPA and specific PCR designs.

Results: Sanger sequencing of PCR amplicons with primers flanking the 7 exons and further analysis with SeqscapeTM detected pathogenic mutations in 173 cases. Whole gene sequencing identified 5 mutations in regulatory regions. MLPA analysis revealed 5 cases with whole or partial deletion of the gene. Moreover, 13 cases had disorders of glycosylation. Interestingly, the analysis of the PCR product and the electropherogram of exon 6 of a 42 year-old male patient (P1) with deep venous thrombosis and 75% of anti-FXa activity

with no apparent gene defect by either Sanger sequencing of 7 exons or by NGS analysis of the whole gene using the Ion Torrent platform, revealed a 193 bp insertion, which corresponded to a tandem duplication involving exon 6. Family studies revealed the same duplication in 5 relatives, all with AT deficiency (60-75%). The first MLPA analysis of this case failed to detect the duplication and only after a fine readjustment, it was detected. MLPA analysis under the new conditions of the remaining 59 cases with unknown molecular base for their AT deficiency identified one additional case, P2, with potential duplication of exon 6. P2 was a 17 year-old female with 41% of anti-FXa activity, who developed deep venous thrombosis. Sanger and NGS sequencing also failed to detect any genetic defect in P2. A set of primers specific to detect tandem duplications of exon 6 was designed with forward primer from 3' end of exon 6, and reverse primer from 5' of exon 6. This set of primers only rendered amplification in the two cases with exon 6 duplication. The second patient (P2) had a new 863 bp duplication in tandem of exon 6. Sanger sequencing of the specific amplicons in the two cases with tandem duplication of exon 6 revealed Alu sequences surrounding these duplications. Finally, one out of 5 cases with gene deletions involved breakpoints affecting intron 5 (deletion of exons 2-5).
Summary/Conclusions: Our study identified a new and relatively frequent *SERPINC1* gene defect causing AT deficiency that is hardly identified by current molecular methods: duplication of exon 6. This genetic defect was detected in 1% of our cohort, and represents nearly half of the total gross gene defects causing AT deficiency. The small size of this exon makes difficult the identification of this defect by MLPA. The presence of 6 Alu elements up and downstream exon 6 makes this region a hotspot for unequal recombination that may cause deletions, tandem duplications and potentially transpositions, which may produce AT deficiency (both severe and mild) by an aberrant splicing. We also developed a simple and specific method to detect duplications in tandem of exon 6.

Stem cell transplantation - Experimental

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CYTOSOLIC NUCLEIC ACID SENSORS PROMOTE INTESTINAL EPITHELIAL INTEGRITY DURING ACUTE TISSUE DAMAGE AND PROTECT FROM GRAFT-VERSUS-HOST DISEASE

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Background: The epithelial lining of the gastrointestinal (GI) tract represents the first line of defense mediating protection from microbial challenge. Next to producing antimicrobial molecules, Paneth cells contribute to this defense by providing a supportive niche for intestinal stem cells (ISCs) maintaining the epithelium. Loss of intestinal barrier function by total body irradiation (TBI) or chemotherapy (CTx) is an essential step in enhancing the development of inflammatory disease associated with epithelial surface such as graft versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Our current limited ability to protect epithelium and promote regeneration in GVHD is at least in part due a limited understanding of ISC function and epithelial regeneration in the allo-HSCT setting. Recent work suggests a protective function of Type I Interferons (IFN-I) at epithelial surfaces and in the preventon of GVHD. Yet, the molecular pathways that trigger those functions during acute tissue damage are poorly understood. Given that the RIG-I-MAVS and STING pathways are important regulators of IFN-I production and IFN-Is can initiate epithelial repair, we hypothesized that activation of these pathways during conditioning therapy may protect epithelial integrity and could be exploited interventionally to promote intestinal barrier function and prevent GVHD.
Aims: We aimed at characterizing the role of of RIG-I/MAVS and STING during allo-HSCT, and at understanding mechanisms by which activation of these pathways can promote barrier function to enhance healing after genotoxic tissue damage.
Methods: We used an integrated approach with pathophysiologic mechanistic studies on IECs in experimental mouse models (MHC-mismatched and minor histocompatibility antigen (miHA)-mismatched transplants to model highly aggressive GVHD; genotoxic stress induced by TBI and CTx) and evaluation of immune-mediated regenerative strategies to promote epithelial barrier function (organoid cultures, barrier function test)
Results: Mice lacking MAVS were more sensitive to total body irradiation (TBI)- and chemotherapy induced intestinal barrier damage, and, like RIG-I-deficient mice, developed worse graft transplantation (allo-HSCT). This phenotype was not associated with changes in the intestinal microbiota, but with reduced epithelial integrity and regeneration. Conversely, targeted activation of the RIG-I pathway during damage promoted these processes and ameliorated GVHD. Mechanistically, IFN-I (RIG-I-induced or recombinant) could promote growth of intestinal organoid cultures and production of RegIIIγ. Importantly,our findings were not confined to RIG-I/MAVS signaling, as interventional engagement of the STING pathway also protected from loss of barrier function and GVHD and led to IFN-I-dependent intestinal organoid growth. Consistent with this, STING-deficient animals suffered from worse GVHD.
Summary/Conclusions: Our studies may have the potential to develop novel targeted therapies (i) to promote intestinal barrier integrity, (ii) to prevent the development of GVHD, and (iii) for the regenerative response of other tissues.

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CD4 T CELLS RECOGNIZING MISMATCHED HLA-DP AFTER ALLOGENEIC STEM CELL TRANSPLANTATION SHOW TISSUE SPECIFIC REACTIVITIES

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Background: Expression of HLA class II molecules is under non-inflammatory conditions predominantly restricted to hematopoietic cells. However, donor CD4 T cells directed against mismatched HLA-DP can cause Graft-versus-Host Disease (GVHD) after allogeneic stem cell transplantation (alloSCT) or donor lymphocyte infusions from HLA 10/10 matched but HLA-DP mismatched donors due to upregulation of HLA class II expression under inflammatory conditions. It is often assumed that allo-HLA-DP directed CD4 T cells recognize peptides encoded by household genes presented in foreign HLA-DP and that every cell that expresses the mismatched HLA-DP allele is a target for these T cells. However, *in vitro* experiments illustrated that allo-HLA-DP directed CD4 T cells were not always recognizing patient derived fibroblasts induced to express HLA-DP. We hypothesized that HLA-DP directed CD4 T cells can have tissue specificity if the presented peptides in HLA-DP are encoded by genes with tissue specific expression.

Aims: The aim of the study is to investigate whether donor CD4 T cells recognizing mismatched HLA-DP show tissue specific reactivities.
Methods: In a randomized clinical trial we treat patients 3 months after T cell depleted alloSCT from HLA 10/10 matched, HLA-DP mismatched, donors with 0.25-0.50 x 10^6/kg donor CD4 T cells to promote immune reconstitution. In 4 patients, Graft-versus-Leukemia reactivity and/or organ specific GVHD occurred after the infusion. To characterize the immune responses in these patients, *in vivo* activated T cells were clonally isolated and tested for reactivity against a panel of target cells, including patient and donor derived hematopoietic cells, third party hematopoietic cells as well as different GVHD target cells (patient skin fibroblasts, third party colon carcinoma cells, biliary epithelial cells and lung fibroblasts) expressing the mismatched, patient variant, HLA-DP molecule.
Results: Allo-HLA-DP directed CD4 T cells showing differential recognition of target cells were found in all 4 patients. A total of 33 HLA-DPB1*04:01 reactive CD4 T cell clones were isolated from patient 1 who suffered GVHD of skin and colon, but not liver. Within these 33 clones, 3 clones recognized only hematopoietic target cells, 9 clones recognized hematopoietic, skin and colon derived target cells and 5 clones recognized hematopoietic and colon derived cells only. None of the T cell clones recognized biliary epithelial cells. From patient 2 total of 230 HLA-DPB1*03:01 reactive CD4 T cell clones were isolated, of which 27 recognized only hematopoietic target cells and 96 clones also recognized GVHD target cells with differences in tissue specificity. 32 HLA-DPB1*03:01 reactive T cell clones were found from patient 3, of which 6 recognized only hematopoietic target cells, whereas other clones again showed various tissue specificities. From patient 4, 26 HLA-DPB1*01:01 reactive T cells could be isolated which all recognized biliary epithelial cells with or without co-recognition of other target cells. In addition, also 11 HLA-DPB1*03:01 reactive T cells were isolated, again with different tissue specificities.
Summary/Conclusions: These results illustrate that donor CD4 T cells directed against mismatched HLA-DP show differential recognition of target cells including restricted specificity for cells of hematopoietic origin. Donor CD4 T cells recognizing hematopoietic target antigens in the context of patient specific HLA-DP alleles can be used to mediate tumor specific immune responses after HLA 10/10 matched unrelated stem cell transplantation.

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MESENCHYMAL STROMAL CELLS STIMULATE THE PROLIFERATION AND IL-22 PRODUCTION BY TYPE 3 INNATE LYMPHOID CELLS

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Background: Infusion of mesenchymal stromal cells (MSCs) is a promising and increasingly applied therapy for patients who suffer from graft-versus-host disease (GvHD), a common and life-threatening complication of allogeneic stem-cell transplantations (ASCT). The therapeutic effect of MSCs is mainly ascribed to their suppression of (alloreactive) lymphocyte proliferation and enhancement of tissue-repair activity. However, only about half of the GvHD patients benefit from MSC therapy, and which factors determine MSC responsiveness is unclear. We recently observed that relatively high frequencies of activated type 3 innate lymphoid cells (ILC3s) before and/or after ASCT were associated with a lower risk to develop GvHD, which may be related to the production of tissue-protective IL-22 by ILC3s.

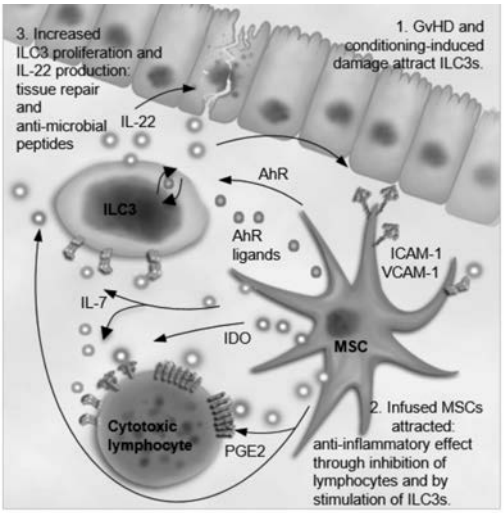

Figure 1.

Aims: To investigate if ILC3s can contribute to the therapeutic effect of MSCs, we studied the interaction between MSCs and ILC3s *in vitro*.
Methods: ILC3s isolated from human tonsils were CellTrace-labeled and co-cultured with bone-marrow derived MSCs for 5 days in the presence of IL-2.
Results: Co-culture with MSCs significantly enhanced the proliferation of ILC3s and their IL-22 production. Reciprocally, ILC3s promoted ICAM-1 and VCAM-1 expression on MSCs. Transwell experiments revealed that for both directions, the interaction is mainly dependent on cell-cell contact or close proximity of MSCs and ILC3s. Addition of blocking antibodies against ICAM-1, VCAM-1, or their integrin ligands, did not affect ILC3 proliferation, suggesting that ILC3 stimulation is ICAM/VCAM independent. Soluble factors also contributed to the interaction, as ILC3s proliferated slightly better in the presence of MSC culture supernatant compared to IL-2 only. Based on experiments with blocking antibodies, we found IL-7 to be the likely candidate for this effect.
Summary/Conclusions: We show that via cell-cell contact and IL-7, MSCs promote the proliferation and IL-22 production by ILC3s *in vitro*, suggesting ILC3s may play a role in the control of GvHD upon MSC therapy.

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ABERRANT T CELL RESPONSES IN THE BONE MARROW MICROENVIRONMENT OF PATIENTS WITH POOR GRAFT FUNCTION AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Poor graft function (PGF) remains a life-threatening complication following allogeneic hematopoietic stem cell transplantation (allo-HSCT), and the underlying mechanisms have not yet been elucidated. Considerable evidence from murine studies has demonstrated that effective hematopoiesis depends on the specific bone marrow (BM) microenvironment, where hematopoietic stem cells reside. In this regard, we previously reported that PGF patients had impaired BM endosteal and vascular microenvironment (***BBMT*** 2013; ***BMT*** 2016; ***Oncotarget*** 2016; ***Blood*** 2016). Moreover, our pilot study found that both $CD4^+$ and $CD8^+$ T cells were polarized towards a type 1 immune response in the BM microenvironment of PGF patients (N=10) compared to those in matched good graft function (GGF) patients(N=20) (***BBMT*** 2016). Nevertheless, whether abnormalities of T cell subsets in the BM immune microenvironment, including Th17, Tc17, Th1, Tc1, Th2, Tc2 cells and regulatory T cells (Tregs), are involved in the pathogenesis of PGF remains to be explored.
Aims: To compare the T cell subsets in the BM immune microenvironment, including Th1, Tc1, Th2, Tc2, Th17, Tc17 cells and Tregs, between patients with PT and GGF after allo-HSCT.
Methods: This prospective nested case-control study enrolled 20 patients with PGF, 40 matched patients with good graft function (GGF) after allo-HSCT, and 20 healthy donors (HD). Th17, Tc17, Th1, Tc1, Th2, Tc2 cells, Tregs and their subsets were analyzed by flow cytometry. The study was approved by the Ethics Committee of Peking University People's Hospital, and written informed consent was obtained from all the patients before study-entry in accordance with the Declaration of Helsinki.
Results: The demographic and clinical characteristics were similar between allo-HSCT patients with PGF and those with GGF. The percentages of Th1 (37% *vs.* 26.4%, P=0.0005) and Tc1 (52.4% *vs.* 19%, P<0.0001) cells were significantly higher in PGF patients than in GGF patients, whereas the percentages of Th2 (0.8% *vs.* 2.4%, P<0.0001) and Tc2 (0.5% *vs.* 1.1%, P<0.0001) cells were markedly lower in the PGF group than in the GGF group. PGF patients showed significantly greater Th1 cell/Th2 cell (31.6 *vs.* 10.8, P<0.0001) and Tc1 cell/Tc2 cell ratios (108.8 *vs.* 18.4, P<0.0001) than those for GGF patients. Moreover, a significantly higher proportion of stimulated $CD4^+$ T cells that produced IL-17 (Th17) was found in the BM of PGF patients than in the BM of GGF patients and HD (3.7% *vs.* 1.6% *vs.* 1.1%, P<0.05), whereas the percentages of Tregs in PGF patients were comparable to those in GGF patients and HD, resulting in a dramatically elevated ratio of Th17 cells/Tregs in the BM of PGF patients relative to those in GGF patients (1.01 *vs.* 0.57, P=0.04).
Summary/Conclusions: The present study revealed that aberrant T cell responses in the BM immune microenvironment may be involved in the pathogenesis of PGF after allo-HSCT. These findings will facilitate the optimization of immune regulation strategies and improve the outcome of PGF patients post-allotransplant.

S450

HIGHER FREQUENCY OF SWITCHED MEMORY B CELLS PREDICTS THE INCIDENCE OF CHRONIC GRAFT-VERSUS-HOST DISEASE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: Aberrant B-cell homeostasis has been described in patients (pts) with chronic graft-versus-host disease (cGVHD) following allogeneic stem cell transplantation (allo-SCT). However, there is no information on the predictive value of specific B-cell subsets of the incidence of cGVHD.
Aims: We sought to determine if B-cell subsets measured around day 100 after allo-SCT predict the subsequent occurrence of cGVHD in a prospective clinical study.
Methods: Peripheral blood (PB) samples were obtained from consented patients (pts) between day 80 and 110 (D100) after allo-SCT at The University of Texas MD Anderson Cancer Center from 2012 to 2015. Only pts who had not been diagnosed with cGVHD or progression of underlying malignancy by D100 were eligible for this study. We analyzed CD19+CD20+ B cell subsets by FACS. Subsets were defined as naïve (CD27-IgD+), unswitched (CD27+IgD+) and switched (CD27+IgD-) memory cells. Receiver Operating Characteristic (ROC) curve was used to identify threshold levels of B cell % and numbers that predict the incidence of cGVHD. cGVHD diagnosis was based on the 2014 National Institutes of Health guidelines.
Results: A total of 80 pts were enrolled in the study. The median age at SCT was 49 years (range 21-75). The majority (80%) of pts received myeloablative conditioning, and 75% received tacrolimus with methotrexate or mycophenolate mofetil for GVHD prophylaxis. Diagnosis was myeloid (61%) or lymphoid (34%) malignancy in the majority of pts. Grafts source was primarily PB or bone marrow from matched- unrelated (61%) or related (24%) donors. Grade 2-4 acute GVHD had occurred in 45% of pts before D100. Thirty-six percent of pts were receiving steroids at D100. Forty-seven (59%) pts had detectable (≥0.1%) CD19+CD20+ signal on D100. In this subset, median B cell % was 3 (range 0.25-34) and median absolute number was 23 (1.8-419) $x10^3$ cells /μL. Median % naïve, unswitched, and switched B cells was 89% (54-99), 1.85% (0.3-8.5), and 2.1% (0-30), respectively. A total of 15 pts were diagnosed with cGVHD within 1 year after D100 including 11 with detectable B cells. ROC analysis did not identify predictive thresholds for overall B cell % or numbers. However, it identified predictive thresholds for each of the B cell subsets analyzed. The area under the curve (AUC) analysis indicated that % naïve (cutoff 87.3%) and % switched (cutoff 4%) B cells were the most significant (AUC 86%) predictors of the incidence of cGVHD. Rounding up the cutoff values, we grouped pts into 3 mutually exclusive groups: 1) naïve B cells ≥90% (n=23, none had >5% switched B cells), 2) naïve B cells <90% and switched B cells ≤5% (n=11), and 3) naïve B cells <90% and switched B cells >5% (n=13). Patients (n=33) with undetectable B cells were considered together as one group. The rate of cGVHD after D100 was significantly higher in pts with <90% naïve and >5% switched B cells (HR=7, p<0.001) with a 1-year cumulative incidence of 61% (Figure). None of the characteristics listed above were significantly associated with the rate of cGVHD. Percent naive and switched B cells did not correlate with receipt of steroids on D100. Patients with undetectable B cells were significantly more likely to have an underlying lymphoid *vs* myeloid malignancy (58% *vs* 33%, p< 0.001); and those with ≥90% naïve B cells were significantly more likely to be ≤55 years of age at the time of allo-SCT (83% *vs* 42%, p=0.004).

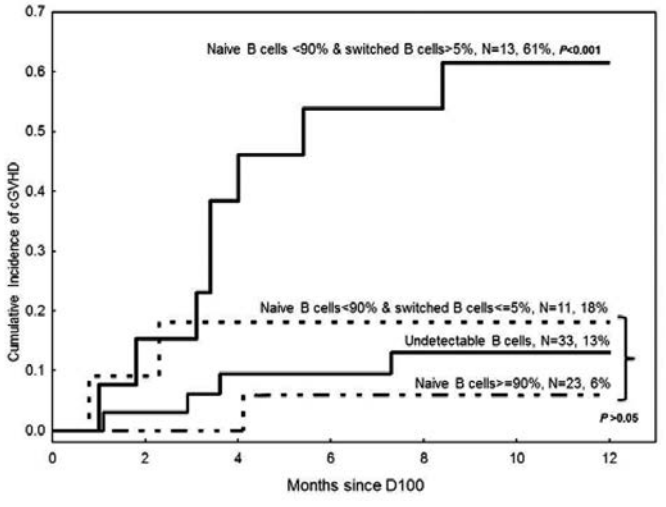

Figure 1.

Summary/Conclusions: In conclusion, D100 frequency of naïve and switched B cells predicts the subsequent development of cGVHD. Lymphoid malignancies and older age may be associated with aberrant B-cell reconstitution. Consideration of D100 B-cell subsets may improve risk stratification models for the development of cGVHD.

Sickle cell disease, enzymes

S451

EFFECTS OF AG-348, A PYRUVATE KINASE ACTIVATOR, IN PATIENTS WITH PYRUVATE KINASE DEFICIENCY: UPDATED RESULTS FROM THE DRIVE PK STUDY

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Background: Pyruvate kinase (PK) deficiency is a congenital hemolytic anemia caused by deficiency of the glycolytic enzyme, red cell PK (PK-R). AG-348 is an orally available, small molecule, allosteric activator of PK-R that activates the wild-type and a range of mutated PK-R enzymes *in vitro*, and increases PK-R activity and restores adenosine triphosphate levels in red blood cells from patients with PK deficiency *ex vivo*.
Aims: To report on preliminary efficacy and safety data from the use of AG-348 in the ongoing DRIVE PK study (NCT02476916), an open-label dose-ranging trial of AG-348 in transfusion-independent adults with PK deficiency.
Methods: After providing informed consent, patients were randomized to AG-348 50mg or 300mg orally twice daily (BID) for 6 months (Core Period). At the end of the Core Period, patients can continue on treatment for another 2 years in the Extension Period. Transfusion independence is defined as ≤3 units of red blood cells transfused in the 12 months preceding the first dose of AG-348 and no transfusions in the 4 months preceding the first dose. Patients are followed weekly for Weeks 1-3, every 3 weeks for Weeks 4-12, monthly for Weeks 13-24 and then every 3 months until the end of the study. Hormone and iron status are evaluated at Baseline, Week 12 and End of Core Period, and then every 6 months in the Extension Period.
Results: As of 18 Jan 2017, goal enrolment has been met and all 52 patients are evaluable for safety and efficacy; 24 have completed the Core Period and 23 are ongoing in the Core Period. Five patients discontinued from the Core Period, owing to adverse events (AEs) (n=2) or consent withdrawal (n=3). Of the 24 subjects who completed the Core Period, 21 entered the Extension Period and 20 are still on treatment; 1 was discontinued by the investigator. Patients are currently receiving doses ranging between <25mg BID and 300mg BID. As of the previous data cutoff date of 23 Sep 2016 (where N=34), AG-348 had an acceptable AE profile, with headache (44%), nausea (41%) and insomnia (38%) being the most frequently reported events. Serum levels of sex steroids measured at Baseline, Week 12 and Week 24 in the Core Period demonstrated increases in free and total testosterone, and decreases in estradiol and estrone, consistent with the known effect of aromatase inhibition by AG-348. Of the 32 patients evaluable for efficacy at 23 Sep 2016, 15 (47%) had a maximal increase in hemoglobin (Hb) >1 g/dL. Hb responses were seen across a range of four doses, and were rapid and sustained. For a subset of patients (n=8), the rate of glycolytic metabolism in peripheral blood samples was assessed before and after treatment, and a positive correlation was observed between increases in glycolytic flux through the PK-R pathway and increases in Hb. Updates on safety, clinical efficacy measures (including Hb levels) and genotype–response correlations will be provided.
Summary/Conclusions: AG-348 is a novel, first-in-class PK-R activator undergoing clinical testing in patients with PK deficiency. The ongoing DRIVE PK study has now met goal enrolment of 52 patients, and data from these patients will be available at the time of presentation. Chronic daily dosing with AG-348 is well tolerated and has demonstrated clinically relevant, durable increases in Hb across a range of doses from <25mg BID to 300mg BID. These data highlight the potential of AG-348 to be the first disease-altering treatment for patients with PK deficiency.

S452

STEM CELL TRANSPLANTATION IN PYRUVATE KINASE DEFICIENCY

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Background: Pyruvate kinase deficiency (PKD) is the most common glycolytic enzyme defect causing hereditary non-spherocytic hemolytic anemia. PKD does not have a specific curative treatment. Therefore treatment is mainly supportive, consisting of regular red blood cell transfusions, splenectomy and chelation therapy for iron overload. This does impact life expectancy and quality of life for affected patients. Hematopoietic allogeneic stem cell transplantation (HSCT) has the potential to cure the disease. However, there is little experience in applying HSCT in PKD and guidelines are not available. To date, only four cases of HSCT have been published. Thus, additional data are required to help the establishment of HSCT guidelines and support future strategies, such as gene therapy.
Aims: The aim of this study was to make a worldwide inventory of all cases of PKD that have been treated by HSCT, and to evaluate indication, procedures employed, and outcome.
Methods: This is an international case series. Queries were send to national and international databanks and to physicians involved in HSCT on PKD patients. The latter were asked to complete a questionnaire on disease characteristics, pre-transplant condition, transplant regimen and post-transplant outcome. Two additional cases were included from a recently published report (Kim. 2016. Bone Marrow Transplantation).

Table 1.

	Survivor	Non-survivor	P value
Age	7,5 *3,0* (0,8-41)	17,4 *15,2* (6-39)	0,036*
Asian origin	8/11 (72,7%)	0/5	0,026*
Splenectomy performed	3/11 (27,3%)	4/5 (80%)	0,106
mean Hb (mmol/L) (N=13)	3,7 *3,4* (2,8-4,9)	4,4 *4,3* (3,7-5)	0,112
Pre-transplant ferritin level (ng/ml) (n=12)	804 *771* (2066-1650)	2167 *675* (596-7026)	0,432
myeloablation	6/11 (54,5%)	4/5 (80%)	0,588
Conditioning techniques			
ATG	10/11 (90,9%)	4/5 (80%)	1
Cyclophosphamide	8/11 (72,7%)	2/5 (40%)	0,299
Fludarabine	9/11 (81,8%)	4/5 (80%)	1
Busulfan	6/11 (54,5%)	3/5 (60%)	1
Busilvex	0/11	1/5 (20%)	0,313
Thiothepa	3/11 (27,3%)	1/5 (20%)	1
Treosulfan	3/11 (27,3%)	1/5 (20%)	1
Thymoglobulin	0/11	1/5 (20%)	0,313
Graft type			0,507
MSD	2/11 (18,2%)	0/5	
MUD	6/11 (54,5%)	3/5 (60%)	
CORD	2/11 (18,2%)	0/5	
MFD	1/11 (9,1%)	2/5 (40%)	
Transplant source			0,333
Bone marrow	4/11 (36,4%)	4/5 (80%)	
Peripheral blood	5/11 (45,5%)	1/5 (20%)	
Cord blood	2/11 (18,2%)	0/5	
Transfusion free after transplant	10/11 (90,9%)	4/5 (80%)	
GvHD			0,015*
None	7/11 (63,6%)	0/5	
Grade 1	1/11 (9,1%)	0/5	
Grade 2	1/11 (9,1%)	0/5	
Grade 3	0/9	1/5 (20%)	
Grade 4	2/11 (18,2%)	4/5(80%)	
(descriptive statistics: mean-median (range) (N), frequencies number/total (percentage) *P=<0.05			

Results: From 1996 to 2016 a total of 16 PKD-patients were reported to have been treated by stem cell transplantation. Eight patients were treated in the EU and eight in Asian centres, respectively. No patient resulted to be transplanted in the US. Median age at transplantation was 6,5 years. (10 patients (62,5%) were <10 years; 6 (37,5%) >10 years), seven patients (43,8%) were splenectomized at the time of HSCT. Fifteen patients (94%) reached engraftment. The sixteenth patient showed mixed chimerism followed by spontaneous transition to full donor chimerism after splenectomy six months post transplantation. Two patients suffered from secondary graft loss. One of these had recovery to 91% donor chimerism after donor lymphocyte infusion. Outcome in the other patient is unknown. GvHD grade 4 was reported in 6/16 cases (38%). There was no obvious relation between GvHD prophylaxis or any other clinical factors and the occurrence of GvHD grade 2-4 in our patients. Two-year cumulative survival was 74%. Two patients did not reach the two-year milestone yet. All five patients who did not survive died of transplant-related causes. Patients who did not survive were significantly older (p=0.036) and were all treated in a European center (p=0.026) (see Table). Also, they had suffered more often from GvHD grade 2-4 (p=0.031). Nine out of ten patients (90%) younger than ten years old survived transplantation, whereas two out of six (33.3%) patients older than ten survived. Patients younger than ten years old were less often splenectomized (p=0.001). All Asian patients (8/8) survived transplantation, whereas three out of eight European patients survived. Patients treated in Asian hospitals differed from European patients in that they were younger (p=0.001), less often splenectomized (p=0.041) and had a lower ferritin level prior to transplantation (p=0.048). They were more often transplanted with peripheral blood stem cells (p=0.014) and more often conditioned on a cyclophosphamide (p=0.007) regimen.
Summary/Conclusions: This is the first study on outcome of HSCT in PKD patients. Due to the still relatively small number of cases no definite conclusions on the safety of HSCT in PKD can be drawn. However, we observed a better survival for patients transplanted before the age of ten. This difference could also explain difference in survival between patients transplanted in Europe *versus* Asia. The high rate of severe GvHD in this cohort is a reason for concern. The strong decline in survival of patients older than ten years of age indicates the need for very careful selection of HSCT-candidates.

S453

HEREDITARY XEROCYTOSIS: CLINICAL AND BIOLOGICAL PRESENTATION AT DIAGNOSIS IN A RETROSPECTIVE SERIES OF 103 PATIENTS

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Background: Dehydrated hereditary stomatocytosis, also called hereditary xerocytosis (HX), is a dominant non-spherocytic chronic hemolytic anemia characterized by an increased leak of monovalent cations through the red cell membrane leading to dehydration and a shortened red cell survival. HX is difficult to diagnose because of its rarity and the heterogeneity in its clinical presentation.
Aims: Our study aims to characterize the clinical and biological features at HX diagnosis in a retrospective multicentric series of 103 patients from 49 families.
Methods: HX diagnosis was based on the typical left-shifted curve of osmolar gradient ektacytometry performed at CHU Bicêtre from 1993 to 2016. All patients were from European origin. They were referred to our center for: chronic non-spherocytic hemolysis (30), thrombotic events after splenectomy (8), hyperferritinemia with hemolytic features (5), unexplained perinatal oedema (6) or family study (54) after diagnosis in a first-degree probands. PIEZO1 and KCNN4 were analyzed by Sanger sequencing of the exons and intron-exon junctions.
Results: Clinical features: Most of HX diagnosis was made in adults (median age 31.5 years, range 0-88), children less than 10 years representing only 21% of all cases. 71 patients (69%) were already followed for unexplained hemolysis. Hyperferritinemia and/or a chelation therapy were noticed for 26 patients among the 55 for whom this data was available (47%). 19 patients were treated for iron overload: phlebotomy (14) and/or Deferasirox (6) and/or Deferoxamine (6) and/or Deferiprone (1). A perinatal edema history was noted in 17 (16,5%) patients. A history of thrombotic complication was reported in 12 (11,6%) patients, corresponding to a total number of 17 thrombotic events including post-embolic pulmonary hypertension (2), arterial events (3), pulmonary embolism (4), portal thrombosis (4), splenic infarcts (2) and deep venous throm-

bosis (2). 10 among these 12 patients (83%) were splenectomized, confirming the very high risk of thrombosis after splenectomy in HX. Two patients underwent a thrombotic event without splenectomy: one cerebral stroke and one splenic infarct.

Biological features: The median hemoglobin level was in the normal range: 135±19 g/L (range 71-195) with a slight macrocytosis (median MCV: 99±8fL) and a marked reticulocytosis (median reticulocyte count: 252±141G/L). Of note, 57 patients (55%) presented a totally compensated hemolysis with a hemoglobin level above 115g/L. MCHC was in the normal range (median 35±1,3g/dL) but was above 36 g/dL for 28 (27,1%) patients. Stomatocytes were noticed on the blood smear in 42 patients over 70 available, numbered as rare (19%), few (60%) or numerous (21%). Genetics could be performed in 45 subjects from 22 distinct families. At least one *PIEZO1* mutation was identified in very affected subjects. No *KCNN4* mutations were found in these typical ektacytometric forms of HX.

Summary/Conclusions: This work represents the largest HX series and highlights the important heterogeneity in the clinical features at diagnosis. One important finding is that most patients were not anemic and presented a compensated hemolysis. In a significant percentage of cases, diagnosis was made in the exploration of extra hematological features including perinatal edema or hemochromatosis occurring despite the absence of any red blood cells transfusion. Moreover, we confirmed the very high risk of thrombotic events after splenectomy, underlining the absolute necessity of formally eliminating HX in any unexplained chronic hemolysis each time splenectomy is considered.

S454

CRIZANLIZUMAB, A P-SELECTIN INHIBITOR, INCREASES THE LIKELIHOOD OF NOT EXPERIENCING A SICKLE CELL-RELATED PAIN CRISIS WHILE ON TREATMENT: RESULTS FROM THE PHASE II SUSTAIN STUDY

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Background: Sickle cell-related pain crises (SCPCs) are a substantial cause of morbidity in patients with sickle cell disease (SCD). At a dose of 5.0mg/kg, the P selectin inhibitor crizanlizumab, delivered intravenously every 4 weeks after loading, was shown to significantly reduce the frequency of SCPC events and was well tolerated in the 52-week SUSTAIN study (Ataga KI *et al. N Engl J Med* 2017;376:429-439).

Aims: This *post-hoc* analysis evaluated patients who did not experience a SCPC for the duration of the trial.

Methods: SUSTAIN was a randomized, double-blind, placebo-controlled, Phase II study (NCT01895361). Patients aged 16–65 years with SCD (including HbSS, HbSC, HbSβ^0–thalassaemia, and HbSβ$^+$–thalassaemia genotypes) and 2-10 SCPC events in the previous 12 months were included. Concomitant use of hydroxyurea (HU) was permitted if the patient had been using it for ≥6 months and at a stable dose for ≥3 months. Patients were randomized 1:1:1 to receive intravenous crizanlizumab 5.0mg/kg, 2.5mg/kg or placebo. Loading doses were administered on days 1 and 15, followed by routine treatment every 4 weeks to week 50, with the final assessment visit at week 52. Descriptive statistics were used to summarize the frequency of patients who were SCPC event-free for the duration of the study, based on the intent-to-treat (ITT) population overall and by prior SCPC events, SCD genotype and HU use at baseline.

Table 1.

Table. SCPC event-free patients by prior SCPC events, genotype and HU use

	Crizanlizumab 5.0 mg/kg N=67	Crizanlizumab 2.5 mg/kg N=66	Placebo N=65
SCPC events in the year prior to study			
2–4	17/42 (40.5)	10/41 (24.4)	10/41 (24.4)
5–10	7/25 (28.0)	2/25 (8.0)	1/24 (4.2)
Genotype			
HbSS	15/47 (31.9)	9/47 (19.1)	8/47 (17.0)
Other	9/20 (45.0)	3/19 (15.8)	3/18 (16.7)
HU use			
Yes	14/42 (33.3)	9/41 (22.0)	7/40 (17.5)
No	10/25 (40.0)	3/25 (12.0)	4/25 (16.0)

All data are n/N (%)

Results: Among the 198 patients included in the study (ITT population), 62.6% and 37.4% had experienced 2-4 and 5-10 SCPC events in the previous year, respectively, and 62.1% were taking HU at baseline. HbSS was the most common genotype (71.2%; HbSC: 16.2%, HbSβ^0–thalassemia: 6.1%, HbSβ$^+$–thalassemia: 5.1%, other: 1.5%). Overall, more patients in the crizanlizumab 5.0mg/kg group (n=24/67; 35.8%) were SCPC event-free than in the 2.5mg/kg (n=12/66; 18.2%) and placebo (n=11/65; 16.9%) groups. In each of the prior SCPC events, SCD genotype and HU use subgroups, a greater proportion of patients treated with crizanlizumab 5.0mg/kg were SCPC event-free compared with those in the crizanlizumab 2.5mg/kg or placebo arms (Table 1). In subpopulations considered to be at increased risk of experiencing a SCPC (patients with 5-10 SCPC events in the previous year and/or with the homozygous HbSS genotype), a higher proportion of patients treated with crizanlizumab 5.0mg/kg were SCPC event-free compared with those in the placebo arm (28.0% *vs* 4.2% and 31.9% *vs* 17.0%, respectively). Additionally, 33.3% of patients who were taking HU and treated with crizanlizumab 5.0mg/kg were SCPC event-free during the study, compared with 17.5% in the placebo arm, possibly suggesting an additive effect.

Summary/Conclusions: Treatment with crizanlizumab 5.0mg/kg appears to increase the likelihood of adult patients with SCD being SCPC event-free while on treatment, even in high-risk subpopulations. Crizanlizumab 5.0mg/kg was also effective in those who had experienced at least two SCPCs in the previous year despite taking HU, suggesting that this dose is effective as a disease-modifying agent that meets an unmet medical need.

S455

FREE IRON IN SERA OF PATIENTS WITH SICKLE CELL DISEASE CONTRIBUTES TO THE RELEASE OF NEUTROPHIL EXTRACELLULAR TRAPS

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Background: Chronic hemolysis is a hallmark of sickle cell disease (SCD). Hemolysis in SCD has been associated with elevated levels of heme in the circulation of both human patients and SCD mice. It was shown that TNF-α treatment induces experimental vaso-occlusive crisis (VOC) in SCD mice, associated with the formation of neutrophil extracellular traps (NETs) as shown by staining of lung sections (Chen *et al. Blood* 2014). In addition, the administration of DNase I to degrade NETs led to improved survival. Furthermore, it was shown that plasma from SCD patients obtained during SCD crisis induced NET formation by TNF-α primed neutrophils. Free heme was suggested to mediate the formation of NETs in SCD, as treatment of SCD mice with the heme-binding protein hemopexin (Hpx) to scavenge free heme led to reduced NET formation (Chen *et al. Blood* 2014).

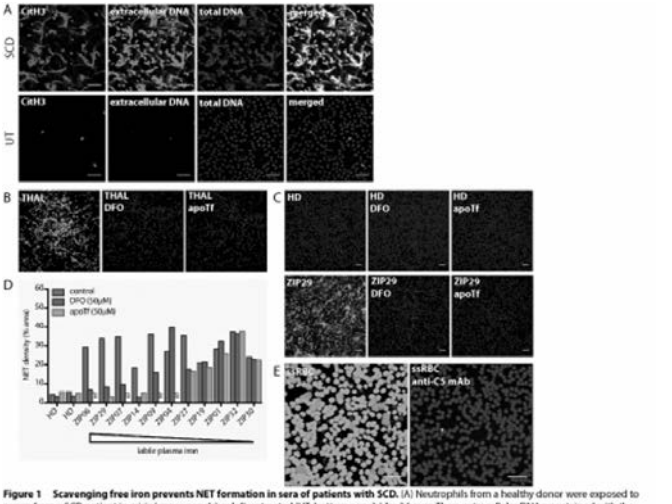

Figure 1 Scavenging free iron prevents NET formation in sera of patients with SCD. (A) Neutrophils from a healthy donor were exposed to serum from a SCD patient in crisis (upper panels) or left untreated (UT, bottom panels) for 3 hours. Then, extracellular DNA was stained with the nonpermeable DNA-binding dye Sytox Green (green). Immunostaining for citrullinated histone H3 (CitH3, red) confirmed the presence of NETs. Cells were counterstained with the cell permeable DNA-binding dye Hoechst 33342 (blue). Merged images (right panels) show co-staining of extracellular DNA with CitH3 when neutrophils were exposed to SCD serum. Scale bar, 50 μm. (B) Neutrophils from a healthy donor were exposed to serum from a thalassemia patient in the presence or absence of deferoxamine (DFO, 50 μM) or apotransferrin (apoTf, 50 μM). Release of extracellular DNA (green in these images) was visualized with confocal fluorescence microscopy using 2 DNA-binding dyes, one cell impermeable (Sytox Green) and the other cell permeable (Hoechst 33342, blue). (C) Neutrophils from a healthy donor were exposed to SCD serum in the presence or absence of DFO or apoTf. Release of extracellular DNA was visualized as in (B). Scale bar, 50 μm. (D) In 3 independent experiments, neutrophils from a healthy donor were exposed to sera of patients with SCD in the presence or absence of DFO or apoTf. NET formation was detected as outlined in (B) and the densities of released DNA were determined after the indicated treatments. Levels of circulating labile iron were measured as indicated in the figure. (E) Neutrophils from a healthy donor were challenged with sickled RBCs in the presence or absence of an anti-C5 blocking monoclonal antibody (80 μg/ml). Release of extracellular DNA (green in these images) was followed over time with live cell imaging over 3 hours. Depicted are images after 3 hours of incubation. Scale bar, 50 μm.

Figure 1.

Aims: To verify the potential therapeutic use of Hpx administration to block NET formation and the occurrence of VOC in human SCD, we aimed to deter-

mine whether *ex vivo* Hpx addition to human SCD sera would prevent NET formation.
Methods: Patient serum and plasma samples were obtained from 32 incidents of VOC in 24 adult SCD patients, with informed consent. Moreover, steady state samples were obtained at least 4 weeks after discharge from the hospital. Patients having had a blood transfusion in the 3 months prior to admission were excluded. NET formation by human neutrophils from healthy donors was studied using confocal fluorescence microscopy and staining for extracellular DNA with the cell nonpermeable dye Sytox Green. The presence of extracellular DNA that stains positive for citrullinated histone H3 confirmed the formation of NETs (Figure 1A).
Results: Indeed, we found that hemin (ferriprotoporphyrin IX) activated neutrophils to generate reactive oxygen species and release NETs, which was prevented by addition of plasma-derived Hpx. Moreover, exposure of neutrophils to sera from patients with SCD promoted NET formation, which was significantly enhanced during VOC. However, we observed that circulating free heme levels were elevated in SCD patient serum irrespective of disease state, and serum concentrations of Hpx were reduced in both VOC and steady state compared to healthy donor serum. Strikingly, addition of Hpx in supraphysiological concentrations failed to prevent the formation of NETs in all SCD sera tested. We and others (Chen *et al. Blood* 2014) have found that, in contrast to hemin, protoporphyrin IX does not trigger NET formation, revealing that the iron atom is required for the release of NETs. This observation led us to investigate whether free iron may directly induce NET formation. When neutrophils were exposed to Fe-NTA or serum from a thalassemia patient with iron overload, NETs were formed. Scavenging of free iron by addition of the iron-chelator deferoxamine or the specific iron-binding protein apotransferrin prevented NET release (Figure 1B). Moreover, we found that sequestration of free iron prevented NET formation induced by a subset (6 out of 11 tested), but not all, sera of patients with VOC (Figure 1C and D). In addition, sickled red blood cells (RBCs) are known to bind to neutrophils in vitro. Here, we found that neutrophils released NETs in response to sickled RBCs, even in the presence of Hpx. By contrast, blocking of complement C5 activation completely prevented the formation of NETs when neutrophils were exposed to sickled RBCs (Figure 1E).
Summary/Conclusions: In summary, we observed that sequestration of free iron with the iron-chelator deferoxamine or the iron-binding protein apotransferrin was effective in more than half of the SCD patient sera, while the binding of heme to Hpx did not prevent NET release in human SCD patient sera. Therefore, we propose that targeting free iron with these iron binding compounds may be explored therapeutically to prevent or treat VOC development in SCD. Finally, complement activation in the presence of sickled RBCs activates neutrophils to release NETs, which may also contribute to VOC and SCD pathogenesis. Therefore, anti-C5 IgG may represent an alternative therapeutic strategy to prevent VOC in SCD.

New drugs for rescue in relapsed/refractory multiple myeloma

S456

PHASE 3 ELOQUENT-2 STUDY: EXTENDED 4-YEAR FOLLOW-UP OF ELOTUZUMAB PLUS LENALIDOMIDE/DEXAMETHASONE *VS* LENALIDOMIDE/DEXAMETHASONE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background: Elotuzumab is an immunostimulatory monoclonal antibody that targets SLAMF7, a glycoprotein highly expressed on multiple myeloma (MM) cells and natural killer cells. Elotuzumab exerts a dual effect, directly activating natural killer cells and mediating MM cell death via antibody-dependent cell-mediated cytotoxicity. In a 3-year follow-up of ELOQUENT-2 (NCT01239797), elotuzumab plus lenalidomide/dexamethasone (ELd) demonstrated a sustained 27% reduction in the risk of disease progression/death and an overall survival (OS) trend towards benefit compared with lenalidomide/dexamethasone (Ld) alone in patients with relapsed/refractory (RR) MM (Dimopoulos et al, ASH 2015).
Aims: To evaluate the long-term efficacy and safety of ELd following extended 4-year follow-up (median 46 months).
Methods: RRMM patients with 1-3 prior lines of therapy randomized 1:1 received ELd or Ld in 28-day cycles until disease progression/unacceptable toxicity or consent withdrawal. Co-primary endpoints were progression-free survival (PFS) and overall response rate (ORR); OS was a secondary endpoint (analysis not prespecified for this data cut) and safety an exploratory endpoint. Written informed consent was obtained for all patients.

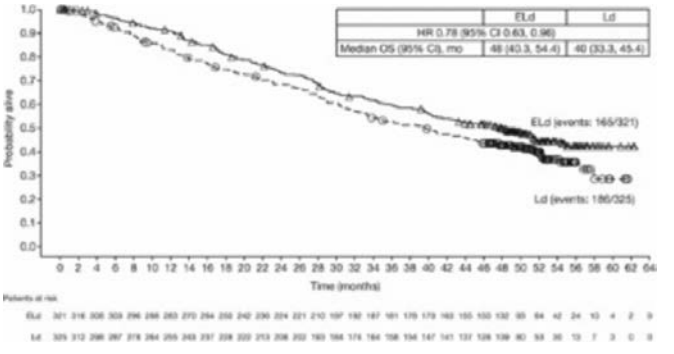

Figure 1. OS Kaplan-Meyer Curve (all randomized patients).

Results: In total, 646 RRMM patients were randomized: 321 to ELd and 325 to Ld. At 4-year follow-up (data cut-off: Oct 18, 2016), nearly twice as many patients remained on ELd therapy *vs* Ld (17% *vs* 9%). With the extended follow-up, ELd demonstrated a sustained relative improvement of 50% in PFS rates *vs* Ld (21% *vs* 14%) and maintained reduction in the risk of progression/death of 29% for ELd *vs* Ld (all randomized patients: HR 0.71; 95% CI 0.59, 0.86). Patients with ≥very good partial response (VGPR) (ELd 112 [35%] *vs* Ld 95 [29%]) had the greatest reduction (35%) in risk of progression/death (HR 0.65; 95% CI 0.46, 0.94). ORR was greater with ELd *vs* Ld (79% *vs* 66%) and the duration of response benefit was maintained over time (HR 0.77; 95% CI 0.62, 0.95). Early separation of the Kaplan–Meier survival curves, which remained consistently separated over time, supports a sustained OS benefit in favor of ELd *vs* Ld (Figure). Grade 3-4 adverse events (AEs) in ≥5% of patients were generally comparable between ELd and Ld arms-vascular diseases (10% *vs* 8%; mostly venous-related), second primary malignancies (SPMs; 9% *vs* 6%) and cardiac disorders (5% *vs* 8%); the exception was a

slightly higher incidence of infection with ELd (33% *vs* 26%). Overall rate (any grade) of infection (84% *vs* 75%) and SPMs (17% *vs* 11%) was also higher for ELd *vs* Ld. However, exposure to ELd was longer than to Ld (median [Q1, Q3] treatment cycles: 19 [9, 42] *vs* 14 [6, 25]). Disease progression and infection were major causes of mortality in both arms; however, fewer deaths were reported with ELd *vs* Ld (165 *vs* 186).
Summary/Conclusions: At 4 years, ELd has the longest median follow-up of an immuno-oncology agent in MM. The data continue to show that adding elotuzumab to Ld results in durable long-term responses, clinically relevant improvement in PFS, sustained reduction in risk of progression/death, and a survival trend in favor of ELd. Overall, these data continue to support the durable efficacy of ELd. Updated safety and tolerability, including rate of SPMs, was consistent with previous findings despite longer exposure, with minimal incremental AEs compared with Ld therapy.

Study funding: BMS. Writing support: C Tomas, Caudex, funded by BMS.

S457

A PHASE IB STUDY OF ISATUXIMAB PLUS POMALIDOMIDE (POM) AND DEXAMETHASONE (DEX) IN RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM)

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Background: Isatuximab (ISA) is an anti-CD38 monoclonal antibody, which kills tumor cells via multiple mechanisms. Here, we report preliminary data from the dose-escalation cohorts, and the first 3 patients (pts) of the expansion cohort, of a Phase 1b study of ISA plus Pom/Dex in pts with RRMM (NCT02283775).
Aims: To evaluate combination therapy with ISA plus Pom/Dex in pts with RRMM.
Methods: Pts with RRMM (≥2 prior MM therapies, including lenalidomide and a proteasome inhibitor) were sequentially enrolled to ISA 5, 10, or 20mg/kg (4 weekly doses, then every 2 wks until disease progression or intolerable toxicity) with Pom 4mg (Days 1–21) and Dex 40mg (Days 1, 8, 15, and 22; 20mg if ≥75 yrs old), in 28-day cycles. An expansion cohort was initiated at ISA 10mg/kg (plus Pom/Dex) based on preliminary safety, efficacy, and PK data. Primary objective: determine maximum tolerated dose (MTD). All patients were required to provide informed consent.
Results: 26 pts were analyzed (5mg/kg [n=8]; 10mg/kg [n=12]; 20mg/kg [n=6]), median age 65 (42-80) yrs. Median 4.0 (2-11) prior treatment regimens, with 20 (77%) pts refractory to prior immunomodulatory drug therapy. At data cutoff (Nov 8, 2016), median duration of ISA treatment was 19.0 wks and 16 pts remained on treatment. 2 pts at 10mg/kg discontinued therapy due to adverse events (AEs) (grade [Gr] 5 perforated bowel; Gr 3 infusion-associated reaction [IAR]). Dose-limiting toxicities reported in 1 pt at each dose level (Gr 4 neutropenia; Gr 4 neutropenic infection; Gr 3 confusional state), and MTD has not been reached. Most common TEAEs, besides IARs, were fatigue (62%), diarrhea (35%), and dyspnea (31%). Most frequent Gr 3/4 hematologic abnormality (laboratory assessment) was neutropenia (Gr 3, 40%; Gr 4, 52%). Gr 3/4 thrombocytopenia was reported in 8 (32%) pts (Gr 3, 16%; Gr 4, 16%). IARs occurred in 12 (46%) pts (Gr ≥3 in 1 pt); only with 1st infusion in 9/12 pts. 16 (62%) pts achieved at least PR (5, 8, and 3 pts at 5, 10, and 20mg/kg), including 1 CR, 8 VGPR, and 7 PR. Clinical benefit rate (≥MR) was 73%. Median time to 1st response, 4.2 wks; median duration of response, 25.6wks. The PK parameters of ISA were not affected by co-administration with Pom/Dex.
Summary/Conclusions: The combination of ISA and Pom/Dex was manageable and clinically active in heavily pretreated RRMM. A Phase III trial of this combination is ongoing.

S458

OVERALL SURVIVAL OF PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA TREATED WITH CARFILZOMIB AND DEXAMETHASONE *VERSUS* BORTEZOMIB AND DEXAMETHASONE IN THE RANDOMIZED PHASE 3 ENDEAVOR TRIAL

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Background: In a phase 3 head-to-head comparison of two proteasome inhibitors (PIs) in patients with relapsed or refractory multiple myeloma (RRMM), ENDEAVOR, progression-free survival (PFS) was shown to be significantly longer with carfilzomib and dexamethasone (Kd) than with bortezomib and dexamethasone (Vd) (median 18.7 months *vs* 9.4 months, hazard ratio [HR], 0.53; 95% confidence interval [CI], 0.44-0.65; *P*<0.0001; Dimopoulos MA *et al. Lancet Oncol*. 2016;17:27-38).
Aims: Results from a planned second interim overall survival (OS) analysis of ENDEAVOR are presented here.
Methods: Patients who had RRMM and had received 1–3 prior lines of therapy were randomized in a 1:1 ratio to receive Kd or Vd. In the Kd arm, carfilzomib was given on days 1, 2, 8, 9, 15, and 16 (20mg/m^2 on days 1, 2 of cycle 1; 56mg/m^2 thereafter) and dexamethasone 20mg was given on days 1, 2, 8, 9, 15, 16, 22, and 23 of 28-day cycles. In the Vd arm, bortezomib (1.3mg/m^2) was given intravenously or subcutaneously on days 1, 4, 8, and 11 and dexamethasone 20mg was given on days 1, 2, 4, 5, 8, 9, 11, and 12 of 21-day cycles. Patients were treated until progression or withdrawal of consent. OS was compared between treatment arms using a stratified log-rank test.
Results: The median treatment duration was 48 weeks for carfilzomib (N=464) and 27 weeks for bortezomib (N=465), with a median follow up of 38 months for Kd and 37 months for Vd. The median OS (95% CI) was 47.6 (42.5-NE) months in the Kd arm and 40.0 (32.6-42.3) months in the Vd arm, and all-cause mortality was significantly reduced with Kd *vs* Vd (HR, 0.791; 95% CI, 0.648-0.964; 1-sided *p*=0.0100). The overall survival benefit was consistent regardless of prior bortezomib therapy (HR 0.75 for Kd *vs* Vd, no prior bortezomib; HR 0.84 for Kd *vs* Vd, prior bortezomib) and across all age groups (HR, 0.85 <65 yo; 0.71, 65-74 yo; 0.84, ≥75 yo), baseline ECOG performance status groups (HR, 0.81, ECOG 0; 0.80, ECOG 1; 0.50, ECOG 2), cytogenetic risk groups (HR, 0.83, high risk; 0.85, standard risk), and number of prior lines of therapy (HR, 0.83, 1 prior line; 0.76, 2-3 prior lines). The most frequent any-grade adverse events in the Kd arm were (Kd *vs* Vd) anemia (42.5% *vs* 28.3%), diarrhea (36.3% *vs* 40.6%), pyrexia (32.4% *vs* 15.4%), dyspnea (32.2% *vs* 13.6%), fatigue (32.2% *vs* 30.7%), and hypertension (32.2% *vs* 9.9%). Grade 3 or higher adverse events were experienced by 81.4% of patients in the Kd arm and 71.1% of patients in the Vd arm.
Summary/Conclusions: ENDEAVOR was the first randomized phase 3 trial to directly compare two different PIs in RRMM. Patients who received Kd had significantly longer OS compared with patients who received Vd. Safety results were comparable with those previously reported in the PFS interim analysis for ENDEAVOR.

S459

EFFICACY AND SAFETY OF DARATUMUMAB, BORTEZOMIB AND DEXAMETHASONE (DVD) *VERSUS* BORTEZOMIB AND DEXAMETHASONE (VD) IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA (RRMM): UPDATED ANALYSIS OF CASTOR

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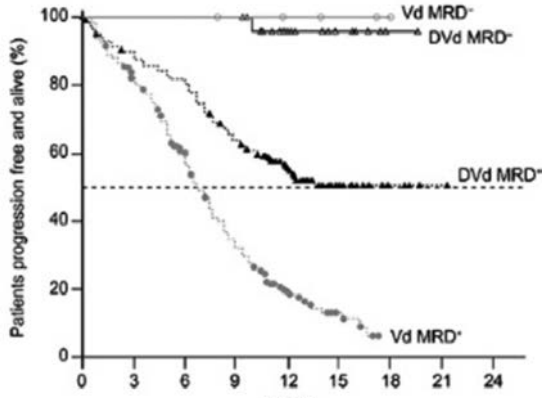
Background: Daratumumab is a human monoclonal antibody targeting CD38 that induces deep and durable responses with significant clinical benefit and is well tolerated as monotherapy and in combination with established standard-of-care regimens in patients with RRMM.
Aims: To provide updated efficacy and safety data from CASTOR, a multicenter, phase 3, randomized, active-controlled study of DVd *vs* Vd in RRMM.
Methods: Eligible patients with ≥1 prior line of therapy were randomly assigned to 8 cycles (every 3 weeks) of Vd (1.3mg/m^2 SC bortezomib on Days 1, 4, 8,

and 11; 20mg PO/IV dexamethasone on Days 1-2, 4-5, 8-9, and 11-12) with or without daratumumab (16mg/kg IV once weekly in Cycles 1-3, every 3 weeks for Cycles 4-8, then every 4 weeks until progression). Patients who were refractory to bortezomib were excluded. Progression-free survival (PFS) was the primary endpoint. Minimal residual disease (MRD) was assessed at suspected complete response (CR) and at 6 and 12 months after first dose at 3 sensitivity thresholds (10^{-4}, 10^{-5}, and 10^{-6}) using the ClonoSEQ™ next-generation sequencing (NGS)-based assay (Adaptive Biotechnologies, Seattle, WA).

Results: A total of 498 patients were randomized with median (range) age of 64 (30-88) years. Patients received a median (range) of 2 (1-10) prior lines of therapy; 66% of patients previously received bortezomib, and 21% were refractory to lenalidomide in their last prior line of therapy. After median follow-up of 13.0 months, DVd significantly prolonged PFS compared with Vd alone (median: not reached *vs* 7.1 months; hazard ratio [HR], 0.33; 95% confidence interval [CI], 0.26-0.43; *P*<0.0001). Twelve-month PFS rates were 60% *versus* 22%, respectively. Significant PFS benefit was observed with DVd over Vd regardless of the number of prior lines of therapy, although the greatest benefit was seen in patients with 1 prior line of therapy (median: not reached *vs* 7.9 months; HR, 0.22; 95% CI, 0.14-0.34; *P*<0.0001). Overall response rate (ORR; 84% *vs* 63%) and rates of very good partial response (VGPR) or better (62% *vs* 29%) and CR or better (26% *vs* 10%) continued to be significantly higher with DVd compared with Vd (*P*<0.0001 for all). MRD-negative rates were more than 4 times higher at all 3 sensitivity thresholds with DVd *versus* Vd: 18.3% *versus* 3.6% at 10^{-4}(*P*<0.0001), 10.4% *versus* 2.4% at 10^{-5} (*P*<0.01), and 4.4% *versus* 0.8% at 10^{-6} (*P*<0.01). MRD-negative patients had prolonged PFS compared with MRD-positive patients at 10^{-5}sensitivity threshold (**Figure**). At the clinical cut-off date, 37 (15%) deaths in the DVd group and 58 (24%) in the Vd group have been observed (HR, 0.63; 95% CI, 0.42-0.96), and follow up is ongoing. Thrombocytopenia was the most common grade 3 or 4 treatment-emergent adverse event (45% with DVd *vs* 33% with Vd). No new safety signals were reported after median treatment duration of 11 months with daratumumab. Updated efficacy and safety data with longer follow up will be presented at the meeting.



No. at risk									
Vd MRD negative	6	6	6	5	3	2	0	0	0
DVd MRD negative	26	26	26	26	15	7	1	0	0
Vd MRD positive	241	176	123	68	20	7	0	0	0
DVd MRD positive	225	189	172	134	76	26	4	1	0

Figure 1.

Summary/Conclusions: DVd is superior to Vd in terms of PFS, ORR, depth of response, and MRD-negative rates, with no new safety signals reported. These updated data further support the use of DVd as a standard of care in RRMM, with the greatest benefit observed in patients with 1 prior line of therapy.

S460

A PHASE 1B STUDY OF VENETOCLAX COMBINED WITH BORTEZOMIB AND DEXAMETHASONE IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background: Venetoclax (VEN) is a potent, selective, orally bioavailable small-molecular inhibitor of BCL-2. When combined, VEN can enhance the activity of bortezomib in multiple myeloma (MM) cell lines and xenograft models.

Aims: The objectives of the study are to evaluate safety and preliminary efficacy of VEN with bortezomib and dexamethasone in relapsed/refractory (RR) MM.

Methods: Phase 1b study of patients (pts) with R/R MM who received daily VEN (50-1200mg for dose escalation cohorts; 800mg in safety expansion) with standard bortezomib (1.3mg/m^2 SC) and dexamethasone (20mg PO).

Results: As of 19Aug2016, 66 pts were enrolled. Median age was 64 years; 9 (14%) pts had t(11;14), 5 (8%) had t(4;14), 15 (23%) had del(17p), and 30 (45%) had del(13q) abnormalities. Median number of prior therapies was 3 (range: 1-13), with 39% of pts refractory to prior bortezomib, 14% to carfilzomib, 53% to lenalidomide, and 21% to pomalidomide. Median time on study was 5.9 months (range: 0.3–29.8). Forty-six (70%) pts discontinued, with 36 due to disease progression (PD). Common AEs in ≥30% of pts were diarrhea (46%), constipation (41%), thrombocytopenia (39%), nausea (38%), peripheral neuropathy (33%), and insomnia (32%). Common grade 3/4 AEs in ≥10% of pts were thrombocytopenia (29%), anemia (15%) and neutropenia (14%). Serious AEs in ≥2 pts were febrile neutropenia, thrombocytopenia, cardiac failure, pyrexia, influenza, lower respiratory tract infection, pneumonia, sepsis, acute kidney injury, respiratory failure, embolism, and hypotension. Dose-limiting toxicities were grade 3 cardiac failure in the 300mg cohort (possibly related to dexamethasone) and grade 3 thrombocytopenia during the first cycle in the safety expansion. No events of laboratory or clinical TLS were reported. Four deaths were due to PD and 1 due to respiratory syncytial virus infection. Overall response rate (ORR) for all pts was 67% (44/66); 28 (42%) pts achieved very good partial response (VGPR) or better (3 stringent complete response [sCR], 10 CR, 15 VGPR). Pts non-refractory to prior proteasome inhibitors (PI) or immunomodulatory drugs (IMiDs) had higher ORR than refractory pts (PI, 92% *vs* 32%; IMiDs, 82% *vs* 57%). Among pts refractory to any 2 or more (n=15), 3 or more (n=7), or all 4 (n=4) prior therapies (bortezomib, carfilzomib, lenalidomide, pomalidomide), ORR was 40%, 43%, and 25%, respectively. Median time to progression (~10 *vs* 3 months) and duration of response (~10 *vs* 7 months) were longer for pts not refractory to any of these therapies *versus* refractory pts. ORR for pts with or without cytogenetic abnormalities, respectively, was as follows: 78% *vs* 65% for t(11;14), 60% *vs* 67% for t(4;14), 47% *vs* 73% for del(17p), and 63% *vs* 69% for del(13q).

Summary/Conclusions: VEN combined with bortezomib and dexamethasone has an acceptable safety profile with promising anti-myeloma activity, and the highest response rates were observed in R/R MM pts who were not refractory to PI or IMiDs. These data support the ongoing phase 3 trial with this regimen in R/R MM.

Improving prognostication and front-line therapy in chronic lymphocytic leukemia

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CYTOGENETIC COMPLEXITY IN CHRONIC LYMPHOCYTIC LEUKEMIA: DEFINITIONS, ASSOCIATIONS WITH OTHER BIOMARKERS AND CLINICAL IMPACT; A RETROSPECTIVE STUDY ON BEHALF OF ERIC

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Background: Recent evidence suggests that complex karyotype (CK) identified by chromosome banding analysis (CBA) may be a relevant biomarker for treatment decisions in CLL, especially regarding the response to signaling inhibitors. However, many challenges towards routine clinical application of CBA still need to be overcome.
Aims: Reappraisal of definitions for CK in CLL and systematic investigation of clinicobiological associations and prognostic impact.
Methods: 3580 CLL and monoclonal B-cell lymphocytosis (MBL) patients (CLL=3322, 93% and MBL=258, 7%, respectively) were analysed with CpG-oligodeoxynucleotides/interleukin 2 (CPG/IL2, n=379, 11%), phorbol-12-myristate-13-acetate (TPA, n=1846, 52%) or both (n=1355, 37%). CBA was mostly performed within the first year from diagnosis and before treatment administration (79% and 88%, respectively). Main features of the studied cohort: median age: 65.6 years/ males: 2252 (63%)/ Binet A/B/C: 2356/357/258 (79%/12%/9%: MBL and Binet A were grouped together)/ IG-unmutated CLL (U-CLL): 829/2051 (40%)/del(13q), 1769/3271 (54%)/ trisomy 12, 507/3260, 16%/ del(11q): 377/3256 (12%)/ *TP53* abnormality (*TP53*abn *i.e.* del(17p) and/or *TP53* mutations): 299/3308 (12%).
Results: Following the current definition for CK *i.e.* ≥3 structural and/or numerical aberrations, 381/3580 cases (11%) displayed CK, with no difference in the detection rate between different cell stimulation protocols. CK was significantly associated ($p<0.05$) with male gender, advanced clinical stage (Binet B/C), U-CLL, del(13q), +12, del(11q) and *TP53*abn. On univariate analysis, CK was associated with inferior OS (median: 11.5 years); CK remained significant also on multivariate analysis along with older age, Binet B/C stage, U-CLL and *TP53*abn. Considering earlier evidence in smaller series that CLL cases with ≥3 structural and/or numerical cytogenetic aberrations are not equivalent, we assessed the relevance of other numerical cut-offs for CK, while also investigating the impact of the type of aberrations (i.e. structural *versus* numerical). CK cases were stratified into those with 3 ('low-CK', n=200, 52%), 4 ('intermediate-CK', n=82, 22%) and ≥5 ('high-CK', n=99, 26%) aberrations. High-CK cases differed significantly ($p<0.05$) from the other two subgroups, being enriched for U-CLL and *TP53*abn (79% and 57%, respectively). The median OS was 5.1 years for the high-CK *vs* not reached for the low- and intermediate-CK groups ($p<0.0001$). We also identified 46 cases (12% of those with ≥3 aberrations) who carried +12,+19 plus other numerical and/or structural abnormalities and displayed extremely indolent clinical course (median OS not yet reached and only 4 deaths at a median follow-up of 5.2 years). When high-CK was assessed as an independent parameter, it was correlated with inferior OS in the univariate analysis, retaining significance also in the multivariate analysis ($p=0.012$) independently of the remaining parameters, including clinical stage, U-CLL and *TP53* status. In contrast, low/intermediate-CK had no impact on OS, not even in univariate analysis ($p=0.57$).
Summary/Conclusions: CK defined by the presence of ≥3 numerical and/or structural abnormalities should not be axiomatically considered unfavorable in CLL, representing a heterogeneous group with variable clinical behavior. High-CK with ≥5 chromosomal aberrations emerges as prognostically adverse, independently of clinical stage, IG somatic hypermutation and *TP53* status. Prospective clinical validation is warranted before finally incorporating high-CK in risk stratification in CLL.

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IS FCR THE TREATMENT OF CHOICE FOR IGHV MUTATED CLL WITHOUT POOR FISH CYTOGENETICS?

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Background: Chemoimmunotherapy (CIT) is the standard treatment for young and fit treatment-naïve patients with CLL. The median progression-free-survival (PFS) in patients treated with CIT is about 5-6 years and the overall survival (OS) is increased by 5-10% compared to those treated with chemotherapy only. Patients with mutated IGHV genes (M-CLL*)* and no unfavorable cytogenetic alterations (i.e. del(17p)/*TP53* mutation, and del(11q)) have a better outcome than those with unmutated IGHV genes (U-CLL) and/or poor FISH cytogenetics and show a plateau in survival curves, suggesting that a fraction of these patients may have a survival similar to general population. Nevertheless, the possibility that some M-CLL patients without unfavorable cytogenetics are overtreated is of concern because of the treatment toxicity related to CIT, particularly FCR.
Aims: The aim of this study was to analyse the outcome of M-CLL patients with no unfavorable cytogenetics CLL according to the type of therapy.
Methods: We analysed 816 CLL patients from Sant Pau Hospital, Barcelona, Spain; Uppsala Universitary Hospital, Sweden and IRCCS San Raffaele Scientific University, Milan, Italy for whom IGHV mutational status was available. Endpoints were OS and TFS.

Table 1.

Clinical and biological characteristics at diagnosis

	m-IGHV (n=488)	*u-IGHV (n=328)*
Median Age	63y (30-88)	63y (27-89)
Male/Female	284/204	181/147
Clinical Stages		
Binet A/B/C	412/32/16	222/62/20
RAI 0/I-II/III-IV	329/105/26	162/14/26
FISH cytogenetics		
del 13q	212/432	92/298
trisomy 12	25/433	56/298
del 11q	8/426	11/293
del 17p	18/433	42/298

Results: 488 patients had mutated IGHV genes (400 without unfavorable FISH cytogenetics; 26 had either del(11q) and/or del(17p), and in 62 cases FISH was not available) and 328 patients carried unmutated IGHV genes. The main clinical and biological characteristics at diagnosis are shown in Table 1. OS at 5 and 10 years was 93% (CI, 95-91) and 81% (CI, 85-77) for M-CLL cases and 78% (CI, 83-73) and 46% (CI, 52-39) for U-CLL cases ($p<0.05$). TFS at 5 and 10 years was 73% (CI, 77-69) and 61% (CI, 66-56) and 28% (CI, 33-23) and 10% (CI, 14-6) for M-CLL and U-CLL, respectively ($p<0.05$). After a median follow-up of 8 years (range, 1-26), 424 patients [161 M-CLL (136 without poor-prognostic FISH cytogenetics, 13 with either del(11q) and/or del(17p) and 12 cases in whom FISH information was not available) and 263 U-CLL] required therapy. Front-line treatment consisted of purine analogues (PA)-based therapy (n=83), alkylating agents (n=212), anti-CD20 moAbs with PA or bendamustine (n=75), anti-CD20 moAbs with alkylating agents (n=21), BCR-signal inhibitors or BCL2 antiapoptotic agents (n=9), others (n=23), and unknown (n=1). The

median duration of response to first therapy was 42 months (range, 33-52) in M-CLL cases *vs* 24 months (range, 18-30) in U-CLL patients (p<0.001). 282 patients received a second line of therapy: PA-based therapy (n=95), alkylating agents (n=82), anti-CD20 moAbs with PA or bendamustine (n=33), anti-CD20 moAbs with alkylating agents (n=16), BCR-signal inhibitors or BCL2 antiapoptotic agents (n=12), others (n=39), and unknown (n=5). In 481 of 816 patients in whom detailed information on treatment regimens beyond second-line was available, 99 patients received a third-line treatment including PA-based therapy (n=15), alkylating regimens (n=20), anti-CD20 MoAbs with PA or bendamustine (n=15), anti-CD20 moAbs with alkylating agents (n=8), BCR or BCL2 inhibitors (n=11), others (n=28) and unknown (n=2); 49 patients received four or more lines of therapy. In M-CLL patients without poor FISH cytogenetics (n=136) the type of therapy did not impact patients´ outcome. Thus, the median survival was not reached in patients treated with CIT as first-line (i.e FCR, BR) as compared to 202 months in those not having received CIT (p=0.317). In contrast, in U-CLL patients the OS was highly dependent on the type of therapy. In detail, U-CLL patients who received anti-CD20 MoAbs with PA or bendamustine either as first line or subsequent lines (60 of 120 patients) showed significantly longer survival than those who did not receive these therapeutic regimens (median survival: 173 *vs* 103 months, p=0.001). On the contrary, in M-CLL cases no differences in survival were observed in those receiving anti-CD20 MoAbs with PA or bendamustine *vs* who did not (p=0.558).

Summary/Conclusions: This retrospective study suggests that OS of CLL patients with mutated IGHV genes and no unfavorable FISH cytogenetics do not depend on the type of therapy. This has important clinical implications and provides background for randomized studies aimed at identifying the optimal treatment strategy for this group of patients.

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IBRUTINIB, FLUDARABINE, CYCLOPHOSPHAMIDE, AND OBINUTUZUMAB (GA101) (IFCG) FOR PREVIOUSLY UNTREATED PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) WITH MUTATED IGHV AND NON-DEL(17P)

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Background: Patients with mutated *IGHV* (*IGHV*-M) have favorable long-term outcomes (10-year PFS of >60%) after receiving first-line FCR.
Aims: To develop an FC-based chemoimmunotherapy regimen of finite duration that included ibrutinib and obinutuzumab. The intent was to limit FC to 3 courses, potentially reducing short- and long-term toxicity, while maintaining efficacy through the addition of ibrutinib and a more potent antibody (obinutuzumab).
Methods: We designed an investigator-initiated phase II trial with ibrutinib, fludarabine, cyclophosphamide, and obinutuzumab (iFCG) for previously untreated pts with *IGHV*-M CLL (NCT02629809). The intent was to limit FC to 3 courses, potentially reducing short- and long-term toxicity, while maintaining efficacy through addition of ibrutinib and obinutuzumab. Key eligibility included age ≥18, *IGHV*-M, no del17p. Pts received 3 courses of iFCG. G-CSF was not mandated. Primary endpoint: CR/CRi with bone marrow (BM) MRD-neg (4-color flow-cytometry) after 3 courses of iFCG. Pts meeting primary endpoint received ibrutinib with obinutuzumab (iG) for C3-6, then ibrutinib C7-12. Pts not achieving primary endpoint received iG (C4-12). All pts who are MRD neg at 1 year will stop all therapy, including ibrutinib. Pts MRD+ at 1 year may continue ibrutinib. Historic C3 BM MRD-neg with FCR in *IGHV*-M 26% (Strati, Blood 2014). Target BM MRD-neg after iFCG x3 is 45%. Sample size 45.
Results: Since activation (April 2016), 26 patients were consented; 23 initiated treatment. We report data on these 23 patients. This is the first report of this trial. Median age is 59 years (range, 25-71); there were 18 men. Prognostic markers included [FISH: del13q (n=17), negative (n=3); trisomy 12 (n=3); CD38+ (n=7); ZAP70+ (n=6/21 evaluated)]. By trial design, all patients had *IGHV*-M. Median B2M was 2.6 (range, 1.4-8.1). Median pretreatment WBC count was 81.1 K/µL (range, 3.1-224), platelet count 120 K/µL (range, 62-292), hemoglobin 12.4 g/dL (range, 9.1-15.6). Eighteen patients have completed 3 courses of iFCG and had initial response assessment (the remaining 5 patients have not yet completed 3 courses of treatment). All 18 patients achieved a clinical response; 14/18 (78%) achieved MRD-negative remission in the marrow at 3 month (Table 1) and 16/18 (89%) achieved MRD-negative remission as their overall best response. Overall, 7/18 achieved CR/CRi with MRD-negative status in bone marrow at 3 months. All patients with PR had bulky adenopathy at baseline, and had residual lymphadenopathy ranging from 1.8 to 3.5 cm after 3 courses of iFCG. No patient has progressed, and all but one continue to receive treatment on protocol. Of the 23 pts, 11 pts had G3-4 neutropenia and 5 pts had G3-4 thrombocytopenia. 4 pt had neutropenic fever. 1 pt who achieved MRD-neg CR developed pulmonary MAC infection, and declined further therapy. 1 pt had atrial fibrillation. G3 ALT developed in 3 pts. FC was dose reduced in 10 pts; ibrutinib dose-reduced in 2 pts.

Table 1.

	N=18	Marrow MRD	N=18	Marrow MRD
ORR	18/18 (100)	14/18 (78) neg	18/18 (100)	16/18 (89) neg
CR/CRi	7 (39)	7/7 (100) neg	9 (50)	9/9 (100) neg
PR	11 (61)	7/11 (64) neg	9 (50)	7/9 (78) neg

Summary/Conclusions: iFCG achieves high rate of MRD-neg remission after 3 courses. Pt enrollment continues, and updated results will be presented at the EHA meeting.

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BENDAMUSTINE (B), FOLLOWED BY OBINUTUZUMAB (G, GA101) AND VENETOCLAX (A, ABT-199) IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): CLL2-BAG PHASE-II-TRIAL OF THE GERMAN CLL STUDY GROUP (GCLLSG)

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Background: Based on the theoretical "sequential triple-T" concept [Hallek M., Blood 2013; 122(23): 3723-34] of a tailored and targeted treatment aiming for total eradication of minimal residual disease (MRD), the GCLLSG designed the CLL2-BAG trial.
Aims: This prospective, open-label, multicenter phase-II trial investigates a sequential treatment with a B debulking, followed by G and A as induction and maintenance therapy in an all-comer population of physically fit and unfit, treatment-naïve (TN) and relapsed/refractory (R/R) CLL pts.
Methods: Pts with an absolute lymphocyte count (ALC) ≥25.000/µl and/or lymph nodes (LN) ≥5cm were to receive 2 cycles of B as debulking (70mg/m^2 d1&2 q28 days), unless contraindicated. In the induction G (1000mg) was administered 3 times in cycle 1 (days 1/2, 8 & 15) and every 4 weeks in cycles 2-6. A was added in cycle 2 with a dose ramp-up (to 400mg daily) over 5 weeks and several safety precautions. In the maintenance therapy, daily intake of A was continued and G administered every 3 months until achievement of a MRD-negative complete response or for up to 24 months. The primary endpoint is the overall response rate (ORR) at the end of induction therapy; secondary endpoints include MRD evaluations, safety and survival parameters. This primary endpoint analysis is based on uncleaned data, the final analysis will be presented at the meeting.

Table 1.

Efficacy (at end of induction treatment)

	all patients	treatment-naive	relapsed/refractory
Response (according to iWCLL guidelines)			
- CR/CRi	6 (9.5%)	3 (8.8%)	3 (10.3%)
- PR	55 (87.3%)	31 (91.2%)	24 (82.8%)
- SD	1 (1.6%)	-	1 (3.4%)
- PD	1 (1.6%)	-	1 (3.4%)
MRD in peripheral blood			
- negative (< 10^{-4})	56 (88.9%)	33 (97.1%)	23 (79.3%)
- intermediate (≥ 10^{-4} and < 10^{-2})	-	-	-
- positive (≥ 10^{-2})	4 (6.3%)	1 (2.9%)	3 (10.3%)
- missing	3 (4.8%)	-	3 (10.3%)
MRD in bone marrow			
- negative (< 10^{-4})	8 (12.7%)	4 (11.8%)	4 (13.8%)
- intermediate (≥ 10^{-4} and < 10^{-2})	-	-	-
- positive (≥ 10^{-2})	-	-	-
- missing	55 (87.3%)	30 (88.2%)	25 (86.2%)

Results: Between May 2015 and January 2016, 66 pts were enrolled. Two R/R pts died of a sepsis and 1 TN pt discontinued due to toxicity during the first induction cycle; these 3 pts with <2 induction cycles were excluded from the analysis as predefined by protocol. 34 pts were treatment-naïve and 29 had R/R CLL (median number of prior therapies: 2, range: 1-8). Median age was 59 (28-77) years, the median CIRS score was 2 (0-14) and 16 pts (25%) had a creatinine clearance of 30-70ml/min. 11 of 59 pts (19%) had a del(17p) and 45 of 61 (74%) had an unmutated IGHV status. Risk categories for TLS at baseline were: low (ALC <25.000/µl & LN <5cm): 9 pts (15%), intermediate (ALC ≥25.000/µl or LN 5-10cm): 35 (58%) and high (ALC ≥25.000/µl & LN 5-10cm or LN >10cm): 16 (27%), 3 missing. 45 pts (71%) received B debulking, 18 (29%) pts immediately started with the induction. 60 pts completed 6 induction cycles with G and A. All TN (100%) and all but two of the R/R pts (93%) respond-

ed (table 1); with an ORR of 97% at the end of induction, the primary endpoint was met. MRD negativity (<10^{-4} by flow cytometry) in peripheral blood (pB) was achieved in 56 pts (89%); MRD assessment from bone marrow was available in 8 pts (4 TN and 4 R/R, among them 4 with a CR and 4 with a PR) and were all negative. As of January 9th2017, 83 serious adverse events (SAEs) were reported in 37 pts, including 69 SAEs (83%) related to study treatment. 66 SAEs (80%) were CTC°3-4 and 1 had a fatal outcome (sepsis in 4th induction cycle). Most SAEs occurred in the R/R cohort (61 SAEs, 74%) and during the induction phase (63 SAEs, 76%). Most common SAEs were infections (27 in 16 pts; including 13 CTC°3-5) and hematological disorders (18 in 10 pts; 10 CTC°3-4), followed by infusion-related reactions (6 in 6 pts), laboratory TLS (5 in 5 pts; 1 during B debulking, 1 in induction cycle 1 with G, 2 in cycle 3 and 1 in cycle 4 with G and A) and ischemic coronary artery disorders (5 in 4 pts). No clinical TLS occured.

Summary/Conclusions: With an ORR of 97% and a MRD negativity rate of 89% in pB at the end of induction phase this sequential treatment of B debulking, followed by G and A was very efficacious in a heterogeneous study population and well tolerated except for 3 fatal septicaemias in R/R pts.

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SAFETY RESULTS OF TERMINATED PHASE 2 STUDY OF IDELALISIB PLUS RITUXIMAB IN TREATMENT NAÏVE CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) WITH DEL(17P)

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Background: Idelalisib (IDELA) is an oral PI3Kδ inhibitor approved in the EU for use with rituximab (R) or ofatumumab in patients (pts) with previously treated CLL or as first-line treatment of CLL with either del(17p) or TP53 mutation in pts unsuitable for other therapies. Prior single arm studies have suggested that front line use of IDELA may be associated with an increased frequency of transaminase elevations compared to relapsed pts.

Aims: To describe: 1) the safety of IDELA plus rituximab in previously untreated CLL pts with del(17p) in this terminated study, and 2) the relation of key AEs and age.

Methods: Treatment-naïve pts with CLL and confirmed del(17p) were treated in a single arm study with R 375mg/m² IV weekly x8 and IDELA 150mg PO BID continuously until disease progression or intolerability. Informed consent was obtained. The study was fully enrolled when terminated early due to infection related safety concerns observed in a pooled analysis of ongoing Ph3 IDELA trials in front/early line therapy; the planned independent efficacy analysis was not performed, but investigator assessment is available.

Results: 102 pts (median age, 66; range, 37-86) were enrolled between Aug 2014 and Jan 2016 and received IDELA for a median (med) duration of 6.4 months (range, 0.7-17.0). The study was terminated in Mar 2016, >8 wks after dosing of the last enrolled pt. 77 pts (75.5%) remained on study at the time of study termination. The reasons for discontinuation from study were death (4.9%), progressive disease (3.9%, 1 fatal), investigator discretion (9.8%), withdrawal of consent (2.9%), other anticancer therapy (2.0%), and lost to follow up (1.0%). The investigator assessed response rate was 79%. 101 pts (99%) had adverse events (AEs); Gr ≥3 occurred in 80.4%, the most frequent Gr ≥3 were ALT increased (27.5%), neutropenia (20.6%), infections (18.6%), and diarrhea (14.7%). Laboratory Gr ≥3 ALT and/or AST elevations were seen in 41.2%, with med time of onset of 8.1 wks (range 4.1-24.1). The med age of pts both with and without Gr ≥3 ALT/AST was 66 years, and the incidence of Gr ≥3 ALT/AST was similar in younger (43.9%, <65yr) and older (39.3%, ≥65yr) pts. Gr ≥3 diarrhea/colitis occurred in 17.1% of pts <65yr and in 14.8% of pts ≥65yr. Dose interruptions due to AEs occurred in 71 pts (70%), most frequently due to transaminase elevations (37.3%), and diarrhea/colitis (15.7%). Discontinuation due to AEs occurred in 27% of pts, most frequently due to ALT/AST elevation (9.8%). Serious adverse events were reported in 46 (45.1%), including pyrexia (10.8%), diarrhea/colitis (11.8%). AEs of special interest included Gr ≥3 infections in 20 pts (19.6%) of whom 5 had CMV and 3 had PJP (none on prophylaxis), Gr ≥3 febrile neutropenia in 5 (4.9%) and any grade pneumonitis in 5 (4.9%). Of the 5 pts with CMV, all were CMV IgG+ at screening and 2 also were IgM+. There were 6 on-study deaths, 3 associated with infection, 2 due to CLL progression and 1 due to heart failure.

Summary/Conclusions: In IDELA plus rituximab treated front-line CLL, the pattern of AEs was similar to that seen in relapsed CLL studies at similar duration of therapy, however the frequency of Gr ≥3 ALT/AST was increased compared to the relapsed setting. There was no significant effect of age on the risk of either ALT/AST elevations or diarrhea/colitis. The occurrence of CMV and PJP infections is consistent with current IDELA labeling and speaks to the potential benefit of risk mitigation through PJP prophylaxis and CMV monitoring during treatment. NCT02044822.

Aggressive Non-Hodgkin lymphoma - Relapsed/refractory

S466

CLINICAL AND BIOLOGIC COVARIATES OF OUTCOMES IN ZUMA-1: A PIVOTAL TRIAL OF AXICABTAGENE CILOLEUCEL (AXI-CEL; KTE-C19) IN PATIENTS WITH REFRACTORY AGGRESSIVE NON-HODGKIN LYMPHOMA (NHL)

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Background: Outcomes for pts with refractory aggressive NHL are poor with current therapies (Crump, ASCO 2016). Results from the interim analysis of (n=62) of ZUMA-1, the 1st multicenter trial of an anti-CD19 chimeric antigen receptor (CAR) T cell, axi-cel, in refractory aggressive NHL, showed an objective response rate (ORR) of 79% (complete response [CR] 52%; *Blood* 2016;128:LBA-6). Here we present results from the primary analysis of ZUMA-1.

Aims: Here we present results from the primary analysis of the ZUMA-1 trial.

Methods: Pts received a target dose of 2 × 10^6 anti-CD19 CAR T cells/kg after low- dose conditioning with cyclophosphamide and fludarabine. Eligible pts (≥18 y) had diffuse large B cell lymphoma (DLBCL), primary mediastinal B cell lymphoma (PMBCL) or transformed follicular lymphoma (TFL); an ECOG performance status (PS) 0-1; and refractory disease (progressive or stable disease as best response to last prior therapy, or relapsed ≤12m of autologous stem cell transplant [ASCT]). The primary endpoint for this analysis was ORR in the combined DLBCL, PMBCL, and TFL population. Key secondary endpoints were duration of response (DOR), overall survival (OS), and frequency of adverse events (AEs). The primary analysis was triggered when 92 pts had at least 6m of follow-up

Results: As of January 27, 2017, 111 pts from 22 institutions were enrolled; 101 pts (91%) received axi-cel. Median age was 58 y (range, 23-76), 67% male, 85% stage III-IV, 47% IPI 3-4, 77% refractory to ≥2nd line of therapy, and 21% relapsed ≤12m of ASCT. Axi-cel was successfully manufactured in 110/111 (99%) pts with an average turnaround time from apheresis to the clinical site of 17 d. With an ORR of 82% (n=92; *P*<.0001) the study met the primary endpoint. The ORR in the mITT analysis set of 101 pts was 82% (CR 54%, PR 28%), and was consistent across key covariates including disease subtype, refractory status, stage, and IPI score. At a median follow up of 8.7 m, 44% of pts were in response and 39% were in CR. The median DOR was 8.2m overall and not reached for pts who achieved a CR. Median OS was not reached; 80% of pts remained alive at 6 m. The most common Gr ≥3 treatment-emergent AEs were neutropenia (66%), leukopenia (44%), anemia (43%), febrile neutropenia (31%), and encephalopathy (21%). Gr ≥3 cytokine release syndrome (CRS) and neurologic events (NE) occurred in 13% and 28% of pts, respectively. All CRS and all NE resolved except 1 Gr 1 memory impairment. As previously reported, there were 3 Gr 5 AEs (3%). Peak CAR T levels and AUC post–axi-cel were associated with durable responses. Additionally, this presentation will include an expanded analysis of efficacy outcomes by novel biologic and clinical covariates including key molecular phenotypes and tocilizumab/corticosteroid interventions used for management of adverse events.

Summary/Conclusions: Axi-cel significantly improved ORR in patients with refractory aggressive NHL. The CR rate was 7-fold higher compared to historical controls (Crump, ASCO 2016) and nearly half the patients had an ongoing response. Axi-cel demonstrated significant clinical benefit with a manageable safety profile in pts lacking curative treatment options.

Drs Locke and Neelapu contributed equally to this study

S467

CC-122 IN COMBINATION WITH OBINUTUZUMAB (GA101): PHASE IB STUDY IN RELAPSED OR REFRACTORY PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA, FOLLICULAR LYMPHOMA, OR MARGINAL ZONE LYMPHOMA

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Background: CC-122 is a cereblon modulating agent that degrades Aiolos and Ikaros, resulting in potent anti-lymphoma and immunomodulatory effects on T- and NK-cell function. Phase I clinical data revealed promising activity of CC-122 against follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL). Preclinical combination of CC-122 with obinutuzumab has shown synergism in FL and additive effects in DLBCL *vs* either single agent (Chiu. ASH 2015), supporting further study of this combination's therapeutic potential.
Aims: The current phase Ib study (EUDRACT 2014-003333-26; NCT02417285) evaluates the safety and efficacy of CC-122 plus obinutuzumab in patients with relapsed or refractory (R/R) B-cell non-Hodgkin lymphoma (NHL).
Methods: Patients at study entry must have R/R CD20+ B-cell NHL after ≥1 prior regimens for FL/marginal zone lymphoma (MZL) and ≥2 regimens and/or ASCT for DLBCL. CC-122 was given orally (5 of 7 d) for 28-d cycles in escalating doses plus a fixed dose of intravenous obinutuzumab 1000mg on d2, 8, 15 of cycle 1 (c1) and d1 of c2-8, upon informed consent. CC-122 was continued until progressive disease (PD) or unacceptable toxicity. CC-122 active ingredient in capsule formulation (AIC) 1, 2, 3, and 4mg and CC-122 formulated capsules (F6) 3 and 4mg were evaluated in separate cohorts. Primary endpoints included safety and tolerability, non-tolerated dose (NTD), and maximum tolerated dose (MTD). Response was assessed using the international Cheson 2007 criteria every 2 cycles to c6, every 3 cycles to c12, and every 6 cycles thereafter.
Results: As of January 12, 2017, 34 R/R B-cell NHL patients with DLBCL (n=18), FL (n=15), or MZL (n=1) were enrolled. At study entry, median age was 60 y (26-81), most patients were male (68%), and Ann Arbor was extended stage III/IV in 76% of patients. Of the 18 DLBCL patients, 8 had transformed FL. Of the 16 FL/MZL patients, 44% relapsed in <12 months after first-line treatment. The median number of prior regimens was 4 (range, 1-11), and 13 (38%) patients had received prior SCT. One patient experienced a dose-limiting toxicity (DLT) of grade 4 neutropenia (CC-122 dose level of AIC 3mg); no dose was yet an NTD. Median CC-122 duration was 22 wks (range, 3-71) equivalent to 6 cycles (range, 1-18). CC-122 dose reduction or temporary interruption occurred in 10 (29%) or 26 (76%) of patients, respectively, primarily due to adverse events (AEs). Most patients (56%) had <1 wk of interruption due to AEs. The most common (≥10%) grade 3/4 treatment-emergent AEs (TEAEs) were neutropenia (50%) and thrombocytopenia (21%). Fifteen patients (44%) had ≥1 serious TEAE, including 2 each of febrile neutropenia (related to CC-122), cytokine release syndrome (related to obinutuzumab), and pneumonia. Three deaths occurred during the study (2 PD; 1 AE-related). Overall response rate (ORR) was 59%, including 26% CR and 32% PR (Table 1). Median time to best response was 57 d, and median duration of response was not yet reached. In evaluable patients, 6-mo progression-free survival (PFS) was 63%.

Table 1.

Table 1. Best responses with CC-122 and obinutuzumab in evaluable patients.

Response Status, n (%)	R/R DLBCL* n=18	R/R FL/MZL n=16	All Patients N=34
ORR	8 (44)	12 (75)	20 (59)
CR	3 (17)	6 (38)	9 (26)
PR	5 (28)	6 (38)	11 (32)
SD	4 (22)	3 (19)	7 (21)
PD	3 (17)	1 (6)	4 (12)

Data cutoff was 10Feb2017. *3 patients were not evaluable for efficacy.

Summary/Conclusions: The combination of CC-122 and obinutuzumab was well tolerated and demonstrates promising response rates and durable remissions in R/R patients with B-cell NHL. CC-122 doses of ≥3mg and obinutuzumab have shown best response rates to date. The study is ongoing to establish the phase II recommended dose.

S468

POLATUZUMAB VEDOTIN PLUS BENDAMUSTINE AND RITUXIMAB OR OBINUTUZUMAB IN RELAPSED/REFRACTORY FOLLICULAR LYMPHOMA OR DIFFUSE LARGE B-CELL LYMPHOMA: UPDATED RESULTS OF A PHASE 1B/2 STUDY

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Background: Transplant ineligible patients (pts) with relapsed/refractory (R/R) FL or DLBCL have poor outcomes. Polatuzumab vedotin (pola) is an antibody drug conjugate that targets delivery of the microtubule inhibitor MMAE to cells expressing CD79b. Pola + rituximab (R) previously showed promising responses in R/R FL and DLBCL. Adding bendamustine (B) to pola-R and substituting obinutuzumab (G) for R could improve outcomes. We report updated results from the Phase 1b/2 (P1b/2) study evaluating pola + BR or BG in R/R FL and DLBCL and the expansion cohorts evaluating pola + BG in R/R FL and DLBCL (ClinicalTrials.gov NCT02257567).
Aims: The primary aim is to assess safety and tolerability of pola + BR/BG in R/R FL and DLBCL. Secondary aims include assessing safety and efficacy of pola + BG in an expansion cohort.
Methods: All pts provided informed consent to participate in the study and were treated with pola (1.8mg/kg) + B (90mg/m^2) and R (375mg/m^2) or G (1000mg) every 28 days (FL) or 21 days (DLBCL) for 6 cycles. Responses were assessed by modified Lugano 2014 criteria after 3 cycles, end of treatment (tx), and every 6 months for 2 years during follow-up (fu).
Results: As of 14 Nov 2016, 65 pts were enrolled: 24 pts (12 FL, 12 DLBCL) in P1b and 41 pts (20 FL and 21 DLBCL) in P2. In safety evaluable pts, FL pts (N=32) were median age of 63 yr (37-86), 82% ECOG 0-1 and 6% ECOG 2, 44% FLIPI1 3-5, 78% Stage III/IV, 2 (1-7) median lines of prior tx, 38% refractory to last tx, 13% prior transplant (BMT). DLBCL pts (N=32) were median age 66 (30-86), 88% ECOG 0-1 and 13% ECOG 2, 59% IPI 3-5, 75% Stage III/IV, 2 (1-7) median lines of prior tx, 82% refractory to last tx, 3% prior BMT. Among 64 pts who received ≥1 dose, the adverse events (AEs) that occurred in >20% of pts were: fatigue (67%), nausea (54%), diarrhea (54%), vomiting (42%), pyrexia (39%) and constipation (39%). As expected, grade (Gr) 3/4 cytopenias were common: neutropenia (34% FL, 28% DLBCL), thrombcytopenia (16% FL, 13% DLBCL), anemia (6% FL, 9% DLBCL). Tx emergent neuropathy occurred in 19/64 (30%) of pts, with 1 Gr 3 event, and led to pola discontinuation in 1 pt, dose reduction in 2 pts, and interruption in 1 pt. In FL, 75% (24/32) had Gr 3/4 AEs and 41% (13/32) had serious AEs (SAEs). The only SAE occurring in ≥10% was infection (22%). The most common Gr 3/4 non-heme AEs were infection (16%) and hypokalemia (9%). AEs led to study tx discontinuation in 6 pts. B was stopped in 2 pts due to Gr 3 thromboctyopenia. Of 4 deaths: 2 were PD and 2 were Gr 5 AEs, 1 tx related (PML), 1 tx unrelated. In DLBCL, 88% (28/32) had Gr 3/4 AEs and 63% (20/32) had SAEs. Most common Gr 3/4 non-heme AEs were febrile neutropenia (13%), fatigue (13%), and diarrhea (13%). SAEs occurring in ≥10% of pts were infection (33%) and pyrexia (22%). AEs led to study tx interruption in 19 pts and discontinuation in 8 pts. There were 13 deaths: 9 PD, 4 AE (all unrelated to tx). Responses by modified Lugano 2014 criteria are shown in Table1. Median duration of response (DoR) for FL P1b pts was 16 months (mo)(median fu 14.5 mo). Median DoR for FL P2 (median fu 6.5 mo) and DLBCL P1b/2 (median fu 13.7 mo P1b, 6.4 mo P2) have not been reached.

Table 1.

Table 1. Best Overall Response by PET/CT*

	P1b				P2	
Response	FL		DLBCL		FL	DLBCL
N (%)	Pola+BR (N=6)	Pola+BG (N=6)	Pola+BR (N=6)	Pola+BG (N=6)	Pola+BG (N=20)	Pola+BG (N=21)**
ORR	6 (100)	6 (100)	3 (50)	5 (83)	16 (80)	12 (57)
CR	4 (67)	5 (83)	2 (33)	4 (67)	12 (60)	7 (33)
PR	2 (33)	1 (17)	1 (17)	1 (17)	4 (20)	5 (24)
SD	0	0	0	0	1 (5)	3 (14)
PD	0	0	2 (33)	1 (17)	1 (5)	3 (14)
Unevaluable	0	0	1 (17)	0	2 (10)	3 (14)

*Using Modified Lugano 2014 criteria **1 pt not dosed

Summary/Conclusions: Updated evaluation of pola + BR shows promising durable responses and an acceptable safety profile in heavily pre-treated R/R FL and DLBCL pts. Safety and efficacy data will be updated at the time of presentation.

S469

SINGLE AGENT ORAL SELINEXOR EXHIBITS DURABLE RESPONSES IN RELAPSED/REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) OF BOTH GCB AND NON-GCB SUBTYPES: THE PHASE 2B SADAL STUDY

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Background: Patients (pts) with persistent DLBCL after two or more lines of therapy have limited effective treatment options. The nuclear export protein exportin 1 (XPO1) is upregulated in hematologic malignancies, including DLBCL, and has pleiotropic effects on tumorigenesis including functional downregulation of tumor suppressor proteins (TSPs) and increased export and translation of mRNAs for oncoproteins c-Myc and key survival proteins such as Bcl-2. Selinexor (SEL), an oral XPO1 inhibitor, causes sequestration of TSPs including p53, p21, and IkBa, the latter of which serves to suppress NF-kB driven transcription, along with reductions in c-Myc and Bcl-2 family proteins. In a Phase I clinical study, pts with relapsed/refractory (R/R) DLBCL treated with SEL presented an overall response rate (ORR) of 32% including 4 CRs. Interestingly, 2 of these pts remain in CR for >1 yr.
Aims: In this clinical study we assess the efficacy of single agent SEL in pts with R/R DLBCL after ≥2 prior regimens.
Methods: Pts with R/R DLBCL were randomized to 60 or 100mg of SEL twice weekly (8 doses) per 28-day cycle. Pts were also stratified by DLBCL subtype (GCB or non-GCB). The primary objectives are to determine the ORR and evaluate the safety of 60 *vs* 100mg doses. Disease response was assessed by an Independent Central Radiological Review (ICRR), using the Lugano Classification (Cheson, 2014).
Results: 72 pts were enrolled: 37 pts on 60mg (24 M/ 13 F, median age 71 yrs) and 35 pts on 100mg (23 M/ 12 F, median age 68 yrs). Both groups had a median of 3 prior treatment regimens. The most common related adverse effects (AEs) across both dosing groups (Grade 1/2) were: fatigue (47%), nausea (46%), anorexia (42%), and vomiting (33%). Common Grade 3/4 AEs were: thrombocytopenia (39%), fatigue (18%), neutropenia (18%), and anemia (13%). These were managed with dose interruption/reduction, platelet stimulators, and/or standard supportive care. Grade 3/4 fatigue (26% v 11%) and thrombocytopenia (46% v 32%) were higher in 100mg arm as compared to the 60mg arm. Among the 63 evaluable pts (9 pts pending response), the ICRR determined ORR was 28.5% (**Table 1**). Nine responders, including 6 pts in CR, remain on treatment. Responders on the 60mg arm have a median time on treatment of 8.9 months as compared with 3.8 months on the 100mg arm.

Table 1. Independent Central Radiological Review-Best Response.

Category	N	ORR (%)	CR (%)	PR (%)	SD (%)	DCR (%)
All Doses	63	18 (28.5%)	7 (11.1%)	11 (17.4%)	9 (14.2%)	27 (42.8%)
60 mg	32	9 (28.1%)	4 (12.5%)	5 (15.6%)	3 (9.3%)	12 (37.5%)
100 mg	31	9 (29%)	3 (9.6%)	6 (19.3%)	6 (19.3%)	15 (48.3%)
GCB-Subtype	32	8 (25%)	3 (9.3%)	5 (15.6%)	6 (18.7%)	14 (43.7%)
Non-GCB Subtype	31	10 (32.2%)	4 (12.9%)	6 (19.3%)	3 (9.6%)	13 (41.9%)

Summary/Conclusions: SEL monotherapy shows activity in pts with R/R DLBCL including in pts with GCB subtype. 60mg SEL twice weekly was more tolerable than 100mg twice weekly, with fewer interruptions due to toxicity. Objective responses to SEL were durable at 60mg BIW, suggesting these responses were associated with clinical benefit.

S470

L-MIND: MOR208 COMBINED WITH LENALIDOMIDE (LEN) IN PATIENTS WITH RELAPSED OR REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA (R-R DLBCL)–A SINGLE-ARM PHASE II STUDY

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Background: The Fc-enhanced CD19 antibody MOR208 and the immunomodulatory drug LEN have demonstrated single agent activity in patients with R-R DLBCL. MOR208 and LEN have shown synergy *in vitro* and *in vivo* in preclinical lymphoma models.
Aims: This ongoing phase II study was designed to assess the safety and efficacy of MOR208 plus LEN in patients with R-R DLBCL.
Methods: Patients >18 years of age with R-R DLBCL, ECOG performance status 0-2, adequate organ function, having previously received at least 1 but not more than 3 prior therapies, including at least 1 CD20-targeting regimen and who are not candidates for autologous stem cell transplant (ASCT), are eligible. Treatment comprises up to 12, 28-day cycles of MOR208 12mg/kg IV, administered weekly during cycles 1-3 (loading dose day 4 of cycle 1) and every second week during cycles 4-12 plus LEN 25mg administered po days 1-21 of each cycle. Patients progression-free after 12 cycles receive up to 12 additional cycles of MOR208 12mg/kg IV, administered every second week. The primary endpoint is the overall response rate (ORR) by central radiology assessment. Secondary endpoints include disease control, duration of response, progression-free and overall survival, safety, and response by cell of origin and other biomarkers. A preplanned safety evaluation was undertaken.
Results: 31 of 80 planned patients were enrolled prior to data cutoff (3 January 2017). Median age was 74 years (range 47–82); 45% of patients received ≥2 prior lines of therapy; 23% had rituximab refractory disease; 74% had Ann Arbor stage ≥III disease; 65% had elevated lactate dehydrogenase level, and 52% had a poor revised International Prognostic Index (3–5). The most common treatment-emergent adverse events (any grade/grade ≥3 [% patients]) were neutropenia (39/26), anemia (23/0) thrombocytopenia (16/6), infections (26/10) diarrhea (13/0), pyrexia (13/0), and rashes (13/6). Of 26 response evaluable patients (median follow-up 3.3 months), ORR (investigator assessed) was 58% (15 patients), with 7 (27%) complete responses. Median time to response was 1.8 months.
Summary/Conclusions: The combination of MOR208 plus LEN is well tolerated and shows promising activity in patients with R-R DLBCL. Accrual and follow-up of patients is ongoing, as are cell of origin and other biomarker analyses.

Targeted treatment of AML

S471

ENASIDENIB (AG-221) IN MUTANT-IDH2 RELAPSED OR REFRACTORY ACUTE MYELOID LEUKEMIA (R/R AML): RESULTS OF A PHASE 1 DOSE-ESCALATION AND EXPANSION STUDY

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Background: Recurrent mutations in *isocitrate dehydrogenase 2* (m*IDH2*) occur in ~12% of AML patients (pts). mIDH2 proteins synthesize an oncometabolite, 2-hydroxyglutarate (2HG), causing DNA and histone hypermethylation and blocked myeloid differentiation. Enasidenib (AG-221) is an oral, selective, small-molecule inhibitor of mIDH2 proteins. Differentiation of myeloblasts, not cytotoxicity, appears to drive the clinical efficacy of enasidenib. In preclinical studies, bone marrow blasts from pts with m*IDH2* AML exposed to enasidenib *ex vivo* were shown to produce mature, fully functioning neutrophils with conserved m*IDH2* allele frequency, indicating differentiation of mature cells from the m*IDH2* blasts (Yen et al, *Cancer Discov*, 2017). Additionally, no apoptosis was observed in m*IDH2*-R140 erythroleukemia (TF-1) cells treated with enasidenib for 7 days *in vitro*.

Table 1.

Table. Investigator-reported hematologic responses, time to response, and response durations

	Relapsed or refractory AML	
	Enasidenib 100mg/day (n=109)	All doses (N=176)
Overall response rate,* no. (%) [95% CI]	**42 (38.5)** [29.4, 48.3]	**71 (40.3)** [33.0, 48.0]
Best response		
Complete remission, *no. (%) [95% CI]*	**22 (20.2)** [13.1, 28.9]	**34 (19.3)** [13.8, 25.9]
CR with incomplete hematologic recovery / CR with incomplete platelet recovery, *no. (%)*	7 (6.4)	12 (6.8)
Partial remission, *no. (%)*	3 (2.8)	11 (6.3)
Morphologic leukemia-free state, *no. (%)*	10 (9.2)	14 (8.0)
Stable disease,† *no. (%)*	58 (53.2)	85 (48.3)
Progressive disease, *no. (%)*	5 (4.6)	9 (5.1)
Not evaluable, *no. (%)*	2 (1.8)	3 (1. 7)
Time to first response, *median (Range)*	1.9 months (0.5-9.4)	1.9 months (0.5-9.4)
Duration of response, *median [95% CI]*	5.6 months [3.8, 9.7]	5.8 months [3.9, 7.4]
Time to complete remission, *median (Range)*	3.7 months (0.7-11.2)	3.8 months (0.5-11.2)
Duration of response in patients who attained complete remission,‡ *median [95% CI]*	8.8 months [5.3, NR]	8.8 months [6.4, NR]

Responses were evaluated by study investigators and classified according to the 2003 revised IWG criteria for AML.
*Includes patients with complete remission (CR), CR with incomplete hematologic recovery, CR with incomplete platelet recovery, partial remission, or morphologic leukemia-free state
†Stable disease was defined as failure to achieve a response but not meeting criteria for disease progression for a period of more than 8 weeks
‡Date of first documented response to date of relapse, disease progression, or death.
95% CI, 95% confidence interval; CR, complete remission; NR, not reached

Aims: Evaluate the maximum tolerated dose (MTD), pharmacokinetic (PK) and pharmacodynamic (PD) profiles, safety, and clinical activity of enasidenib in pts with m*IDH2* advanced myeloid malignancies.
Methods: This phase 1/2 study included pts aged ≥18 years (yrs) with m*IDH2* WHO-defined AML, or with m*IDH2* MDS with refractory anemia with excess blasts, and ECOG PS scores ≤2. Pts were relapsed or refractory (R/R) to prior anti-cancer therapy, or had untreated AML if aged ≥60 years and not eligible for standard-of-care treatment (Tx). Safety for all pts and clinical efficacy in the largest pt subgroup, those with R/R AML, from the phase 1 dose-escalation and expansion phases are reported.
Results: In all, 239 pts received enasidenib. Median age was 70 yrs. In the dose-escalation phase (n=113), pts received daily enasidenib doses of 50-650mg. The MTD was not reached. Median 2HG reductions from baseline at cycle 2 day 1 were 92%, 90%, and 93% for pts receiving <100mg, 100mg, and >100mg/day, respectively. Enasidenib 100mg QD was chosen for the expansion phase (n=126) based on PK/PD profiles and demonstrated efficacy. Median number of enasidenib cycles was 5 (range 1-25). Grade 3-4 investigator-reported Tx-related adverse events included indirect hyperbilirubinemia (12%) and IDH-inhibitor-associated differentiation syndrome (IDH-DS; ie, retinoic acid syndrome) (7%). Of 176 R/R AML pts, 94 (53%) had received ≥2 prior AML-directed Tx. Overall response rate (ORR; complete remission [CR] + CR with incomplete hematologic recovery + morphologic leukemia-free state + partial remission) in R/R AML pts was 40.3%, including 34 pts (19.3%) who attained CR (**Table**). Median time to 1st response was 1.9 months (mos); 87.3% of responding pts attained a 1st response by cycle 5. Median response duration was 5.8 mos. Of pts who achieved CR, 7 pts (21%) did so by cycle 3, 23 (68%) by cycle 5, and 28 (82%) by cycle 7. Median duration of CR was 8.8 mos. ORR with enasidenib 100mg/day was 38.5% (**Table**). Seventeen pts (11%) proceeded to stem cell transplant. Response was associated with cellular differentiation, typically with no evidence of aplasia. Median overall survival (OS) of R/R AML pts was 9.3 mos. For pts who attained CR, OS was 19.7 mos. Pts who had received ≥2 prior AML Tx had a median OS of 8.0 mos.
Summary/Conclusions: Enasidenib was well tolerated, induced CRs in R/R AML pts, and was associated with OS of >9 mos in pts who had failed prior AML Tx. A randomized phase 3 study of enasidenib *vs* conventional care in older pts with late-stage R/R AML is ongoing (NCT02577406).

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SAFETY AND EFFICACY OF VENETOCLAX (VEN) IN COMBINATION WITH DECITABINE OR AZACITIDINE IN TREATMENT-NAIVE, ELDERLY PATIENTS (≥65 YEARS) WITH ACUTE MYELOID LEUKEMIA (AML)

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Background: Newly diagnosed patients (pts) with AML aged ≥65 years and ineligible for standard induction therapy have limited treatment options, and low overall survival. VEN is an orally bioavailable, selective BCL-2 inhibitor that has displayed single-agent activity in pts with relapsed/refractory AML. VEN at escalating doses combined with hypomethylating agents (HMAs) has demonstrated antileukemic activity, with an overall response rate (ORR) including complete remission [CR], and CR with incomplete marrow recovery of 60%. Combining VEN with HMAs, such as decitabine (DEC) or azacitidine (AZA), may provide a novel low-intensity approach for treating AML. Preliminary results from the expansion stage of a phase 1b trial comparing 2 doses of VEN plus either DEC or AZA (NCT02203773) are reported.
Aims: To evaluate the safety and efficacy of VEN at 400-mg *vs* 800-mg doses plus DEC or AZA.
Methods: This open-label, nonrandomized, two-stage phase 1b study evaluated the safety and efficacy of VEN plus DEC or AZA in treatment-naive pts ≥65 years with AML. Eligibility included: ECOG PS ≤2; ineligible for standard induction therapy; intermediate- or poor-risk karyotype. Pts received DEC (Arm D, 20mg/m^2/day [d];intravenous [IV]) on d 1–5, or AZA (Arm E, 75mg/m^2/d; subcutaneous or IV) on d 1–7 of each 28-d cycle (C) in combination with once-daily oral VEN. The dose-expansion stage consisted of 2 VEN dose cohorts (continuous 400-mg and interrupted 800-mg dosing) in each arm (D1, D2, E1, and E2, respectively) to determine optimal dose. Tumor lysis syndrome (TLS) prophylaxis was administered in C1 to all pts during VEN dose ramp-up until final dose was reached. All pts provided informed consent.

Results: As of 13/09/16, 100 pts were enrolled in the expansion stage: 25 pts in each arm. Overall, 61% pts were male; 59% had ECOG PS 1 and 15% ECOG PS 2; mean age was 73.9 (range 65–86); 53% had adverse karyotype; and 22% had secondary AML. Median time on study was 6 (4-9), 6 (0.2-9), 5 (0.5-9), and 4 (1-8) mo for arms D1, D2, E1, and E2, respectively. The incidence of adverse events (AEs) was generally comparable between the 4 arms. Overall, the most common treatment-emergent AEs (TEAEs; in ≥30% of pts) were nausea (59%), diarrhea (42%), febrile neutropenia (FN; 41%), constipation (39%), fatigue, and decreased white blood cell count (31% each). The most frequent grade 3/4 TEAE and serious AE was FN (41% and 29%, respectively). No TLS was observed. Overall, 29 pts discontinued the study for ≥1 reason, including progressive disease (PD) per protocol (n=10), "other" (n=10; 9/10 proceeded to stem cell transplantation) and AEs not related to progression (n=10). A total of 16 deaths occurred; 12 pts died within 30 d of initiating VEN and HMA due to AEs (n=12) and PD (n=1). The ORR was 68%, with rates of 76% (19/25), 71% (17/24), 68% (17/25), and 60% (15/25) observed in arms D1, D2, E1, and E2, respectively. The Kaplan-Meier survival curve for all pts with a median follow-up time of 5.4 mo is shown.

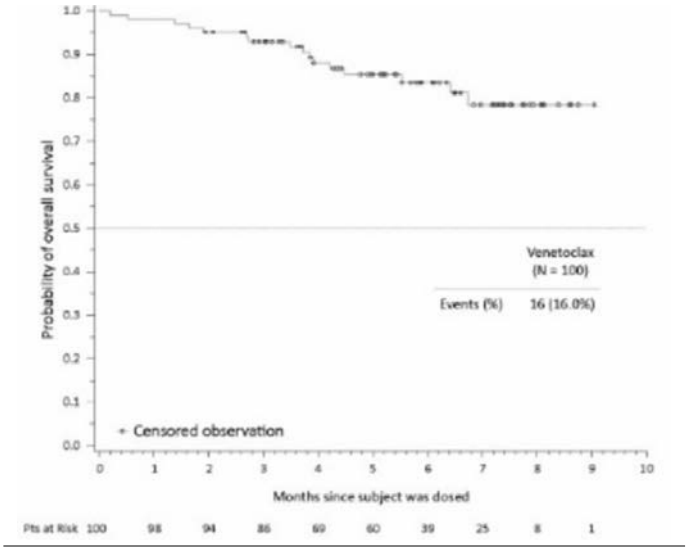

Figure 1.

Summary/Conclusions: Overall, the safety profile was favorable when combining VEN at either dose with DEC or AZA in treatment-naive elderly AML pts. Promising activity with high ORRs was observed at the lower 400-mg VEN dose in both HMA arms. A Phase 3 study of VEN plus AZA is planned.

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UPDATED SAFETY AND EFFICACY RESULTS OF PHASE 1/2 STUDY OF VENETOCLAX PLUS LOW-DOSE CYTARABINE IN TREATMENT-NAÏVE ACUTE MYELOID LEUKEMIA PATIENTS AGED ≥65 YEARS AND UNFIT FOR STANDARD INDUCTION THERAPY

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Background: Incidence of acute myeloid leukemia (AML) increases with age, and patients (pts) ≥65 years have a poor prognosis, with 5-year survival rates of <10%. Treatment with low-dose cytarabine (LDAC) in this population results in modest complete remission (CR)/CR with incomplete blood count recovery (CRi) rates of 11-19% and median survival of 5-6 months. Venetoclax (VEN), an orally bioavailable, potent, selective BCL-2 inhibitor, has shown single-agent activity in heavily pretreated pts with relapsed/refractory AML (Konopleva et al 2016). In combination with LDAC, the recommended phase 2 dose (RP2D) of VEN is 600mg QD (Lin et al, ASCO 2016); preliminary data show the combination is tolerable and exhibits significant and durable activity in older AML pts ineligible to receive intensive induction chemotherapy (Wei et al, ASH 2016). Updated safety and efficacy data from the RP2D 600-mg dose cohorts of this study (NCT02287233) are presented.

Aims: Evaluate the safety and efficacy of VEN+LDAC in older pts with untreated AML.

Methods: In this open-label phase 1/2 study, pts ≥65 years with untreated AML, ineligible for standard induction chemotherapy, with an ECOG performance status of 0-2 received oral VEN QD on days (d) 1-28 and subcutaneous LDAC 20mg/m^2 QD on d 1–10 of each 28-d cycle. VEN target dose evaluation followed a 3+3 design, ranging from 600–800mg; 18 pts were enrolled and the RP2D was established as 600mg. Safety and efficacy of VEN at RP2D were evaluated in the expansion phase. All pts were hospitalized and received prophylaxis before a dose ramp-up of VEN during cycle 1 to mitigate the risk of tumor lysis syndrome (TLS). Adverse events (AEs) were graded by NCI CTCAE V4.0. Pts enrolled as of May 2016 are included in this analysis; data cutoff was August 2016. All pts provided informed consent.

Results: In total, 61 pts, including 8 from phase 1, were treated at the RP2D of 600mg (median age 74 years; ECOG 1–2 70%; adverse karyotypes 31%; secondary AML 44%; prior hypomethylating agent [HMA] 28%). AEs (all grade; ≥30% pts; excluding cytopenias) were nausea (72%), hypokalemia (46%), diarrhea (44%), fatigue (43%), and decreased appetite (41%). Grade 3/4 AEs (≥10% pts) were febrile neutropenia (34%), hypokalemia (15%), hypophosphatemia (13%), and hypertension (10%). No pts had clinical TLS; 1 pt had laboratory TLS, which was managed. The 30-d and 60-d mortality rates were 3% and 15%, respectively. The CR/CRi rate was 54% (33/61; 21% CR and 33% CRi). The overall response rate (ORR; CR+CRi+partial remission) was 61% (37/61). VEN+LDAC was shown to be active across a wide range of cytogenetic mutations and pt profiles (ORR: 70% in pts ≥75 years; 52% in secondary AML; 47% in pts with adverse karyotypes; 53% in pts with prior HMA). Among response-evaluable pts, those achieving an objective response have longer survival than pts who do not achieve an objective response (Figure 1).

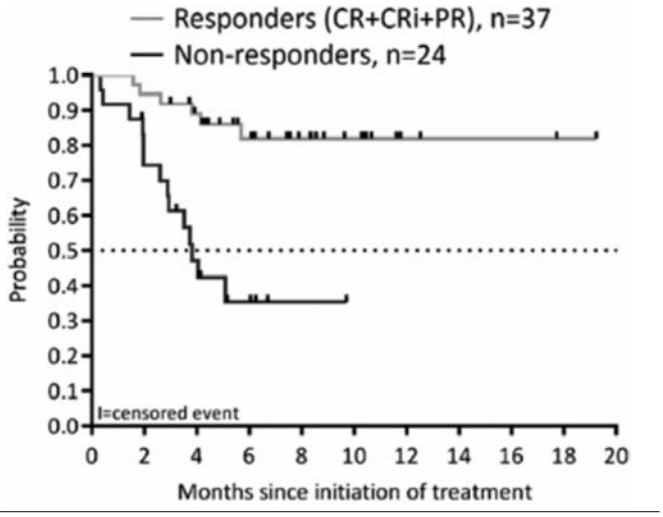

Figure 1.

Summary/Conclusions: VEN (RP2D 600mg) and LDAC exhibited an acceptable safety profile and durable efficacy in pts aged ≥65 years with untreated AML who are ineligible for or unable to receive intensive induction chemotherapy. ORR highly correlated with overall survival, with better survival observed in responders compared with nonresponders. A planned phase 3 randomized trial has commenced.

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PHASE IB/II STUDY OF NIVOLUMAB IN COMBINATION WITH AZACYTIDINE (AZA) IN PATIENTS (PTS) WITH RELAPSED ACUTE MYELOID LEUKEMIA (AML)

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Background: Blocking PD-1/PD-L1 pathways enhances anti-leukemia responses in murine AML (Zhang et al, Blood 2009). PD-1 positive CD8 T-cells are increased in bone marrow (BM) of pts with AML (Daver et al, AACR 2016). AZA up-regulates PD-1 and interferon-gamma signaling in AML and the up-regulation of PD-1 has been associated with emergence of resistance to AZA (Yang *et al.*, Leukemia 2013).

Aims: To assess the best response to Aza+Nivo at the end of 3 courses of combination therapy.

Methods: Pts were eligible if they had AML and failed prior therapy, had adequate performance status (ECOG ≤2), and organ function. The first six pts

received AZA 75mg/m^2 Days 1-7 with nivolumab 3mg/kg on Day 1 and 14. Courses were repeated every 4-5 weeks indefinitely. Only one of six pts had a dose limiting toxicity (grade 3 pneumonitis) and this dose was RP2D. 60 additional pts have been treated at the RP2D.

Results: 66 pts with a median age of 71 years (range, 44-90), secondary AML (39%), poor risk cytogenetics (35%), median number of prior regimens 2 (range, 1-7) have been enrolled. All 66 pts had baseline next generation sequencing: TP53 (n=14), DNMT3A (n=12), ASXL1 (n=10), TET2 (N=9), and RAS (n=9), IDH2 (n=9), IDH1 (n=6), CEBPA (n=7). 63 pts are evaluable for response: 14 (22%) achieved complete remission (CR)/complete remission with insufficient recovery of counts (CRi) (3 CR, 11 CRi), 7 (11%) had hematologic improvement (HI), 13 (21%) had ≥50% BM blast reduction, 5 pts (8%) had stable disease >6 months, and 24 (38%) had progression. 3 pts are too early for response assessment (<3 courses). The median number of courses to CR/CRi/HI was 2 (range, 1-4+). The med OS among the CR/CRi patients was 15.3 months (range, 2.29-17.25+), HI pts was 9.7 months (range, 4.67-17.45+), and NR was 5.0 months (range, 0.29-16.16). The 4- and 8-week mortality were 5% and 11%, respectively. The median OS for the 63 evaluable pts on Aza+Nivo compares favorably to historical median OS with AZA-based salvage protocols in similar pts treated at MDACC (P=0.10) (Fig 1A and Fig 1B). Grade 3/4 and Grade 2 immune toxicities were observed in 8 (12%) and 7 (11%) pts, respectively. The most common Grade 3/4 AEs on treatment included pneumonitis, colitis, nephritis, skin rash, and hypophysitis. One pt died from grade 4 pneumonitis/epiglottitis. In the remaining 14 cases the toxicities responded rapidly to steroids and 13 of these pts were successfully rechallenged with nivolumab. Time to onset of toxicities ranged from 4 days to 3.5 months. Multicolor flow-cytometry studies and Mass-cytometry (CyTOF) studies are conducted by the Immunotherapy Platform on baseline and on-treatment BM aspirate (end of cycle 1, 2, 4, 8). Baseline and end of cycle (EOC) 1 and 2 BM was evaluated in 6 responders and 19 non-responders. Pts who achieved a response had a baseline higher live total CD3 (P=0.10), CD8$^+$ T-cells (P=0.02), and lower live CD4$^+$Foxp3$^+$PD1$^+$ T-regulatory (T-reg) cells (P=0.01) infiltrate in BM. Patients who had a response had progressive increase in BM CD3+ cells and BM CD8+ cells, with increased ICOS (activation) marker on BM CD4-effector cells at EOC 1 and EOC 2 as compared to those who had no response. The CTLA4 on CD8 T-cells went up in both responders and non-responders after PD1 based therapy.

Figure 1. OS with Aza+Nivo compared to historical survival with AZA-based salvage protocols in similar pts treated at MDACC in (a) all salvage and (b) first salvage only

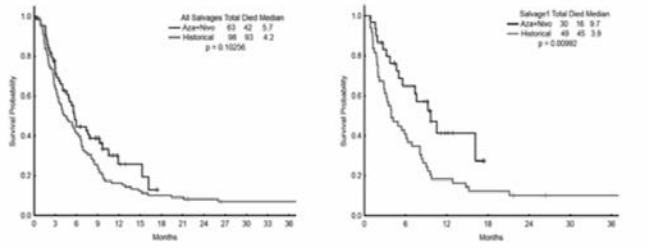

Figure 1.

Summary/Conclusions: Full dose AZA and nivolumab are tolerable and produce an encouraging response rate with durable responses in relapsed AML with poor risk features. Immune mediated toxicities occur and may be adequately managed with early recognition and systemic steroids. Up-regulation of CTLA4 may be a mechanism of resistance to PD1 based therapies in AML and suggest role for combination therapy.

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QUIZARTINIB AND BRIDGE TO TRANSPLANT IN FLT3-ITD AML PATIENTS AFTER FAILURE OF SALVAGE CHEMOTHERAPY: A HISTORICAL COMPARISON WITH UK NATIONAL CANCER RESEARCH INSTITUTE (NCRI) DATA

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Background: The presence of a FMS-like tyrosine kinase 3 (FLT3) Internal Tandem Duplication (ITD) mutation in pts with AML is associated with an increased early relapse rate and a dismal prognosis. Quizartinib is a potent, selective oral FLT3 receptor tyrosine kinase inhibitor that conferred median OS (mOS) of 23 weeks and remission rate of 46% in a single-arm phase 2 study (AC220-002) in pts with AML with a FLT3-ITD mutation who were relapsed or refractory (R/R) to second line therapy. (Levis, *et al.*, ASH 2012) As context, a study of AML pts, regardless of FLT3 mutation status, receiving second-salvage therapy reported mOS of only 1.5 months. (Giles F, *et al. Cancer* 104 (3), 2005). Such poor-risk pts may benefit from a stem cell transplant (SCT), if available.
Aims: The primary aim was to compare SCT rates and outcomes of pts on quizartinib from an exploratory selected cohort in the AC220-002 study with those from a historical cohort of 1388 AML pts with confirmed FLT3-ITD mutations in the UK NCRI database.
Methods: Within AC220-002, 58 pts with a FLT3-ITD mutation were identified who had received intensive chemotherapy, and were relapsed (n=53), or refractory (n=5) to salvage therapy prior to entry. Applying the same entry criteria to the NCRI database, we identified 118 pts who received only recognized chemotherapy regimens prior to eligibility (relapsed n=99; refractory n=19). To avoid biases where those dying early would predominantly contribute to the NCRI group (reflecting that pts in AC220-002 had to be fit enough to be enrolled), pts in this cohort entered analysis 14 days following being identified as R/R. Multivariable Cox/logistic regression was used to compare remission rates and survival stratified for known prognostic factors. A landmark analysis excluding deaths before day 90 (allowing for those too unfit for SCT) was performed on the pooled sample (n=176) of the AC220-002 and NCRI cohorts to compare survival between transplanted and non-transplanted pts.
Results: Overall, quizartinib-treated pts had significantly greater remission rates, consisting mainly of complete remission without normal blood counts (CRi), *vs* NCRI pts (40% *vs* 3%, adjusted OR 0.05 (0.01-0.21), p<0.0001) and improved mOS (140d *vs* 54d, adjusted HR 0.38 (0.25-0.58) p<.0001). A greater proportion of pts in AC220-002 proceeded to SCT: 23/58 (40%) *vs* 9/118 (8%). Comparing survival in SCT *vs* no-SCT in a landmark analysis, 18-month survival was significantly greater in the SCT group (29% *vs* 7%, adjusted HR 0.36 (0.20-0.65) p=0.0005). Significance persisted in sensitivity analyses with the landmark set at 120 or 150 days indicating an association between long-term survival and SCT. A similar analysis in an unmatched cohort consisting of SCT-naïve pts in first relapse also found better survival for SCT *vs* no-SCT, confirming a potential benefit of SCT in this poor risk population.
Summary/Conclusions: When compared to a large historical cohort, quizartinib was associated with greater remission rates and opportunity to receive SCT in pts who relapsed after salvage therapy. While varying practice patterns and patient factors obviously influence treatment choices and outcomes, pts with AML with FLT3-ITD mutation appeared to benefit with longer survival observed with SCT. This data suggests quizartinib may show promise in potentially improving long-term survival by bridging patients to SCT.

Immunotherapy in ALL

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GLOBAL REGISTRATION TRIAL OF EFFICACY AND SAFETY OF CTL019 IN PEDIATRIC AND YOUNG ADULT PATIENTS WITH RELAPSED/REFRACTORY (R/R) ACUTE LYMPHOBLASTIC LEUKEMIA (ALL): UPDATE TO THE INTERIM ANALYSIS

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Background: The CD19-targeted chimeric antigen receptor (CAR) T-cell therapy CTL019, an investigational therapy that reprograms cytotoxic T cells to eliminate target cells, resulted in high response rates and a manageable safety profile in pediatric/young adult patients (pts) with R/R B-cell ALL in a single-center trial.
Aims: We report an updated interim analysis from the first multicenter global pivotal registration trial of a CAR T-cell therapy (ELIANA; NCT02435849) including data for 68 pts infused with CTL019, 50 of whom were followed for ≥6 mo.
Methods: This is a single-arm, open-label, multicenter, global, phase 2 study of CTL019 in pediatric/young adult pts with CD19+ R/R B-cell ALL with ≥5% bone marrow lymphoblasts by morphology. CTL019 was manufactured from leukapheresed autologous peripheral blood T cells at a centralized manufacturing facility. The primary endpoint was overall remission rate (complete remission [CR] + CR with incomplete blood count recovery [CRi]) within 3 mo. Secondary endpoints included duration of remission (DOR), overall survival, safety, and cellular kinetics.
Results: As of November 2016, 88 pts were enrolled. There were 7 (8%) manufacturing failures, 9 (10%) pts were not infused due to death or adverse events (AEs), and 4 pts (5%) were pending infusion at the time of data cutoff. Following lymphodepleting chemotherapy in most pts (fludarabine/cyclophosphamide [n=64]) or other [n=1]), 68 pts were infused with a single dose of CTL019 (median dose, 3.0×10^6 [range, 0.2-5.4×10^6] transduced CTL019 cells/kg), with a median study follow-up of 6.4 mo. Median age was 12 y (range, 3-23 y); 59% of pts had prior allogeneic stem cell transplant (alloSCT). Five infused patients had not reached 3 mo of follow-up; among 63 evaluable pts, 52 (83% [95% CI, 71%-91%]) achieved CR/CRi within 3 mo of CTL019 infusion, all of whom had minimal residual disease–negative marrow. The relapse-free probability at 6 mo after remission onset was 75% (95% CI, 57%-87%; median DOR not reached). The probability of survival was 89% (95% CI, 77%-94%) at 6 mo and 79% (95% CI, 63%-89%) at 12 mo. Seven pts (13% of responders) proceeded to alloSCT within 6 months while in remission. Cytokine release syndrome (CRS) was graded using the UPenn scale and managed using a protocol-specific algorithm; CRS occurred in 78% of pts (21% grade 3; 27% grade 4); no CRS-associated deaths occurred. 38% of pts received tocilizumab for treatment of CRS with or without other anti-cytokine therapy. Most common grade 3/4 nonhematologic AEs (>15%) other than CRS were hypotension (22%), hypoxia (18%), and increased aspartate aminotransferase (16%). The incidence of serious AEs within 8 weeks of infusion was 69%. 15% of pts experienced grade 3 neuropsychiatric AEs, with no grade 4 events and no cerebral edema reported. Grade 3/4 neutropenia with high (>38.3°C) fever occurred in 60% of pts. 2 pts died within 30 days of infusion (ALL progression, n=1; cerebral hemorrhage, n=1), and 9 pts died >30 days after infusion (ALL relapse/progression, n=6; HHV-6 encephalitis, pneumonia, systemic mycosis, n=1 each). CTL019 expansion *in vivo* correlated with CRS severity, and persistence of CTL019 along with B-cell aplasia in peripheral blood was observed for ≥1 year in some responders.
Summary/Conclusions: The ELIANA study confirmed the efficacy of a single infusion of CTL019, without additional therapy, observed in a previous interim analysis and a prior single-center CTL019 trial. AEs were effectively and reproducibly managed globally by appropriately trained personnel at study sites.

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CTL019 CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS IN PEDIATRIC PATIENTS (PTS) WITH RELAPSED OR REFRACTORY (R/R) ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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Background: CTL019 is an investigational therapy whereby autologous T cells are genetically engineered with a chimeric antigen receptor (CAR) to identify and eliminate CD19-expressing malignant B cells. Data from 2 phase 2 studies (ELIANA; NCT02435849 and ENSIGN; NCT02228096) in pediatric and young adult R/R B-cell ALL were pooled to evaluate cellular kinetics of CTL019.
Aims: We report cellular kinetics, humoral immunogenicity, AUC0-28d (exposure)-response analysis and impact of intrinsic/extrinsic and manufacturing factors on CTL019 expansion.
Methods: Cellular kinetic parameters of CTL019 post infusion were derived using traditional pharmacokinetic principles and reported by response category (complete response [CR]/CR with incomplete blood count recovery [CRi] *vs* no response [NR]) using 2 assays of peripheral blood cells: qPCR and flow cytometry. AUC0-28d-response relationships were evaluated by logistic regression. Relationships between manufacturing specifications, therapies for cytokine release syndrome (CRS) management, and anti-CAR19 antibodies on cellular kinetics were explored using summary statistics and graphical- and model-based analyses.

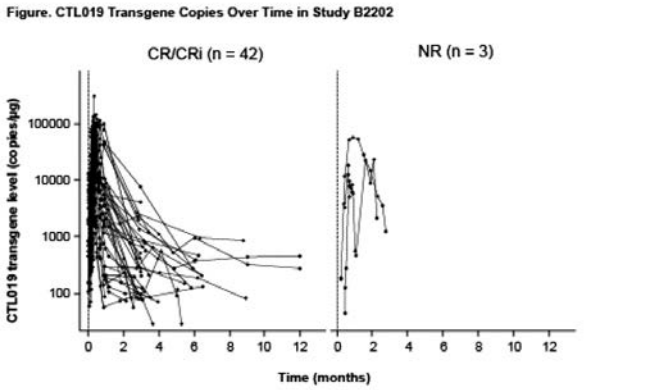

Figure 1.

Results: Data from 79 pts (ELIANA, n=50; ENSIGN, n=29) were pooled for analysis. Using qPCR, pts with CR/CRi (n=62) had ≈ 2-fold higher CTL019 expansion than pts with NR (n=7) (Cmax, 73.5% higher geometric [geo] mean; AUC0-28d, 104% higher geo mean; **Table 1**). Pts with NR had delayed Tmax compared with pts with CR/CRi (20 *vs* 10 days). Intrinsic pt factors including baseline cytogenetics, disease characteristics, and disease status did not appear to affect Cmax or AUC0-28d with the exception that pts with a higher tumor burden at enrollment generally had higher expansion, based on box plots and summary statistics. Extrinsic factors (prior lines of therapy, stem cell transplant) and parameters related to the manufactured product (% T cells, transduction efficiency, cell viability, total cell count), did not appear to impact cellular kinetics, based on graphical analysis. AUC0-28d increased with pres-

ence and severity of CRS. Pts who received anti-cytokine agents for grade 3/4 CRS also had higher expansion. CR/CRi pts treated with tocilizumab and steroids (n=17) had 89% higher AUC0-28d than CR pts who did not receive tocilizumab and steroids (n=45). Experience is limited in NR pts with (n=4) and without (n=4) tocilizumab. Moderate correlation was observed between transgene levels and CAR surface expression in peripheral blood (r^2=0.592) by qPCR and flow cytometry, respectively, when matched by time points from the cellular kinetic profile. Slower B-cell recovery was observed in pts with AUC0-28d above the median. Post-dose anti-CAR19 antibody responses were determined from the fold change of anti-CAR19 antibodies above the baseline predose value. Pts with treatment-induced or boosted anti-CAR19 antibody responses generally had lower expansion, based on box plots, compared with pts with treatment-unaffected anti-CAR19 antibody responses, although AUC0-28d was variable. The boosted levels of anti-CAR19 did not impact clinical response or relapse.

Table 1.

Table. Summary of Cellular Kinetic Parameters for CTL019 by qPCR by Day 28 Response

Parameter	Statistics	CR/CRi (n = 62)	NR (n = 7)
AUC0-28d (copies/µg DNA × days)	n	61	6
	CR/CRi fold change over NR	2.0	
Cmax (copies/µg)	n	61	7
	CR/CRi fold change over NR	1.7	
Tmax (days)	n	61	7
	Median (range)	9.91 (0.00764-27.0)	20.0 (0.0278-62.7)
T1/2 (days)	n	54	3
	Median (range)	14.8 (2.03-206)	1.48 (1.12-9.65)

• Unknown response, n = 10

Summary/Conclusions: There was increased expansion of CTL019 in pts with higher tumor burden at enrollment, which correlated with higher CRS grade. There was no relationship between dose and expansion, supporting the wide dose range used. Expansion was not attenuated by tocilizumab or steroids, indicating therapies for CRS do not abrogate CTL019 proliferation. Cellular kinetics are important to understand the determinants of tumor response with CAR T-cell therapy.

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BLINATUMOMAB *VS* SOC CHEMOTHERAPY IN FIRST SALVAGE COMPARED WITH SECOND OR GREATER SALVAGE IN A PHASE 3 STUDY

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Background: Adults with B-cell precursor (BCP) acute lymphoblastic leukemia (ALL) often relapse following standard induction/consolidation chemotherapy (CTX). Prognosis following second and successive CTX salvage regimens (S2+) is poor compared with first salvage (S1) or frontline therapy, with less favorable outcomes among patients with shorter CR duration. Blinatumomab links cytotoxic CD3-positive T cells and CD19-positive B cells to induce tumor cell lysis. In a randomized phase 3 trial of blinatumomab *vs* investigator's choice of 4 standard of care CTX (SOC) regimens, median OS was 7.7 months in the blinatumomab group *vs* 4.0 months with SOC (Kantarjian H, *et al.*, NEJM 2017). Here, we evaluate outcomes by salvage status for patients in this study (NCT02013167).
Aims: To evaluate responses to blinatumomab *vs* SOC in patients with relapsed/refractory ALL by prior salvage therapy status.
Methods: Patients with relapsed/refractory (R/R) BCP-ALL in this international multicenter trial were randomized 2:1 to blinatumomab (n=271) or SOC (n=134). For this analysis, salvage status was adjudicated separately from prior randomization strata. Blinatumomab was given by continuous IV infusion (9 µg/d in week 1 of cycle 1, then 28 µg/d) in cycles of 4 weeks on, 2 weeks off. The primary endpoint was overall survival (OS), determined from time of randomization until death due to any cause. Adverse events (AE) of interest were coded according to MedDRA version 16.0.
Results: At baseline, patient characteristics were balanced between groups within salvage designations. The rate of complete remission, with or without full hematologic recovery (CR/CRh/CRi) in both the S1 and S2+ groups was higher in the blinatumomab arm compared with the SOC arm (Table 1). Patients randomized to blinatumomab had a median (95% CI) of 11.1 (8.2, NR) months *vs* 5.1 (3.2, 7.1) months overall survival for S1 *vs* S2+ subgroup, compared with 5.5 (3.7, 9.0) months *vs* 3.0 (2.1, 4.0) months in the SOC arm (Figure 1). For both S1 and S2+ subgroups, blinatumomab patients had longer median survival time. Grade 3 or worse AEs were experienced by 61% and 83% of S1 patients in the blinatumomab and SOC group, respectively. These percentages were 68% and 75%, respectively, in S2+ patients. Grade 4 or worse AEs occurred in 34% and 51% S1 patients, and in 36% and 54% S2+ patients. Neurologic events of grade ≥3 occurred in 9% and 9% of S1 patients, and in 10% and 9% S2+ patients, respectively. Grade ≥3 cytokine release syndrome (CRS) was observed in 4% S1 and 5% S2+ patients receiving blinatumomab, and in no SOC patients.

Table 1.

	No prior salvage (S1)		Any prior salvage (S2+)	
	Blinatumomab (n=104)	SOC (n=63)	Blinatumomab (n=167)	SOC (n=71)
Age ≥35 years, n (%)	65 (62.5)	37 (58.7)	82 (49.1)	37 (52.1)
Prior HSCT, n (%)	29 (27.9)	20 (31.7)	65 (38.9)	26 (36.6)
First relapse with remission duration <12 mo, n (%)	58 (55.8)	30 (47.6)	51 (30.5)	19 (26.8)
Maximum blasts ≥50% by central/local lab, n (%)	78 (75.0)	45 (71.4)	123 (73.7)	59 (83.1)
K-M Median OS, mo (95% CI)	11.1 (8.2, NR)*	5.5 (3.7, 9.0)	5.1 (3.2, 7.1)	3.0 (2.1, 4.0)
	HR 0.59 (95% CI 0.38, 0.91) P=0.016		HR 0.72 (95% CI 0.51, 1.01) P=0.055	
Best response (CR/CR/CRi), n (%) [95% CI]	53 (51.0) [41.0, 60.9]	23 (36.5) [24.7, 49.6]	66 (39.5) [32.1, 47.4]	10 (14.1) [7.0, 24.4]
	P=0.07		P<0.001	

**NR=not reached*

Figure. Overall survival among patients with no (S1) or prior (S2+) salvage treatment

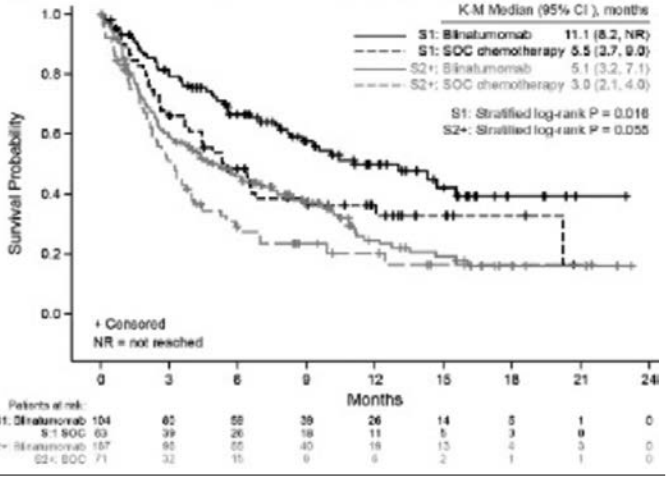

Figure 1.

Summary/Conclusions: Patients in this trial receiving blinatumomab for R/R ALL achieved improved OS and remission rates compared with SOC regardless of prior salvage therapy. Improved OS compared with SOC in S1 patients supports earlier use of blinatumomab.

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DURABLE LONG-TERM SURVIVAL OF ADULT PATIENTS WITH B-ALL AFTER CD19 CAR (19-28Z) T CELL THERAPY

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Background: CD19-specific chimeric antigen receptor (CAR) T cells have demonstrated high initial responses in patients with relapsed B-ALL. However, clinical characteristics associated with the durability of response remain undefined.
Aims: We performed a retrospecive analysis of our phase I clinical trial of 19-28z CAR T cells in adult patients with relapsed B-ALL (NCT01044069) with a focus to identify those patients who optimally benefit from 19-28z CAR T cell therapy with durable long-term survival and reduced toxicities.
Methods: Adults with relapsed B-ALL were infused with autologous T cells expressing the 19-28z CAR following conditioning chemotherapy. Disease burden was assessed by bone marrow biopsy immediately prior to T cell infusion; patients with <5% blasts were classified as minimal residual disease (MRD) cohort *vs* patients ≥5% blasts as morphologic disease cohort. Response assessment occurred at 4 weeks. Median follow-up duration was 18 months (range, 0.2-57.3).
Results: 51 adults received 19-28z CAR T cells; 20 in the MRD and 31 in the morphologic cohort. Complete remission (CR) rates were comparable (95% and 77%, respectively). However, median event-free and overall survivals widely diverged among the 42 patients who achieved MRD-negative CR: not reached (NR) (95% confidence interval [CI]: 4.2-NR) *vs* 6.3 months (95% CI, 4.8-9.0) (p=0.0005), and NR (95% CI, 15.3-NR) *vs* 17 months (95% CI, 8.5-36.2) (p=0.0189), in the MRD and morphologic cohorts, respectively. Subsequent allogeneic HSCT in either cohort did not improve survival (p=0.8). MRD cohort patients developed substantially less severe cytokine release syndrome (CRS) and neurotoxicity, and both toxicities significnatly correlated with peak

CAR T cell expansion (p=0.0326 and p=0.0001, respectively). No case of cerebral edema was observed.
Summary/Conclusions: Despite comparable initial CR rates regardless of pre-treatment disease burden, durability of 19-28z CAR T cell mediated remissions and survival in adult patients with relapsed B-ALL positively correlated to a low disease burden and do not appear to be enhanced by allogeneic transplant. Our findings strongly support the early incorporation of CD19 CAR therapy before morphologic relapse in B-ALL.

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STANDARD-RISK RANDOMIZATION OF PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA IN TRIAL AIEOP-BFM ALL 2000 INDICATES EQUAL OUTCOME WITH REDUCED-INTENSITY DELAYED INTENSIFICATION IN ETV6-RUNX1-POSITIVE PATIENTS

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Background: *ETV6-RUNX1* fusion is a common genetic aberration in childhood acute lymphoblastic leukemia (ALL) and is associated with good prognosis in the context of contemporary treatment regimens. The required treatment intensity for this well-described biologic subgroup with low risk of relapse is not known so far. In trial AIEOP-BFM ALL 2000, feasibility of reduction of delayed intensification treatment to reduce the burden of chemotherapy was tested in a randomized approach in the standard-risk group. Treatment reduction was not successful in the total cohort (8-year probability of disease-free survival (8y-pDFS, ± standard error) 89.2±1.3% for reduced delayed intensification treatment, 92.3±1.2% for the standard treatment (log-rank *P*=0.04) due to evidence of more relapses observed in patients treated less intensively.
Aims: The retrospective subgroup analysis presented here focuses on the *ETV6-RUNX1*-positive patients included in the group of randomized standard-risk patients.
Methods: From 07/2000 to 06/2006, 4741 eligible patients with ALL (age range 1-17 years) were enrolled in the trial AIEOP-BFM ALL 2000 (NCT 00430118 (BFM) and NCT 00613457 (AIEOP)). Of those, 1164 patients were considered at standard risk of relapse, defined by lack of genetic high-risk criteria and absence of minimal residual disease at day 33 and week 12 of treatment (tested by immunoglobulin/T-cell receptor gene rearrangement polymerase chain reaction). They were randomly assigned to either receive the reduced-intensity protocol III (P-III), or standard protocol II (P-II) for delayed intensification. P-III is shorter than P-II (duration 29 *vs* 49 days), the dose of dexamethasone in P-III is 30% lower, and the dose of vincristine, doxorubicin, and cyclophosphamide are reduced by 50% as compared to P-II. The intention was to prove non-inferiority of the reduced-intensity treatment compared to standard treatment.
Results: *ETV6-RUNX1*-positive patients (n=367) accounted for 34% of randomized standard-risk patients (Age: <6 years n=260, 6 to <10 years n=79, ≥10 years n=28; early cytologic response evaluation in bone marrow on day 15 of induction treatment: M1 n=218, M2 n=74). Of those, 188 were treated with the experimental P III, 179 received the standard P-II. With a median follow-up of 8.6 years, the as-treated analysis showed an 8y-pDFS of 94.5±1.7% for P-III, and 94.4±1.8% for patients with P-II (log-rank *P*=0.74). Cumulative incidence of relapse at 8 years was 3.3±1.3% and 4.3±1.6% (Gray *P*=0.09), and 8-year overall survival was 96.9±1.4% and 98.8±0.9% (*P*=0.27) for P III and P II, respectively. Analysis of *ETV6-RUNX1*-positive patients by age groups or treatment response on day 15 allowed no further refinement of prognostic subgroups.
Summary/Conclusions: There was no evidence of prognostic disadvantage in *ETV6-RUNX1*-positive standard-risk patients when treated with the reduced-intensity experimental arm. No clear age- or response-dependent differences could be revealed for this group, which is in line with the biologic understanding of this genetic subgroup. Hence, it might be postulated that treatment reduction might be feasible in this clearly defined biologic subgroup. However, the present data is not result of a sufficiently powered non-inferiority study question focused on the subgroup of *ETV6-RUNX1*-positive patients, but reflects a subgroup analysis with descriptive character. Therefore, any decision for treatment reduction should be considered carefully.

Biology and disease monitoring in CML

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A SECOND GENERATION LYSOSOMOTROPIC AGENT DRIVES LEUKAEMIC STEM CELL DIFFERENTIATION AND SENSITIZES THEM TO TYROSINE KINASE INHIBITOR TREATMENT *IN VITRO* AND *IN VIVO*

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Background: Autophagy is a conserved catabolic process that delivers cytoplasmic constituents to the lysosomes. We have previously shown that the lysosomotropic agent hydroxychloroquine (HCQ) inhibits autophagy and sensitizes Chronic Myeloid Leukaemia (CML) stem cells (LSCs) to tyrosine kinase inhibitors (TKIs) treatment. However, the biological effects of autophagy inhibition in LSCs *in vivo* are currently unknown and remain to be investigated. Furthermore, recent clinical studies showed that maximum tolerated dose of HCQ does not achieve consistent autophagy inhibition in cancer patients. Therefore further pre-clinical studies using more potent 2nd generation lysosomotropic agents, alone and in combination with TKIs, are vital.
Aims: Here we aim to investigate the functional effects of autophagy inhibition in LSCs both *in vitro* and *in vivo* using the highly potent lysosomotropic agent Lys05. Additionally, we aim to address whether Lys05 achieves autophagy inhibition in the most primitive LSC populations *in vivo* and whether it targets LSCs more effectively than HCQ when combined with TKIs.
Methods: In this study, we used primary stem-cell enriched samples ($CD34^+$ cells) derived from CML patients at diagnosis. For *in vivo* studies, we used a human patient-derived xenograft (PDX) model and an inducible transgenic CML model in which the expression of BCR-ABL is induced at a stem/progenitor level (Scl-tTa-BCR-ABL). To accurately measure autophagy flow in long term LSCs *in vivo*, we generated the transgenic mouse Scl-tTa-BCR-ABL/GFP-LC3 by crossing the Scl-tTa-BCR-ABL model with a mouse bearing the autophagy marker LC3 fused to GFP.
Results: Firstly, we show that Lys05 targets LSCs more potently than HCQ *in vitro* by achieving a 60% and a 35% reduction in number of $CD34^+CD38^-$ and $CFSE^{max}/CD34^+CD133^+$cells respectively. Interestingly, Lys05 promoted a 40% loss of quiescent cells and induced myeloid differentiation of $CD34^+$ cells. Functional long-term culture initiating cell (LTC-IC) assay demonstrated that, while HCQ had moderate effects, Lys05 decreased the number of LSC-derived colonies by 80%. Additionally, we show that Lys05 inhibits autophagy flow more efficiently than HCQ both in the Scl-tTa-BCR-ABL/GFP-LC3 model and in patient-derived progenitor cells. Analysis of bone marrow (BM) cells from Lys05-treated leukaemic mice (but not from HCQ-treated mice), showed a statistically significant 35% decrease (p=0.0469) in the most primitive population $Lin^-Sca^+c\text{-}kit^+CD48^-CD150^+$ followed by a 50% increase (p=0.0231) of progenitors $Lin^-Sca^+c\text{-}kit^+$. This result indicates differentiation of LSCs towards a more progenitor phenotype following potent autophagy inhibition. Finally, to test the *in vivo* effect of Lys05 on the most primitive human LSCs we transplanted $CD34^+$ CML cells into irradiated NSG mice. Remarkably, using this PDX model we show that while 3 weeks *in vivo* treatment with HCQ had no effects when combined with TKIs, Lys05 and TKI treatment nearly eliminated engrafted primitive Philadelphia positive $CD34^+CD38^-$ and $CD34^+CD133^+$cells.
Summary/Conclusions: Overall, we demonstrate that lysosomal inhibition induces loss of quiescence and drives differentiation of LSCs *in vitro* and *in vivo*. Furthermore, our results show that Lys05 achieves autophagy inhibition in LSCs and effectively sensitizes LSCs to TKIs *in vitro* and *in vivo*. Therefore, 2nd generation lysosomotropic agents should be considered as a potential alternative to HCQ in order to eliminate LSCs and achieve cure for CML patients.

S482

FC GAMMA RECEPTOR 2B IS CRITICAL FOR BCR-ABL MEDIATED LEUKEMOGENESIS

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Background: Chronic myeloid leukemia (CML) is provoked by the chromoso-

mal translocation t(9;22) that gives rise to the oncogenic tyrosine kinase Bcr-Abl. Implementation of tyrosine kinase inhibitor (TKI) therapy resulted in significant clinical success but with TKIs failing to eradicate the disease initiating leukemic stem cell population (LSC), this treatment is not curative in the vast majority of patients. By using a transgenic CML mouse model, we previously showed that LSC persist despite complete Bcr-Abl kinase inhibition due to a lack of oncogene-addiction. Subsequently, we identified the ITIM carrying Fc gamma receptor IIb (FcγRIIb; CD32) to be 2.8-fold upregulated in Bcr-Abl$^+$ *versus* control LSK (lin$^-$;Sca-1$^+$;c-kit$^+$) cells using microarray and qRT-PCR.

Aims: In this study, we first aimed to validate Bcr-Abl mediated FcγRIIb upregulation on mRNA and protein level in leukemic cells. Next, we tested the effect of shRNA mediated FcγRIIb knock-down and depletion on CFU (colony forming unit) capacity, proliferation and leukemic signaling *in vitro*. Finally, we studied the disease-initiating potential of primitive CML stem and progenitor cells upon FcγRIIb knock down.

Methods: qRT-PCR and western blot analyses were applied using cell lines, primary murine cells and HoxB8 immortalized murine bone marrow (BM) cells for studying FcγRIIb expression and signaling. In order to test the biology of CML cells *in vitro,* we performed CFU and proliferation assays. Moreover, we performed viral infection of 5-FU treated SCLtTA/Bcr-Abl BM using FcγRIIb:shRNA or scrambled control and subsequent transplantation, followed by analyses of the disease, including immune-phenotyping, RNA and protein expression as well as histological analysis.

Results: Bcr-Abl increased FcγRIIb mRNA (13.2-fold, p≤0.001) and protein expression in primary murine lineage negative (lin$^-$) BM cells. Reduction of FcγRIIb in immortalized SCLtTA/Bcr-Abl progenitor cells significantly reduced CFU potential by nearly 10-fold (p≤0.01) and it impaired the proliferation rate in these cells (2.27-fold, p≤0.001). Moreover, transplantation of SCLtTA/Bcr-Abl-shRNA:FcγRIIb BM cells (CD45.1$^+$) into FVB/N wildtype (WT) CD45.2$^+$ recipients reduced spleen weight (352 ± 59.13mg), as compared to scrambled shRNA (568.1 ± 101.72mg). FACS analysis revealed a decrease in GFP$^+$;CD45.1$^+$ BM cells (1.43-fold, p≤0.001) upon FcγRIIb knock down. Likewise, donor-derived Gr-1$^+$ cells (Gr-1$^+$;CD45.1$^+$;GFP$^+$) were reduced in the BM (1.28-fold, p≤0.01) of these mice. Flow-cytometric analysis of the stem cell compartment revealed decreased leukemic BM LSK cells (lin$^-$, c-kit$^+$, Sca-1$^+$, CD45.1$^+$, GFP$^+$, 1.38-fold, p≤0.05) in mice transplanted with shRNA:FcγRIIb *vs* scrambled control. We previously observed similar effects upon FcγRIIb depletion (FcγRIIb$^{-/-}$) *vs* wildtype (FcγRIIb$^{+/+}$), combined with virally induced Bcr-Abl expression. Interestingly, Bcr-Abl signaling induces FcγRIIb phosphorylation in leukemic cells. Analysis of downstream signal pathways showed decreased levels of p-ERK, p-BTK, p-PLCγ1 in FcγRIIb$^{-/-}$, compared to FcγRIIb$^{+/+}$ Bcr-Abl transduced immortalized primary murine BM cells.

Summary/Conclusions: FcγRIIb is upregulated in LSC derived from transgenic CML mice upon Bcr-Abl expression. Complete depletion or knock down of the receptor reduces CFU capacity and cell growth in CML cells and significantly impairs CML development and LSC burden *in vivo,* presumably due to impaired leukemic downstream signaling. Our data demonstrate that FcγRIIb is critical and disease specific making it a potential novel therapeutic target in CML stem cells.

S483

MYC-DEPENDENT REPRESSION MECHANISM OF THE MIR-150 TRANSCRIPTIONAL REGULATION IN CHRONIC MYELOID LEUKEMIA

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Background: The expression of miRNAs is regulated at transcriptional and posttranscriptional levels. Dysregulation of miRNAs could directly induce or be a consequence of oncogenic pathways. Chronic myeloid leukemia (CML) is characterized by reduced miR-150 levels leading to an insufficient repression of its target, oncogene MYB. CML treatment with imatinib normalizes miR-150 levels. Thus miR-150 is crucial for CML biology, however, little is known about its upstream transcriptional regulation. MiR-150 is an inhibitor of oncogene MYB, which is required for BCR-ABL1-dependent leukemogenesis in CML blast crisis. Our recent work brought an evidence of mutual BCR-ABL1/MYC/miR-150/MYB regulatory links in CML, sustained in CML resistant cells. We found low levels of miR-150 to be a hallmark of CML and impaired signaling pathway MYB/BCR-ABL1/MYC/miR-150/miR-155/PU.1 leading to a progressive cell differentiation block.

Aims: To delineate potential mechanisms of the miR-150 transcription regulation via the oncogenic transcription factor MYC in CML.

Methods: Primary bone marrow cells from CML (N=28), CML cell lines K562 and KCL-22 and imatinib resistant (K562R, KCL-22R). Expression analysis: RT-PCR. Protein levels: WB. ChIP: chromatin from the cell lines. SiRNA inhibition: AMAXA electroporation. DNA methylation analysis: Methylated DNA immunoprecipitation.

Results: We observed that unlike MLL-AML diagnosis (Jiang *et al.* 2012), CML is not characterized by a block of miR-150 maturation and that miR-150 levels negatively correlated with MYC mRNA levels in CML HSPCs (p<0.001). Role of MYC in CML was further strengthen by imatinib induced MYC downregulation and restored miR-150 levels in K562 and KCL22. Imatinib resistance in K562R and KCL-22R was characterized by further miR-150 downregulation. To assess the MYC role on regulating miR-150 levels we tested the MYC binding sites upstream the miR-150 gene. We detected MYC binding to the upstream CpG of the MIR150 gene in K562 and KCL-22. We also found a depletion of MYC from the miR-150 locus after the imatinib treatment. We suggested potentially synergistic route for imatinib-induced BCR-ABL1 inhibition. This could be processed not only directly but also through an inhibition of a mutual positive regulatory loop between MYC and BCR-ABL1 (Xie *et al.* 2002). We also noticed MIR150-neighboring gene FCGRT (which is adjacent to the studied miR-150 CpG) to become activated by imatinib. We observed MYC levels dependent regulation of both genes, but FCGRT is activated by MYC. This different MYC regulatory role may be facilitated by the detected transcription factor CTCF binding to an insulator site between miR-150 promoter and the CpG. An activation of the insulator via CTCF binding changes an interaction between enhancers and promoters (Bell *et al.* 2000). CTCF was previously described to be an inhibitor of MYC transcription and we show CTCF transcription to be induced by imatinib. CTCF binding to DNA is prevented by DNA methylation. We did not detect DNA methylation within MIR150 upstream region.

Summary/Conclusions: A miR-150 transcription regulation involves transcription factors-dependent epigenetic changes within the promoter and distal enhancers. We outlined a new insight into MYC/MIR150/BCR-ABL1/imatinib regulation loop in CML. Our work revealed a MYC role in miR-150 repression underlying the CML leukemogenesis, where miR-150 functions as a pivotal gatekeeper and its repression is probably required for CML establishment and is enforced in imatinib resistant CML.

Support: LH15104 of MSMT and 00023736 of MZCR

S484

COMPARISON OF GENOMIC DNA AND REVERSE TRANSCRIPTASE Q-PCR FOR THE MONITORING OF FIRST-LINE IMATINIB TREATMENT: AN ALLG CML9 SUB-STUDY

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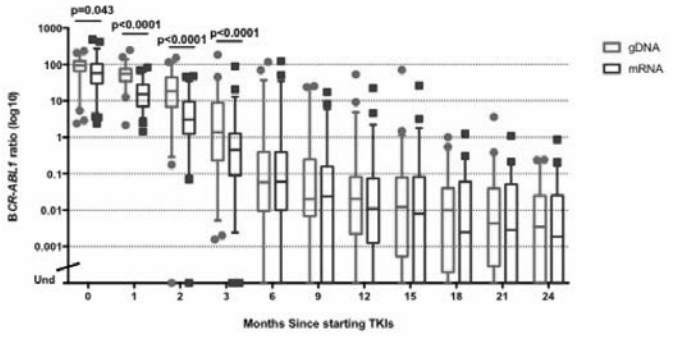
Background: Real-time reverse transcriptase quantitative PCR (RQ-PCR) for *BCR-ABL1* mRNA is widely used for the monitoring of chronic myeloid leukaemia (CML). Pre-analytical factors, such as the rate of degradation of the target mRNA, and methodological factors, such as the choice of control gene, may influence the final result. In contrast the genomic DNA is stable, and the number of copies of *BCR-ABL1* DNA is directly proportional to the number of CML cells. Measuring both DNA and RNA may enable us to understand the contribution of expression and cell number to the RQ-PCR response.

Aims: To compare *BCR-ABL1* DNA Q-PCR and routine RQ-PCR monitoring of CML.

Methods: Fifty-nine newly diagnosed chronic phase CML patients from the ALLG CML9 (TIDEL II) trial were included in this sub-study. Samples were tested prior to commencing TKI treatment (baseline), at 1, 2, and 3 months, and every 3 months to 24 months (total 568 samples). Since we wanted to compare the sensitivity of the Q-PCR methods we selected 19 patients who had achieved undetectable minimal residual disease (UMRD) by RQ-PCR within 24 months, and an additional 40 patients unselected for response. RQ-PCR results were expressed on the International Scale (IS), whereas DNA results were expressed relative to the individual patient's baseline. Quantification of *BCR-ABL1* DNA was performed using *GUSB* as the control gene by real-time Q-PCR (n=40) or by digital PCR (dPCR, n=19) using the Fluidigm BioMark HD System. The mean detection limit of RQ-PCR was 4.5-log, and 5.4-log for DNA methods.

Results: We first demonstrated that DNA dPCR and real-time Q-PCR gave comparable results: 45 samples from 6 patients were quantified by both methods and logarithmically transformed. The mean bias was -0.15 (1.4-fold) with 95% limits of agreement ranging from -1.19 to 0.88. Subsequently, DNA and mRNA values were compared in paired samples. The median *BCR-ABL1*^{IS} at baseline was 58% (range, 2.4% - 487%) *versus* 93% by DNA methods (range, 2.4% - 235%). Interestingly, *BCR-ABL1* DNA was significantly higher than mRNA at 1, 2, and 3 months (Figure). There was good agreement between positive results from 6 months of TKI therapy onwards (mean bias -0.02; 95% limits of agreement from -1.15 to 1.11). Comparing the limit of detection, *BCR-ABL1* DNA was detectable in 60/148 (41%) samples with undetectable mRNA

(median 0.002%, range 0.0003-0.07%). Finally, 88% (15/17) of patients with e14a2 transcripts achieved MMR by 12 months, in comparison with 63% (20/32) of patients with e13a2 transcripts. *BCR-ABL1* mRNA expression levels were significantly lower in e13a2 patients than in e14a2 patients in all follow-up samples analysed (ratio of *BCR-ABL1* mRNA:DNA for e13a2 0.44 *vs* e14a2 0.57; p=0.016).



Comparison of *BCR-ABL1* DNA and mRNA[IS] levels in 59 TKI-treated patients. DNA values in red are expressed as *BCR-ABL1/GUSB* % and compared with *BCR-ABL1/BCR*[IS] %. Und: undetectable.

Figure 1.

Summary/Conclusions: In the first 1-3 months *BCR-ABL1* mRNA fell more rapidly than DNA, likely reflecting the time taken for normal haematopoietic cells to recover. At later time-points there was good agreement between methods, indicating that later reduction in *BCR-ABL1*[IS] is closely related to depletion of leukaemic cells. Normalised to *BCR-ABL1* DNA the expression of e13a2 *BCR-ABL1* mRNA was lower than that of e14a2, an observation that requires confirmation. DNA methods were more sensitive: following the achievement of UMRD by RQ-PCR patients could, on average, be monitored by DNA Q-PCR for an additional 5 months.

S485

ESTABLISHING A NATIONAL NETWORK OF LABORATORIES USING NEXT GENERATION AMPLICON DEEP SEQUENCING FOR BCR-ABL1 KINASE DOMAIN MUTATION SCREENING: THE 'NEXT-IN-CML' STUDY

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Background: Benchtop next generation sequencers are gradually replacing Sanger sequencers in diagnostics labs because of greater throughput, better sensitivity and increasing cost-effectiveness. In chronic myeloid leukemia (CML) patients (pts) on tyrosine kinase inhibitor (TKI) therapy, BCR-ABL1 kinase domain (KD) mutation screening is a precious tool for timely and rational therapeutic reassessment and is recommended in case of Failure and Warning. A multicenter, multilaboratory prospective study ('NEXT-IN-CML') has been conducted to assess the feasibility, cost, turnaround times and clinical utility of a next generation amplicon deep sequencing (Deep Seq) strategy for routine BCR-ABL1 KD mutation screening.

Aims: The first phase of the study was aimed to i) create a network of 4 labs sharing a common protocol, a joint database for clinical and mutational data storage and a common pipeline of data analysis, interpretation and reporting, and ii) verify accuracy and inter-laboratory reproducibility of results. The second phase of the study, involving 39 Italian Hematology Units, was meant to prospectively assess the frequency of low burden mutations in CML pts with Failure or Warning to any TKI.

Methods: In the first phase, centrally prepared identical batches of 32 blinded samples (24 clinical samples with known mutation status/load as assessed by Sanger Seq plus 8 T315I+ BaF3 cell line dilutions simulating mutation loads between 20% and 1%) were distributed and analyzed in parallel by each of the 4 participating labs. In the second phase, 159 consecutive CML pts were prospectively studied in parallel by Sanger Seq and by Deep Seq: 101 Failures (57 pts on 1st-line TKI [IM, n=38; DAS, n=12; NIL, n=7] therapy; 35 pts on 2nd-line TKI [DAS, n=14; NIL, n=17; IM, n=2; BOS, n=1; PON, n=1] therapy; 5 pts on 3rd-line TKI [DAS, n=4; NIL, n=1] therapy and 4 pts on 4th-line TKI [BOS, n=1; PON, n=3] therapy) and 58 Warnings (38 on 1st-line TKI [IM, n=28; DAS, n=4; NIL, n=5; BOS, n=1] therapy and 20 on 2nd-line TKI [NIL, n=10; DAS, n=9; PON, n=1] therapy).

Results: In the first phase, 504/512 amplicons were successfully generated and sequenced, with a median number of forward and reverse reads of 1,757 (range 544-5,838). In the 128 samples analyzed, 51/52 expected mutations were consistently detected by all 4 labs and quantitation of mutation load was highly reproducible across a wide range of frequencies (2%>100%). Three out of 4 labs failed to detect the 1% T315I+ dilution. In clinical samples, additional low burden mutations <3% were occasionally called by one or two labs only, suggesting that this value should be taken as a threshold below which mutation detection is not reproducible and sequencing artifacts and errors cannot be ruled out. In the second phase of the study, pts positive for mutations were 25/159 (16%; 23 Failures and 2 Warnings) by Sanger Seq and 52/159 (33%; 44 Failures and 8 Warnings) by Deep Seq. Among the pts with low burden mutations detectable by Deep Seq, 4 had a T315I; 34 had other known TKI-resistant mutations; 14 had only mutations with unknown clinical significance. Pts positive for mutations by Deep Seq were more frequent in the High and Intermediate Sokal risk group. The number of positive pts and the number of mutations per pt were not significantly higher in those receiving 2nd- or subsequent-line TKI therapy than in those receiving 1st-line TKI therapy. Compound mutations were found only in 2 out of 52 mutated pts (both in blastic phase).

Summary/Conclusions: 1) Results of the 'NEXT-IN-CML', the first prospective study evaluating the routine diagnostic use of Deep Seq of BCR-ABL1, show that this technology can successfully be implemented in national lab networks and is feasible, robust and reproducible; 2) in a relatively large, nonselected cohort of CML pts analyzed for mutations because of a Failure or Warning response, Deep Seq confirmed that enhancing sensitivity enables to detect BCR-ABL1 KD mutations in twice as many pts as compared to Sanger Seq (33% *vs* 16%); 3) all the pts who need to be switched to another TKI would benefit from sensitive BCR-ABL1 KD mutation screening by Deep Seq.

Prognostic markers and new treatment in MDS

S486

PATIENTS WITH IDIOPATHIC CYTOPENIA OF UNDETERMINED SIGNIFICANCE SHOW SIMILAR SURVIVAL PATTERNS AS LOW RISK MDS PATIENTS

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Background: Cytopenia is a hallmark in myelodysplastic syndrome (MDS), however, many patients with persistent cytopenia do not fulfill the criteria for MDS. These patients are now classified as idiopathic cytopenia of undetermined significance (ICUS) or if a mutation is detected as clonal cytopenia of undetermined significance (CCUS). Little is known about these new entities in regards to survival and prognostication.
Aims: In this study we want to compare ICUS patients with MDS patients having low- or very low-risk disease according to the IPSS-R. We also wanted to investigate if sequencing of the cohort could bring additional information in regards to overall survival.
Methods: All patients underwent a bone marrow biopsy, cytogenetics and a broad range of blood tests. Furthermore, all ICUS patients underwent a blinded morphology review by two experienced pathologists; these review data will be ready for presentation at EHA. ICUS was defined as persistent cytopenia for more than six months, no chromosomal aberrations and common causes of cytopenia were ruled out. The patients were sequenced with a targeted sequencing panel, either using a customized Haloplex panel or a customized sequencing panel for the Ion Torrent platform. We analyzed 20 genes which are the most commonly mutated genes in MDS.
Results: So far we included 157 patients, 122 were classified as ICUS and 35 as MDS and the median age is 65 and 68 years, respectively (p=0.27). We have sequenced 78% of the ICUS patients and 74% of the MDS patients. In total 53% and 73% of the ICUS and MDS patients had at least one mutation detected, respectively. If the patients carried a mutation, the median number of mutations was two in both the CCUS and the MDS group. The most commonly mutated genes were *TET2*, *SRSF2*, *DNMT3A* and *ASXL1* in 38 patients (31%), n=16 (13%), n=10 (8%), n=10 (8%), respectively. There were no significant differences in the distribution between the two groups. Mutations in *NRAS*, *KRAS*, *TP53* were only identified in one patient each. The overall survival between the ICUS and the low-risk MDS patients did not differ (p=0.18) (figure 1). We also subdivided the ICUS patients into non-clonal ICUS and CCUS, but observed no difference between these two groups (p=0.355).
Eight of the patients categorized as ICUS progressed to a myeloid neoplasm during the follow up, and of these seven had a detectable mutation at time of enrollment, only one ICUS patient without a detectable mutation progressed (p=0.06).

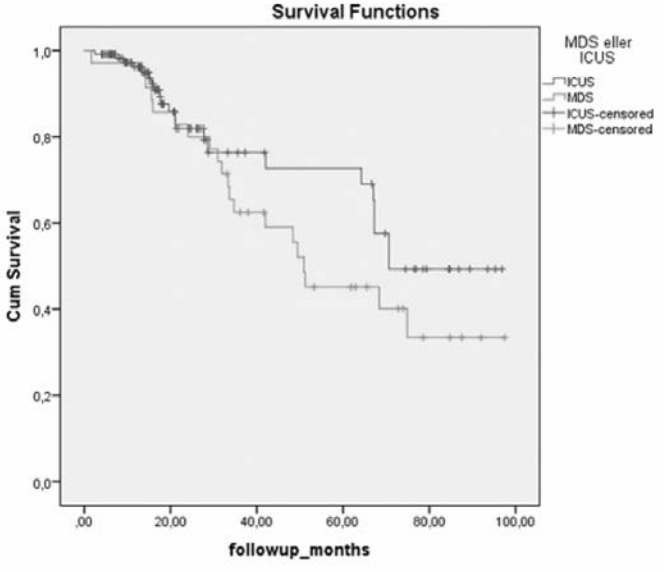

Figure 1.

Summary/Conclusions: We here demonstrate that low-risk MDS and ICUS patients share similar survival patterns, however, larger studies with longer follow up are needed. Mutations are most commonly found in the epigenetic regulators in this cohort of ICUS and low-risk MDS, while mutations in classical tumor suppressors and oncogenes such as *TP53* and *NRAS* are rare. Mutational screening seems promising in detecting patients at risk of progression, however, other biomarkers for prognostication are warranted.

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AN UPDATE OF A PHASE II STUDY OF NIVOLUMAB (NIVO) OR IPILIMUMAB (IPI) WITH AZACITIDINE IN PTS WITH PREVIOUSLY TREATED OR UNTREATED MYELODYSPLASTIC SYNDROMES (MDS)

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Background: Outcomes of pts with MDS after hypomethylating agent (HMA) failure remain poor. Upregulation of PD-1, PD-L1 and CTLA-4 in MDS CD34+ cells after exposure and loss of response to HMA have been reported. Nivo and Ipi are monoclonal antibodies targeting PD-1 and CTLA-4, respectively, with clinical activity in solid tumors.
Aims: To evaluate the potential activity of immune checkpoint antibodies in patients with previously treated or untreated MDS.
Methods: We designed a phase II study of Nivo or Ipi in monotherapy or combination for pts with MDS. Pts with prior therapy with HMA were to be treated in one of 3 consecutive cohorts: cohort #1: Nivo 3mg/kg iv days 1 and 15 of a 28 day cycle; cohort #2: Ipi 3mg/kg iv on day 1 of a 21 day cycle; cohort #3: Nivo 3mg/kg iv on days 1 and 15 + Ipi 3mg/kg iv on day 1 of a 28 day cycle. The study design allowed for AZA add-back after 6 cycles of therapy if there was no response or progression. Pts with previously untreated MDS were to be treated in one of 3 consecutive cohorts combining AZA 75mg/m^2 iv daily days 1-5 of a 28 day cycle with: cohort #4: Nivo 3mg/kg iv Days 6 and 20; cohort #5: Ipi 3mg/kg iv on day 6; and cohort #6: Nivo 3mg/kg iv on days 6 and 20 + Ipi 3mg/kg iv on day 6. The maximum size per cohort is 20 pts. The primary endpoint is to determine the safety of Nivo or Ipi as single agents or in combination with AZA. Secondary objectives included overall response rate (ORR) and assessment of biological activity. Responses were evaluated following the revised 2006 IWG criteria. The study included stopping rules for response and toxicity.
Results: A total of 63 pts have been enrolled, 54 (86%) are evaluable for response and toxicity including 21 treated with frontline AZA+Nivo, and 15 and 18 with Nivo or Ipi after HMA failure, respectively Median age is 69 years (range 39-85). The median number of treatment cycles was 3 (range 1-11). A total of 3 (27%) pts in the AZA+Nivo cohort, 6 (40%) in the Nivo cohort, and 3 (33%) in the Ipi cohort having related grade ≥3 non-hematologic AEs. Therefore, the stopping rule for toxicity was not met in any of the cohorts. Delays of therapy due to AEs were required in 9 pts due to: rash (N=1), adrenal insufficiency (N=1), colitis (N=1), thyroiditis (N=2), pneumonitis (N=3), and nephritis (N=1). Early 8-week mortality occurred in 1 patient due to a non-related intracranial hemorrhage. The ORR was 80% (13/21) in the AZA+Nivo cohort including 6 CR. The ORR was 0% and 30% (5/18) in the Nivo and Ipi arms, respectively. Therefore, the stopping rule for response was met on the Nivo arm, and enrollment after patient 15 was stopped. Immunophenotypic analysis of stem cell and progenitor compartments was performed in 27 pts, including PD-1 and PD-L1 expression analysis in 16 pts. Increased PD-1 and PD-L1 expression on progenitor and stem cell compartments was observed in 3 and 4 pts, respectively. Treatment with PD-1 inhibitors could not overcome the aberrant differentiation patterns. No differences in response were observed based on PD-1 bone marrow expression.
Summary/Conclusions: Preliminary results indicate that PD-1 blockade with Nivo in combination with AZA in untreated higher-risk MDS pts is associated with a tolerable safety profile and clinical activity. Single-agent Ipi is capable of inducing responses in previously treated MDS pts. Single-agent Nivo did not show clinical activity.

S488

ORAL RIGOSERTIB COMBINED WITH AZACITIDINE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) AND MYELODYSPLASTIC SYNDROMES (MDS): EFFECTS IN TREATMENT NAÏVE AND RELAPSED/REFRACTORY PATIENTS

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Background: Azacitidine (AZA) is first line therapy for patients (pts) with higher risk MDS and demonstrated efficacy in older pts with AML (Dombret et al, Blood

2015; Fenaux et al, J Clin Oncol 2010). Rigosertib (RIG) interferes with the RAS-binding domains of RAF kinases and inhibits the RAS-RAF-MEK and the PI3Ks pathways. In vitro, the combination of RIG with AZA synergistically inhibits growth and induces apoptosis of leukemic cells in a sequence-dependent manner (Skidan et al, AACR 2006). RIG's effective inhibition of human hematopoietic tumor cell lines in vitro, favorable clinical adverse event (AE) profile, and its synergy with AZA suggests the potential value of combination treatment.

Aims: Phase I/II results are presented for pts with MDS, either hypomethylating (HMA) treatment naïve or progressing on or failing to respond to prior HMA, and those with AML (per WHO 2002 criteria) with relapsed or refractory disease.

Methods: Oral RIG was administered twice daily on Day 1-21 of a 28-day cycle in escalating cohorts and then at the recommended Phase II dose (560mg qAM and 280mg qPM). AZA 75mg/m^2/d SC or IV was administered for 7 days starting on Day 8. A CBC was performed weekly, and a bone marrow aspirate/biopsy were performed at baseline, D29, and then every 8 weeks thereafter. Response was determined by IWG criteria for MDS (2006) and AML (2003). Stable disease in AML was defined as not meeting criteria for any other treatment response (CR, CRi, PR, disease progression, or treatment failure).

Results: The combination of oral RIG and AZA was administered to 54 pts, of whom 40 had MDS; HMA-treatment-naïve (N=23) and previously HMA-failed pts (N=17). 17 MDS pts received prior HMA therapy: 12 AZA, 4 decitabine, and 1 both. Ten pts had AML, and 6 had CMML. 2 MDS patients with 20-<30% marrow blasts were also included in the AML analysis. Median age was 68 years; 67% of pts were male; and ECOG performance status was 0-1 in 95% of pts. Pts have received 1-37+ cycles of treatment (median, 3.5 cycles), with a median duration of treatment of 17 weeks (range 4 to 158+ weeks). Of the 10 pts with AML, 6 had relapsed AML, 2 secondary AML and 2 with AML transformed from MDS. Eight pts were evaluable for response. There were 3 responses seen, for an ORR of 37.5%, with responses in both secondary and refractory AML. Two additional pts had stable disease (25%). Responses were durable, with the longest response approaching one year (Table 1). Among 33 evaluable MDS pts, overall response by IWG criteria was 76%: complete remission (CR) in 8 (24%), concurrent marrow CR (mCR) and hematologic improvement (HI) in 10 (30%), mCR alone in 6 (18%), and HI alone in 1 (3%). Overall response was 85% in HMA naïve pts and 62% in HMA resistant pts. Correlative studies suggest that RIG has chromatin modifying effects in combination with AZA which may overcome clinical AZA resistance (Chaurasia EHA 2017). Median duration of CR was 8 months for the combination. Median time to initial response was 2 cycles, and median time to best response was 3 cycles. The most frequently reported AEs were diarrhea (70%), nausea (50%), back pain (40%), constipation (40%), fatigue (40%), and peripheral edema (40%).

Table 1.

UPN	Age (years)	Cohort*	DoT (weeks)	Previous Therapy	Status at Study Entry	IWG Response (DoR - weeks)	Reason for Discontinuation
101-003	61	140 bid	4.0	1. Induction 2. Investigational	AML - Refractory	NE	Patient request
101-002	70	140 bid	29.6	Growth Factors	AML - Secondary	MoCR (25.3)	Progressive Disease
102-001	76	140 bid	4.0	Growth Factors	MDS/AML	NE	Toxicity/AE
102-003	78	140 bid	55.1	Growth Factors	MDS/AML	MoCR (43)	Progressive Disease
101-005	73	280 bid	4.0	1. Induction 2. DEC x5	AML – 1st Relapse	TF/I	Death
102-009	71	560/280	12.9	1. Induction x2 2. AZA x25	AML - Relapsed	TF/R	Death
102-007	80	560/280	32.0	AZA x5	AML - Secondary	TF/R	No Hem Response
101-008	57	560/280	8.1	Induction	AML - Refractory	MLFS (4.1)	Inv. Decision
101-009	60	560/280	24.4	Induction	AML - Relapsed	SD	Death
101-007	77	560/280	16.0	1. Induction 2. DEC x12	AML - Relapsed	SD	Progressive Disease

MDS/AML – 20 to <30% blasts
NE – patients off study prior to 12 weeks of combination
MoCR – morphologic complete remission
TF/I – treatment failure/indeterminate
TF/R – treatment failure/resistant
MLFS – morphologic leukemia-free state
SD – stable disease
* Oral rigosertib dose

Summary/Conclusions: The combination of oral RIG and standard-dose AZA was well tolerated in repetitive cycles in pts with AML and MDS. Response was observed both in HMA-treatment-naïve pts (85%) and in pts failing HMA therapy (62%), suggesting the addition of RIG can overcome HMA clinical resistance by acting as a chromatin modifying agent. In AML, responses were seen in 37.5% of evaluable pts. Based on these results, continued study in AML is warranted. A Phase III study of the combination of oral RIG and AZA in pts with treatment naïve MDS is planned.

S489

IMPACT OF THE MUTATIONAL PROFILE AT THE TIME OF DIAGNOSIS IN RESPONSE OUTCOMES IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES AND CHRONIC MYELOMONOCYTIC LEUKEMIA TREATED WITH HYPOMETHYLATING AGENTS

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Background: Hypomethylating agents (HMA) such as azacitidine and decitabine remain the standard of care for the treatment of myelodysplastic syndromes (MDS) however, loss of response to therapy is associated with poor outcomes. Multiple studies have tried do identify biomarkers of response but the impact of the mutational architecture present at the time of diagnosis in response outcomes is unclear.

Aims: To evaluate the impact of the mutational architecture present at the time of diagnosis in response outcomes is unclear.

Methods: We evaluated 222 previously untreated patients with MDS or CMML that received HMA therapy at The University of Texas MD Anderson Cancer Center. Next generation sequencing analyzing a panel of 28 genes was performed prior to therapy with HMA. VAF estimates were used to evaluate clonal and subclonal relationships within each individual sample with clonal heterogeneity being defined in cases with Pearson goodness-of-fit p-values <0.05. Generalized linear models were used to study association of response rates (ORR=overall and CR=complete) and risk factors. Response was defined following 2006 IWG criteria.

Results: A total of 143 patients (79%) had MDS and 43 (19%) had CMML, including 108 (49%) with lower-risk based on IPSS and 114 (51%) with higher-risk disease. Therapy consisted in azacitidine monotherapy in 60 (27%) patients, decitabine monotherapy in 57 (26%), guadecitabine in 46 (21) and combinations in 59 (27%). The ORR was 61% (135/222) with 80 (36%) patients achieving CR. A total of 161 (73%) patients had at least one detectable mutation. Median number of mutations was 1 (range 0-5). Frequencies of detected mutations are shown in Figure 1A. Among 70 (32%) patients evaluable for clonal heterogeneity testing, 38 (55%) where clonally heterogeneous and carried at least 1 subclone. Pairwise associations of mutations revealed distinct and significant co-mutation patterns (Figure 1B). Within these co-mutation associations, there were no clear hierarchical patterns of clonality in patients evaluable for clonal heterogeneity, as indicated in Figure 1B. By univariate analysis, presence of mutations in *ASXL1* (OR 0.45, CI 0.22-0.93, p=0.03) and *RUNX1* (0.44, CI 0.20-0.96, p=0.038) as well as that of *TP53* mutations with VAF ≥0.31 (OR 0.21, CI 0.05-0.8, p=0.024) predicted for a lower likelihood of response. Analysis of functional pathways revealed that patients with mutations in chromatin (OR 0.43, CI 0.21-0.86, p=0.017) and signaling genes (OR 0.48, CI 0.23-1.00, p=0.049) had lower likelihood of achieving response. Additionally, patients with *ASXL1* mutations (OR 0.24, CI 0.09-0.64, p=0.005), particularly in the absence of co-occurring *TET2*, as well as those with increased number of mutations, particularly if more than 3 (OR 0.21, CI 0.06-0.73, p=0.014), or signaling gene mutations (OR 0.32, CI 0.13-0.80, p=0.015), had a lower likelihood of achieving a CR. A longer time to response was observed in patients with *DNMT3A* mutations with VAF ≥0.35 (3.4 *vs* 1 months, OR 0.22, CI 0.06-0.76, p=0.017). Among patients who achieved CR, presence of 3 or more mutations (2.6 *vs* 1.3 months, OR 1.35, CI 1.00-1.83, p=0.049) and *TP53* mutations with VAF ≥0.31 (0 *vs* 3.7 months, OR 2.03, CI 1.03-3.98, p=0.040) predicted for shorter CR duration. Presence of clonal heterogeneity, as well as the identified pairwise co-mutation patterns did not predict for any of the response outcomes.

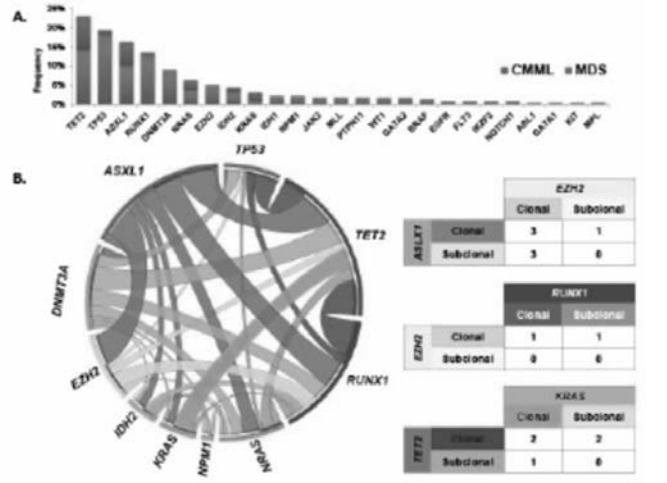

Figure 1.

Summary/Conclusions: The type, number and burden of mutations at the time of diagnosis may predict response to therapy with HMA in patients with MDS and CMML.

S490

STUDY OF THE EFFECT OF MIRNAS TARGETING RPS14 ON CELLULAR BIOLOGICAL BEHAVIOR OF MYELODYSPLASTIC SYNDROMES

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Background: As key factors in gene post-transcriptional regulation, microRNAs(miRNAs) have been identified to play important roles in carcinogenesis in various tumors. Myelodysplastic syndrome(MDS) is a group of clonal myeloid disorders characterized by refractory quantitative and qualitative abnormalities of hemocytes and its pathogenesis is poorly understood. Some studies have showed that abnormal expressions of some miRNAs have close relationship with the pathogenesis of MDS. Recently, low RPS14 expression is found common in all kinds of myelodysplastic syndromes including patients without 5q deletion, but its mechanism remains unclear.
Aims: To determine the cause of RPS14 reduction in MDS except 5q- syndrome, influence of miRNAs on RPS14 expression was analyzed, and the role of specific miRNA on proliferation, differentiation and apoptosis of hematopoietic stem cells were evaluated. This research will help reveal the pathogenesis of MDS from a new angle and provide new ideas for the diagnosis, treatment and prognosis evaluation of MDS.
Methods: Firstly, we predicted that miR-223 may target 3'UTR of RPS14 by bioinformatics software, then verified if the special miRNA could target RPS14 by assay of luciferase activity. Secondly, the mRNA expression level of miR223 were detected in the bone marrow BM selected from 28 MDS patients including ten RCUD patients, ten RCMD patients, four RAEB-1 patients and four RAEB-2 patients, meanwhile, the miR223 expression status were tested in four kinds cell lines including SKM-1, HL-60,K562 and THP-1 cell lines through qRT-PCR and RPS14 expression was detected by means of immunofluorescence(IF). Thirdly, constructing lentivirus which carried miR223 overexpression vector and inhibitor were infected to the SKM-1 cell line and k562 cell line which had the highest level of RPS14, then apoptotic analysis was detected by flow cytometry method and proliferation was tested by CCK-8 assay. Fourthly, hemin (50μM,) was used to induce erythroid differentiation of K562 cells which carried miR223 overexpression We used flow cytometry method CD71 and CD235a makers and qRT-PCR(CD235 and r-globin) to detect the erythroid proliferation.
Results: 1.We verified miR223 could target RPS14 by assay of luciferase activity. 2.MDS patients had higher miR-223 expression compared with health controls especially the types of RAEB-1 and RAEB-2 (P□0.05). In MDS patients, RAEB patients expressed higher level of miR223 than other types of MDS.Meanwhile, in cell lines, K562 cell line showed the highest level of RPS14 and lowest level of miR223. 3. Infecting miR223 overexpression lentivirus could promote cell proliferation and inhibit cell apoptosis while infecting miR223 inhibitor lentivirus had the opposite effect in SKM-1 and K562 cell lines. 4. We found that forced expression of miR-223 suppresses commitment of r-globin, CD235a and CD71 labeling, in contrast, underexpression of miR-223 promoted terminal erythropoiesis in K562 cell line.

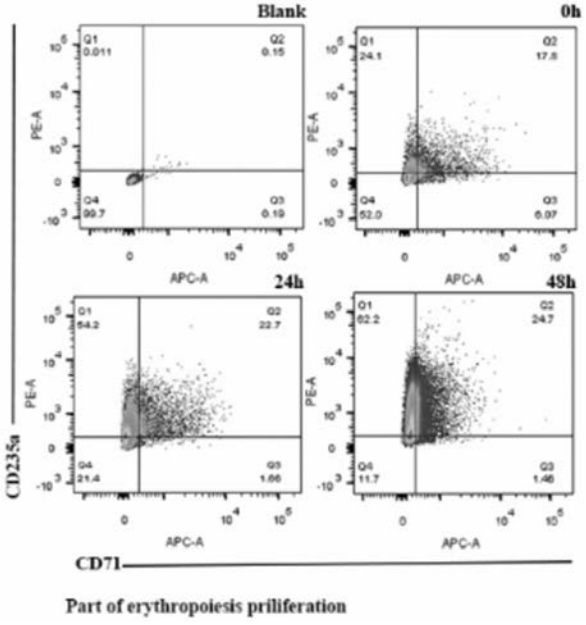

Figure 1.

Summary/Conclusions: MDS patients had higher miR-223 expression compared with health controls. We demonstrated that miR223 could promote cell proliferation, inhibit cell apoptosis and suppress terminal erythropoiesis through target RPS14.

Stem cell transplantation - Clinical 1

S491

SERIAL SEQUENCING REVEALS CLONAL ORIGINS AND STRATEGIES FOR EARLY DETECTION OF POST-ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HCT) RELAPSE IN ACUTE MYELOID LEUKEMIA (AML)

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Background: Clinical applications of next generation sequencing (NGS) in allogeneic hematopoietic stem cell transplantation are a topic of interest. Mutation dynamics post-HCT using longitudinal NGS have not been thoroughly examined. We hypothesized that serial sequencing of pre-HCT and post-HCT in AML patients could provide a much deeper and broader understanding of clonal origin/hierarchy of relapse after allogeneic HCT. The present study aimed to evaluate mutation dynamics in AML using serial samples from pre- and post-HCT with respect to transplant outcomes, particularly overall survival (OS) and relapse.
Aims: To track origins of post-HCT relapse in AML using serial sequencing
Methods: 88 AML patients were enrolled and sequenced using an Illumina HiSeq 2000 sequencer (84 myeloid custom gene panel) on 419 bone marrow samples at diagnosis (n=88), pre-HCT (n=88), 21 days after HCT (n=88), and at relapse (n=20). Two patients relapsed by day 21. T-cell (n=80) and donor samples (n=57) were also sequenced. All computational and statistical analyses were performed using Python and R.

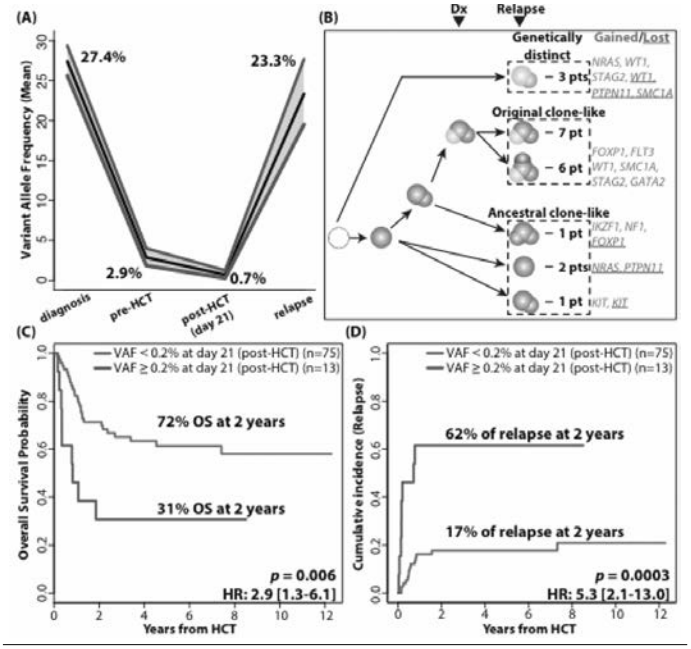

Figure 1.

Results: The mean on-target coverage in 419 samples was 1773.7x. In total, we detected 217 mutations throughout the course of treatment in 79/88 patients (89.8%). *NPM1 (26.1%)*, *DNMT3A (26.1%)*, *CEBPA (13.6%)*, *IDH2 (13.6%)*, *FLT3 (12.5%)*, and *PTPN11 (11.4%)* were commonly mutated at diagnosis. Unsurprisingly, most mutations appeared at initial diagnosis (200/217, 92.1%). Only 1, 2, and 14 mutations were acquired/selected at pre-HCT (0.5%), day 21 (0.9%), and relapse (6.5%), respectively. Most mutations were cleared at pre-HCT (mean mutation allele frequency (VAF) from 27.4% to 2.9%) and were further reduced after HCT (mean VAF from 2.9% to 0.7%) **(Fig A)**. Leveraging

serial samples, we inferred the clonal relationships between original and relapse samples in 20 patients **(Fig B)**. Mutations from initial diagnosis re-appeared in 17 patients. The relapse clone of 13 patients was identical to or clonally evolved from the initial AML clone (7 and 6 patients, respectively). Relapse clones of 4 patients evolved from an inferred ancestral clone, distinct from the initial AML clone. The remaining 3 patients' relapse clones appear to be genetically distinct from the initial AML clone **(Fig B)**. Among 61 mutations from relapsed patients, 37 were stable, whereas 9 were cleared and 15 acquired (or selected) at relapse. Overall, serial samples and donor samples de-convoluted origins of relapse clone from all 20 patients. Among the 13 patients whose donor samples were sequenced, no mutation that was transferred from donor to recipient expanded at relapse. We then assessed whether the mutation status at pre- and post-HCT has any impact on OS and relapse after HCT. With a follow-up duration of 6.9 years, patients with VAF ≥0.2% at day 21 in any gene showed worse OS (HR 2.9, p=0.006) as well as increased risk of relapse (HR 5.3, p=0.0003) **(Fig C-D).** Multivariate analyses verified that VAF ≥0.2% at day 21 was a prognostic factor independent of age and cytogenetics risk group for OS (HR 3.2, p=0.003). Non-relapse mortality did not show significant difference (p=0.3). Thirteen patients carried 20 mutations at day 21 (≥0.2%), 16 of which originated from the initial AML clone. Noticeably, 9 of these mutations (from 9 patients) were in *DNMT3A*, where one of them was a transferred mutation from the donor through HCT (donor VAF: 8.18%, VAF at day 21: 3.42%, non-relapse case). Mutation status at any other time points in any gene, defined with a hard cut-off (VAF >2%), was neither significantly associated with overall survival nor relapse incidence after HCT.

Summary/Conclusions: This study revealed origins of mutations detected at post-HCT relapse. It also revealed that the presence of mutations immediately after HCT, within 21 days, with low VAF (0.2%) can be used to predict relapse after HCT, illustrating the value of longitudinal NGS-based monitoring strategies for AML patients after allogeneic HCT.

S492

IBRUTINIB FOR CHRONIC GRAFT-VERSUS-HOST DISEASE AFTER FAILURE OF FRONTLINE CORTICOSTEROIDS: RESULTS OF A MULTICENTER OPEN-LABEL PHASE 2 STUDY

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Background: There are no approved therapies for chronic GVHD (cGVHD) after failure of steroids. Both B and T cells play a role in the pathophysiology of cGVHD. In preclinical models, ibrutinib (ibr) reduced the severity of cGVHD through inhibition of Bruton's tyrosine kinase (BTK) and interleukin-2–inducible T-cell kinase (ITK).

Aims: This phase 2 study evaluated the efficacy and safety of ibr in patients (pts) with steroid dependent/refractory cGVHD in need of additional therapy.

Methods: Eligible pts had ≤3 prior regimens for cGVHD and either >25% body surface area erythematous rash or a NIH mouth score >4. Informed consent was obtained from all pts. Pts were treated with ibr 420mg/d until cGVHD progression or unacceptable toxicity. The primary end point was cGVHD response based on 2005 NIH consensus response criteria. Secondary end points included rate of sustained response, change in Lee cGVHD symptom scale, change in steroid dose over time, and safety. The pharmacodynamics (PD) of ibr and its effects on biomarkers associated with GHVD, inflammation, and fibrosis were evaluated.

Results: A recommended phase 2 dose of 420mg was identified in phase 1b (n=6). For 42 pts in phase 2, the median number of prior cGVHD regimens was 2 (range, 1–3). At a median follow-up of 13.9 mo, overall response rate (ORR) was 67% (CR, 21%), with 71% of responders showing a sustained response of ≥20 weeks; 79% responded by the first response assessment. Median steroid dose decreased in responders from 0.29mg/kg/d at baseline to 0.12mg/kg/d at week 49. Overall, 62% of pts achieved steroid doses <0.15mg/kg/d while on ibr; 5 responders discontinued steroids. Organs with cGVHD involvement including skin, mouth, and gastrointestinal system showed similar responses (~90%). Of 25 responders with ≥2 involved organs, 20 (80%) showed a response in ≥2 organs. Improvement in Lee cGVHD symptom score was reported for 43% of responders by month 6 and 61% overall, compared with 11% of nonresponders by month 6 and overall. Ibr blocked BTK-driven basophil activation in an ex vivo IgE stimulation assay and ITK-mediated activation of PLCγ1-Y783 in CD4 T-cells. Analysis of soluble plasma factors associated with inflammation, fibrosis, and cGVHD from all treated pts showed a significant decrease over time with ibr. Adverse events (AEs) were largely grade 1 or 2 events; AEs occurring in ≥20% of pts were fatigue, diarrhea, muscle spasms, nausea, and bruising. Grade ≥3 AEs occurring in ≥10% of pts were pneumonia, fatigue, and diarrhea. Serious AEs (SAEs) occurred in 52% of pts; grade ≥3 SAEs were reported in 40% of pts and included pneumonia, septic shock, and pyrexia. Two fatal events (multilobular pneumonia and bronchopulmonary aspergillosis) were reported. Fourteen pts discontinued ibr for AEs, 5 pts for progressive cGVHD, and 2 pts after resolution of cGVHD symptoms; 29% continued ibr.

Summary/Conclusions: With an ORR of 67% and a sustained response rate of ≥20 weeks of 71%, treatment with ibr resulted in clinically meaningful and durable responses in pts who failed at least 1 prior treatment for cGVHD. Most responders were able to reduce steroid dose. PD and biomarker changes support a beneficial effect of ibr on immune cell subsets in pts with cGVHD. The safety profile was consistent with those previously reported for pts with B cell malignancies and those seen in cGVHD pts on concomitant steroids. Responses in this pretreated, high-risk population support study of ibr for frontline treatment of cGVHD.

S493

OUTCOMES OF NON T CELL-DEPLETED HAPLOIDENTICAL HSCT *VERSUS* HSCT FROM MATCHED SIBLING DONORS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA IN FIRST COMPLETE REMISSION, AN ALWP-EBMT STUDY

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Background: Allogeneic hematopoietic stem cell transplantation (HSCT) is the standard of care for patients (pts) with intermediate (int-AML) or high-risk AML. In pts lacking matched sibling (MSD), HSCT from haploidentical donors (HAPLO) is an emerging option

Aims: The aim of the study was to compare outcomes of non T cell-depleted HAPLO HSCT to those from MSD

Methods: Included were adults with AML in first CR undergoing transplantation from HAPLO *vs* MSD from 2007-2015. Due to significant interaction between karyotype and donor type, int- and high-risk AML were studied separately. In addition because of some characteristic differences between the 2 groups the propensity score technique was used: 2 MSD were matched with each haplo. The following factors were included in the propensity score model: patient, year of HSCT, time from diagnosis to HSCT, conditioning (RIC), source of stem cells (BM/PB), cytogenetic group, patient and donor CMV serology status

Results: We identified 2654 pts (HAPLO=185; MSD=2469) for int-AML (HAPLO=122; MSD=1888) or high risk-AML (HAPLO=63; MSD=581). Median follow up was 30 (1-116) months. Median age at HSCT was 50 (18-74)years. Among HAPLO recipients, 74% received PTCY and 26% ATG. Conditioning regimen was myeloablative in 50% *vs* 52% (p=0.52) of HAPLO and MSD pts, respectively. HAPLO pts had a longer interval from diagnosis to HSCT (6 *vs* 4 months; p<0.01), had more often high risk-AML (34% *vs* 23%; p<0.01), bone marrow as stem cell source (49% *vs* 19%; p<0.01) and CMV positive donors (72% *vs* 61%; p<0.01). Graft failure occurred more frequently after HAPLO (3% *vs* 1%; p=0.002).For pts with int-AML CI of aGVHD and cGVHD was 29% *vs* 20% (p<0.03) and 30% *vs* 36% (p<0.02) in HAPLO and MSD pts, respectively. At two years, NRM and RI were 26% *vs* 10% (p<0.01) and 17% *vs* 20% (p=0.52) while LFS and OS were 56% *vs* 70% (p<0.01) and 68% *vs* 79% (p<0.01) in HAPLO and MSD pts, and GRFS was 45% *vs* 53% (p<0.05), respectively. In multivariate analysis HAPLO was associated with reduced LFS (HR 1.74; 95% CI 1.30-2.33; p<0.01), OS (HR 1.80; 95% CI 1.32-2.45; p<0.01) and GRFS (HR 1.32; 95% CI 1.01-1.72; p<0.05) and higher NRM (HR 3.03; 95% CI 1.98-4.62; p<0.01). Incremental age was independently associated to lower LFS, OS, GRFS and higher NRM and cGVHD. MAC was associated with lower RI and higher GVHD. A female donor into male recipient was associated to higher GHVD and lower GRFS. A longer interval from diagnosis to HSCT was asso-

ciated to lower LFS. Donor CMV seropositivity was associated with lower GRFS and higher NRM and aGVHD. In high risk-AML aGVHD and cGVHD were 36% *vs* 24% (p=0.03) and 39% *vs* 33% (p=0.80) for HAPLO and MSD pts, respectively. At two years, NRM and RI were 18% *vs* 10% (p=0.16) and 21% *vs* 36% (p<0.02) while LFS and OS were 61% *vs* 55% (p=0.14) and 67% *vs* 66% (p=0.26) in HAPLO and MSD pts; GRFS was 49% *vs* 40% (p=0.17). In multivariate analysis risk of grade II-IV aGVHD (HR: 2.20; 95% CI: 1.29-3.74; p<0.01) was increased after Haplo as compared to MSD and no difference was observed in LFS, OS and GRFS, respectively. Conditioning regimen was associated with lower NRM and higher GRFS, while younger age and donor CMV status were associated with lower RI, higher LFS and OS. Results were confirmed in the analysis done with the the propensity score technique as for RI, NRM, LFS, OS and GRFS

Summary/Conclusions: As per our registry based study in intermediate risk AML results of HSCT from matched sibling donor are superior to those of HAPLO-HSCT, while in high risk-AML relapse is lower in the HAPLO transplants and NRM, LFS and OS is similar

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INDIVIDUAL OUTCOME PREDICTION FOR MDS AND SECONDARY AML AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION BASED ON GENETIC, PATIENT- AND TRANSPLANTATION-ASSOCIATED RISK FACTORS

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Background: Prediction of individual outcomes after allogeneic hematopoietic cell transplantation (alloHCT) is difficult, as it is influenced by a multitude of risk factors.
Aims: To develop a tool that predicts individual outcomes of patients with myelodysplastic syndrome (MDS) or secondary acute myeloid leukemia from MDS (sAML) after alloHCT.
Methods: We integrated molecular data with available prognostic factors in patients undergoing alloHCT for MDS and sAML to evaluate their impact on prognosis. 304 patients with MDS or sAML who underwent alloHCT were sequenced for mutations in 54 genes. We used a Cox multivariate model and competing risk analysis with internal and cross validation to identify factors prognostic of overall survival (OS), cumulative incidence of relapse (CIR) and non-relapse mortality (NRM).

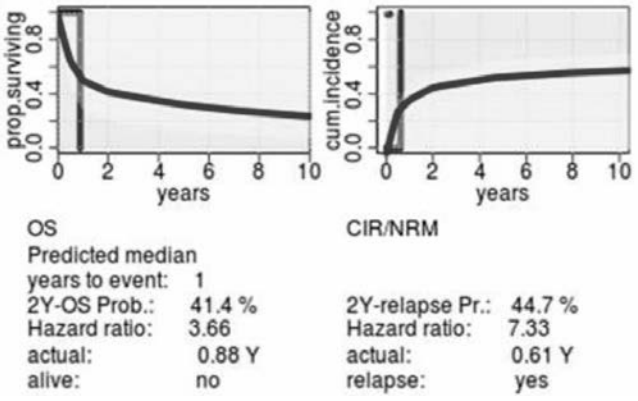

Figure 1.

Results: In multivariate analysis, mutated *NRAS, U2AF1, IDH2, TP53* and/or a complex karyotype were significant prognostic markers for OS besides age above 60 years, remission status treated but not in CR, IPSS-R cytogenetic risk, HCT-CI >2 and female donor sex. Mutated *NRAS, IDH1, EZH2* and *TP53* and/or a complex karyotype were genetic aberrations with prognostic impact on CIR. No molecular markers were associated with the risk of NRM. The addition of molecular information significantly improved the risk prediction for OS and CIR as assessed by the Akaike information criterion. Internal and cross validation confirmed the robustness of our comprehensive risk model. We developed an interactive risk prediction tool to provide personalized predictions for OS, CIR and NRM outcome after alloHCT. An individualized prediction for a 53-year-old male with sAML with trisomy 11, mutated *NRAS, IDH2* and *DNMT3A* and complete remission after double induction is shown in Figure 1. The probability of CIR at 2 years was 45% and the patient relapsed after 0.61 years. The probability of OS at 2 years was 41% and the patient died after 0.88 years.

Summary/Conclusions: We combine molecular, cytogenetic, patient- and transplantation associated risk factors into a comprehensive risk score to provide personalized predictions for outcome after alloHCT. Upon validation in larger patient cohorts, this will improve patient information before alloHCT and provide a platform to improve treatment strategies for patients with high risk of CIR or NRM.

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IMPACT OF POST-TRANSPLANT INFUSION OF DONOR T CELLS GENETICALLY MODIFIED WITH INDUCIBLE CASPASE 9 SUICIDE GENE (BPX-501 CELLS) ON CHILDREN WITH LEUKEMIA GIVEN ALPHA-BETA T-CELL DEPLETED HAPLO-HSCT

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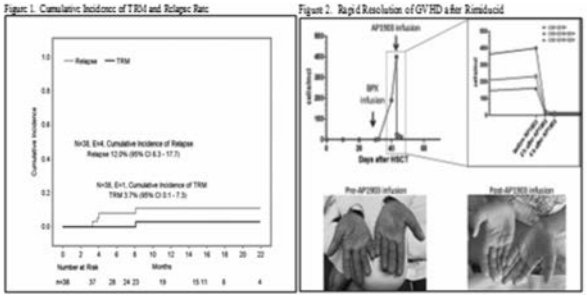
Background: HLA-haploidentical allogeneic hematopoietic stem cell transplant (haplo-HSCT) offers an option for children with acute leukemia in need of a transplant and lacking an available HLA-identical donor. However, performing haploidentical-HSCT without any graft manipulation has historically been associated with a high risk of acute and chronic graft-versus-host disease (GVHD). T cell depletion reduces the risk of GVHD, but leads to delayed immune reconstitution, predisposing to serious infection and leukemia relapse due to the lack of a T-cell mediated graft-versus-leukemia (GvL). To address these challenges, we have infused mature BPX-501 T cells (donor peripheral lymphocytes which have been modified with the iCasp9 suicide gene) after αβ T-cell depleted haplo HSCT to facilitate immune reconstitution and GvL effect. BPX-501 T-cells are genetically modified with the iCasp9 suicide safety switch and a truncated CD19 marker. In the event of GvHD, the switch is activated by an infusion of the drug rimiducid (AP1903) resulting in rapid T cell apoptosis and GvHD reversal. CD3+/CD19+ T-cells can be tracked by flow cytometry.
Aims: This study was performed to evaluate both safety and efficacy of BPX-501 T cell infusion post αβ T-cell depleted haplo HSCT in pediatric patients with high risk ALL and AML in CR1 and CR2.
Methods: : A prospective Phase I-II study enrolling children with hematopoietic disorder who lack a matched donor. 38 patients have been enrolled and treated with αβ TCR depleted haplo HSCT after a myeloablative preparative regimen followed by BPX-T cell infusion to date; of them, 24 had ALL and 14 AML (21% CR1, 79% CR2). Median follow-up is 11 months (range 3-24).
Results: All patients engrafted and no secondary graft failure was recorded. Median time to neutrophil and platelet recovery was 16 days (range 8-33) and 11 days (range 7-19), respectively. With a median follow-up of 11 months (range 3-24 months), the cumulative incidence of NRM and relapse was 3.7% and 12.0%, respectively, while the disease-free survival probability was 84.2% (Fig 1). All aGVHD resolved (5 Grade I skin, 5 Grade II skin, 2 Grade III GI). One child received rimiducid to treat steroid-resistant grade II skin with complete resolution in 24 hours (Fig 2). There were 3 cases of chronic GvHD, 2 were mild; 1 severe and fatal in a patient whose donor had VZV reactivation during mobilization. CD3+ T cells reached 500 cells/μl by day 90, with normalized CD4/CD8 T cell ratio by day 180.



Figures.

Summary/Conclusions: Engraftment was brisk and T cell recovery normalized by 6 months. Overall incidence of severe aGVHD was low and the safety switch was successfully activated with rimiducid infusion. Cumulative incidence of NRM compares favorably to historic controls at the lead center, where a value of of 2.4% for matched related donors (MR), 11.8% for matched unrelated donors (MUD) and 5% for αβ T cell depletion haplo HSCT (Haplo αβ) without BPX-501 infusion was recorded (Bertaina, 2015 ASH). The cumulative incidence of relapse was 12.0% for BPX-501, 32.3% for MR, 22.2% for MUDs and 21.9% Haplo-αβ. Disease-free survival in the BPX-501 treated patients was 84.2% compared to 65.4% for MR, 66.1% for MUDs and 73.1% for Haplo-αβ. However, length of follow-up on the control cohorts differed from that of BPX-501 treated patients. These data suggest that BPX-501 T cells modified with the iCasp9 safety switch, infused after selective αβ T-cell depletion, are safe and result in a rapid immune reconstitution and a potentially stronger GvL effect in children with high-risk leukemia who lack a matched donor.

Bone marrow failure and PNH

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HEREDITARY HEMATOLOGIC MALIGNANCIES: GENETIC COUNSELING IMPLEMENTATION IN A LARGE LEUKEMIA CENTER

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Background: Hematologic malignancies have rarely been targets for genetic evaluation, even in familial cases. Over the past decade, more than 12 genes have been identified to cause inherited predispositions to hematologic malignancies. Genetic counseling, testing, and surveillance protocols for these families are not well-established. Additionally, many families with high incidence of blood cancers do not have described syndromes suggesting additional genes remain to be identified.
Aims: To identify individuals with inherited susceptibilities to hematologic malignancies, the Hereditary Hematologic Malignancy Clinic (HHMC) was established in April 2014 at The University of Texas M. D. Anderson Cancer Center. The clinic provides genetic counseling, clinical and research testing for patients with hematologic malignancies suspected to have inherited predisposition syndromes.
Methods: Individuals were referred to the HHMC for several indications: (1) bone marrow failure/aplastic anemia/hypocellular MDS, (2) personal history of hematologic malignancy with ≥1 first-degree relative or ≥2 second-degree relatives with hematologic malignancy, (3) personal history of multiple primary cancers, (4) germline evaluation of presumed somatic mutations identified on next-generation leukemia prognostication panels, (5) management and/or surveillance of a previously-identified genetic syndrome, or (6) solid tumor hereditary syndrome evaluation in patients with active hematologic malignancy. Over the past 3 years, 152 probands were evaluated (n=152). Skin biopsies were performed to obtain germline DNA, and next-generation sequencing approaches on both a clinical and research basis were utilized.
Results: Clinical genetic testing was performed in 97/152 individuals (64%). Research testing was performed in 46/152 (30%), particularly in patients negative for known susceptibility genes or without features suggestive of a clinical syndrome. Nine (6%) individuals did not undergo genetic testing. Clinical testing identified 23/97 (24%) individuals with a germline susceptibility to hematologic malignancy. Seven probands (7%) were identified to have *RUNX1* mutations associated with familial platelet disorder with myeloid malignancy (FPD-AML). Six (6%) were identified to have the telomere disorder dyskeratosis congenita; only one of them met clinical diagnostic criteria with the "classic triad" of symptoms. Three (3%) patients were identified to have Li-Fraumeni syndrome due to constitutional *TP53* mutations. Two adults (2%) were diagnosed with Diamond-Blackfan anemia; both of these individuals developed adult-onset myelodysplastic syndrome after a long latency period and prior spontaneous remission of their childhood anemia. Two young adults (2%) with Fanconi anemia were diagnosed, and one patient each with *DDX41* mutation and *CBL* (Noonan-like syndrome with JMML) were identified. Counseling, testing, and surveillance of identified mutation carriers in many affected families is ongoing.
Summary/Conclusions: Individuals with hereditary susceptibilities to hematologic malignancies are not as rare as previously thought. Clinical evaluation of these patients through genetic counseling and testing is high yield for identified at-risk families. Research-based sequencing for novel mutations is indicated and ongoing.

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SECONDARY LEUKEMIAS IN GENETIC SUBTYPES OF CONGENITAL NEUTROPENIA (ELANE, HAX1, WASP, G6PC3, ETC.): A LONG-TERM ANALYSIS OF THE SCNIR EUROPE

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Background: Leukemia predisposition is well known in congenital neutropenia (CN) subtypes. By taking all patients with known and unclassified CN together the incidence of secondary leukemia accounts for more than 10 percent. Advanced molecular diagnostics and the identification of inherited and acquired gene mutations have improved our understanding of leukemic transformation in CN patients.
Aims: In the European SCNIR 449 patients with congenital neutropenia and 91 patients with cyclic neutropenia (CyN) have been enrolled since 1994. These 449 patients can be differentiated by causative gene mutation in more than 10 genetic subtypes: *ELANE, HAX1, G6PT, G6PC3, WAS, SBDS, TAZ1* and *p14* or no identified mutation, respectively. Our aim is to assess the risk of leukemic transformation within these genetic subgroups.
Methods: Here we report the leukemia incidence of genetic subtypes analyzing all available long-term data from the European Branch of the Severe Chronic Neutropenia Registry (SCNIR). In addition, we analyzed 91 patients with CyN with or without *ELANE* mutations.
Results: Results from genetic testing were available for 314 of 449 CN patients, of whom 118 patients revealed *ELANE*, 48 *HAX1*, 71 *SBDS*, 28 *G6PT*, 9 *G6PC3*, 7 *WAS*, 5 *TAZ1* mutations and 27 other rare gene mutations (*e.g.* p14, CXCR4). 135 patients remain unclassified. In addition, 48 of 91 patients with CyN revealed *ELANE* mutations. Secondary myelodysplastic syndrome (MDS) or leukemia occurred in 49 of the 449 CN patients and in 1 of the 48 ELANE-CyN patients. Acquired *CSF3R* nonsense truncating mutations have been detected in the bone marrow cells of about 80% of CN patients who progress to MDS or acute myeloid leukemia (AML) and around 30-35% of non-leukemic CN patients, supporting the association between the acquisition of *CSF3R* mutations and leukemic transformation. These mutations have been shown to be acquired in hematopoietic cells only and therefore are not the primary cause of CN. The time between first detection of *CSF3R* mutations and signs of malignant transformation is highly variable. Some patients progressed to MDS/AML within a few months. In others, *CSF3R* mutant clones persisted for many years without progression to leukemia. The distribution by genetic subtypes and the frequency of CSF3R mutations is shown in the table below:

Table 1.

Gene Mutation	Patients N	MDS/Leukemia n/ (%)
Total CN	**445**	**49 (11,0)**
ELANE	118	17 (14,4)
HAX1	48	6 (12,5)
SBDS	71	6 (8,5)
SLC37A4	28	1 (3,6)
WAS	7	2
JAGN1	2	1
gene mutations without leukemia*	36	0
unclassified	135	16 (11,8)
Total CyN	**91**	**1 (0.2)**
ELANE CyN	48	1 (0,2)
CyN unclassified	43	**1 (2,3)**

**Gene mutations without leukemia :(G6PC3 n=9, TAZ1 n=5, p14 n=4, digenic mutations n=4, COH1 n=4, CXCR4 n=3, germline extracellular CSF3R n=2, C16ORF57 n=2, Pearson syndrome n=2, LYST n=1)*

All subgroups benefit from G-CSF treatment. However, patients requiring maintenance doses of G-CSF above 8µg/kg/day are at greater risk of leukemic transformation.
Summary/Conclusions: Conclusion: The incidence of secondary AML reflects the genetic heterogeneity of CN.

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EFFECT OF ECULIZUMAB IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) PATIENTS WITH OR WITHOUT HIGH DISEASE ACTIVITY: RESULTS FROM THE INTERNATIONAL PNH REGISTRY

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, progressive, life-threatening disease caused by somatic phosphatidylinositol glycan class A (*PIGA)* gene mutation in bone marrow stem cells. The International PNH Registry (NCT01374360) is a prospective, multinational, observational study to record the natural history of PNH and collect data on long-term efficacy and safety of treatment with eculizumab (ecu), a humanized monoclonal antibody approved for treatment of PNH.
Aims: Evaluate the effect of ecu in patients with PNH with or without high disease activity (HDA).
Methods: Patients enrolled in the Registry as of December 5, 2016, were stratified by HDA and ecu treatment status into 4 groups: HDA/ecu-treated; HDA/never ecu-treated; no-HDA/ecu-treated; no-HDA/never ecu-treated. HDA is defined as lactate dehydrogenase (LDH) ratio ≥1.5x upper limit of normal within 6 months of baseline and history of any of the following: fatigue, hemoglobinuria, abdominal pain, dyspnea, anemia (hemoglobin <100 g/L), major adverse vascular event (MAVE; including thromboembolism [TE]), dysphagia, or erectile dysfunction. Patients were assessed at baseline (date of enrollment in never ecu-treated patients; date of initiation of ecu in ecu-treated patients) and at last follow-up. Outcomes include changes from baseline to last follow-up in LDH ratio, GPI-deficient granulocytes, red blood cell transfusions received, MAVE, and Functional Assessment of Chronic Illness Therapy (FACIT)-Fatigue score in patients with at least 6 months of follow-up.

Results: 4717 patients were enrolled; of these, 2670 had non-missing data on ecu and HDA status, and were included in the current analysis (HDA/ecu-treated, n=785; HDA/never ecu-treated, n=636; no-HDA/ecu-treated, n=111; no-HDA/never ecu-treated, n=1138). Median (min, max) duration of follow-up after baseline was longer for the ecu-treated patients compared with the never ecu-treated patients for both the HDA and no-HDA groups (see Table). Results for changes from baseline to last follow-up in outcomes of interest are summarized in the **Table**. Data show that patients in the ecu-treated cohort had high burden of disease at baseline. Specifically, in the HDA population, a higher proportion of ecu-treated patients had a history of MAVE (33.3%) *vs* never ecu-treated patients (13.7%). A similar disparity at baseline was also observed in the no-HDA population (33.0% *vs* 11.0%, respectively). Following ecu treatment, the divergence in the proportion of patients with MAVE has substantially narrowed for the HDA patients (3.9% for ecu-treated *vs* 3.3% for never ecu-treated) despite longer follow-up for the treated patients. Similar findings were seen in no-HDA patients (5.3% *vs* 2.1% respectively). In patients with HDA status, treatment with ecu was associated with meaningful improvement in mean (standard deviation [SD]) reduction from baseline in LDH ratio (-5.0 [3.7] *vs* -0.4 [2.3]) and proportion of red blood cell transfusion-free patients (37.6% *vs* 15.8%). The FACIT-Fatigue data, while limited, showed the HDA/ecu-treated group experienced greater mean (SD) score improvement than the HDA/never ecu-treated group (4.1 [10.3] *vs* 0.5 [6.8] points).

Table 1.

Outcome Measure*	HDA/Ecu-treated (n=785)	HDA/Never Ecu-treated (n=636)	No-HDA/Ecu-treated (n=111)	No-HDA/Never Ecu-treated (n=1138)
	n=767	n=636	n=108	n=1135
Years from baseline to last follow-up, median (min, max)	3.1 (0.0, 11.0)	1.5 (0.0, 10.0)	2.1 (0.1, 11.0)	1.5 (0.0, 9.5)
	n=583	n=356	n=67	n=582
Change from baseline in LDH ratio, mean (SD)	−5.0 (3.7)	−0.4 (2.3)	−0.4 (2.0)	0.2 (0.9)
	n=210	n=107	n=25	n=248
Change from baseline in % GPI-deficient granulocytes, mean (SD)	3.5 (23.9)	−0.5 (20.2)	6.3 (21.8)	1.3 (17.6)
Change from baseline in number of patients requiring blood transfusions, n (%)	n=599	n=425	n=74	n=747
Yes to no	225 (37.6)	67 (15.8)	21 (28.4)	161 (21.6)
No change	332 (55.4)	317 (74.6)	47 (63.5)	549 (73.5)
No to yes	42 (7.0)	41 (9.6)	6 (8.1)	37 (5.0)
Change from baseline in number of patients with MAVE, n (%)	n=699	n=460	n=94	n=766
Previous history of MAVE and occurrence of MAVE after baseline	17 (2.4)	3 (0.7)	3 (3.2)	4 (0.5)
Previous history of MAVE and no occurrence of MAVE after baseline	216 (30.9)	60 (13.0)	28 (29.8)	80 (10.4)
No previous history of MAVE and occurrence of MAVE after baseline	10 (1.4)	12 (2.6)	2 (2.1)	12 (1.6)
No previous history of MAVE and no occurrence of MAVE after baseline	456 (65.2)	385 (83.7)	61 (64.9)	670 (87.5)
Change from baseline in FACIT-Fatigue score, mean (SD)	4.1 (10.3)	0.5 (6.8)	−3.8 (14.5)	0.3 (7.7)

*All analyses of change from baseline to last follow-up were restricted to patients with at least 6 months of follow-up and who had data at both baseline and last follow-up time points. Abbreviations: Ecu, eculizumab; FACIT, Functional Assessment of Chronic Illness Therapy; GPI, glycosylphosphatidylinositol; HDA, high disease activity; LDH, lactate dehydrogenase; MAVE, major adverse vascular event; SD, standard deviation.

Summary/Conclusions: Our analysis of real-world data from the International PNH Registry has demonstrated that treatment with eculizumab was associated with improved outcomes in patients with HDA. Our findings are consistent with the notion that patients with HDA, including those with a history of MAVE, should be treated with eculizumab.

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CONGENITAL AMEGAKARYOCYTIC THROMBOCYTOPENIA: FUNCTIONAL RESCUE OF A NOVEL MPL MUTANT IN PRIMARY HEMATOPOIETIC CELLS USING CRISPR-CAS9

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Background: Thrombopoietin (Tpo) and its receptor, Mpl, are the principal regulators of early/late thrombopoiesis and hematopoietic stem cells maintenance. Mutations in *MPL* can drastically impair its function and be a contributing factor in multiple hematologic malignancies, including congenital amegakaryocytic thrombocytopenia (CAMT). CAMT is a rare inherited syndrome characterized by thrombocytopenia at birth, progressing to bone marrow failure and pancytopenia. The functional impact of CAMT mutations on Mpl is yet to be determined. Here we report unique familial cases of CAMT presenting with a previously unreported *MPL* mutation: T814C (W272R) in the background of the activating *MPL* G117T (K39N or Baltimore) mutation.
Aims: This study focuses on the functional characterization of this novel Mpl mutant and the use of genome editing as a novel therapeutic option for CAMT.
Methods: Human megakaryoblastic UT-7 and murine Ba/F3 cells stably expressing human wild-type (WT) Mpl or mutant Mpl fused to mNeonGreen were used as models. Confocal microscopy, proliferation and surface biotinylation assays, as well as co-immunoprecipitation and western blotting analysis, were used to elucidate the function and trafficking of Mpl mutants. Multiplex, flow-based, CRISPR-Cas9 gene editing was used to repair mutant *MPL* and rescue its function. Cord blood from the younger male sibling was used as a source of primary homozygous Mpl K39N/W272R CD34$^+$ cells. CD34$^+$ cells were edited using ribonucleoproteins electroporation followed by sequencing and functional assays such as flow cytometry and single colony assays.
Results: Consanguineous parents and their eldest daughter, all heterozygous for Mpl K39N/W272R, do not present any signs of disease. Their monozygotic twin daughters presented at birth with severe thrombocytopenia leading to a diagnosis of CAMT type I. Whole blood sequencing revealed the presence of a homozygous double Mpl K39N/W272R mutation, as their younger male sibling. One of the twins died after bone marrow transplant. Confocal microscopy shows that a significant fraction of chimeric WT Mpl protein reaches the cell surface. Significant surface expression is also noted for Mpl K39N. In contrast, the chimeric Mpl protein bearing the W272R mutation, alone or together with the K39N mutation, showed no detectable surface expression of the Tpo receptor while being strongly co-localized with ER marker calreticulin. Both WT and K39N-mutated Mpl were found signaling competent, while single or double mutants bearing W272R were unresponsive to Tpo. Tpo-induced signaling was partially restored via GRASP55 over-expression (forcing ER-trapped Mpl to traffic to the cell surface). Genome editing performed on cells carrying the W272R mutation restored the WT sequence and the response to Tpo, with similar cell proliferation as WT Mpl cells. Finally, when applied to primary Mpl K39N/W272R CD34$^+$ cells, CRISPR-based gene editing rescued surface expression of Mpl and response to Tpo, as assessed by flow cytometry. Furthermore, edited CD34$^+$ cells were able to generate a similar number of megakaryocytic colonies as control CD34$^+$ cells in a single colony assay. Non-edited cells failed to do so.
Summary/Conclusions: We report a new double *in cis* mutation of Mpl (K39N/W272R) in the context of CAMT. Function of the deficient Mpl receptor could be rescued using two separate approaches: GRASP55 over-expression and CRISPR-Cas9 genome engineering. Successful editing of primary hematopoietic stem cells indicates direct therapeutic applications for gene editing in this disease.

S500

DISCOVERY OF ORALLY BIOAVAILABLE SMALL MOLECULES FOR INHIBITION OF COMPLEMENT C5

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic uremic syndrome (aHUS) are well-characterized diseases of complement dysregulation. The only approved therapeutic for these diseases is Soliris® (Eculizumab, Alexion), a monoclonal antibody that binds and inhibits the cleavage of complement C5. Soliris® requires lifelong intravenous administration by a medical professional every two weeks. An orally bioavailable small molecule inhibitor of complement C5 to treat these and other complement mediated diseases represents a potential paradigm shift in the treatment of diseases of complement dysregulation.
Aims: To demonstrate the utility of an orally available, small molecule Complement C5 inhibitor for the treatment of complement mediated disorders.
Methods: Surface Plasmon Resonance (SPR) and Fluorescent Polarization assays (FP) were used to evaluate the affinity and specificity of the binding interaction between complement C5 and small molecule inhibitors. Determination of binding site, mechanism of action and potency were achieved by X-ray crystallography studies, Wieslab ELISA, and a sheep erythrocyte hemolysis based assay. The ability of the small molecules to prevent the hemolysis of PNH erythrocytes was evaluated using a modified Ham test. Pharmacokinetic studies were performed in rodents.
Results: Here we describe a series of first in class, orally bioavailable small molecules that bind to C5 with high affinity and inhibit its cleavage into C5a and C5b. These molecules demonstrate desirable drug-like properties with molecular weights under 500 amu and tPSA<100 Å2. A high-resolution co-crystal structure with C5 shows a unique binding site on the 188 kDa C5 protein, and specific binding of these molecules to C5 has been demonstrated by surface plasmon resonance (SPR) and fluorescence polarization (FP) assays. The position of the binding site suggests that these molecules will inhibit C5 cleavage in patients with the R885H/C polymorphism, which confers resistance to eculizumab. The molecules inhibit the terminal complement complex activity with single digit nanomolar IC50 as measured by inhibition of hemolysis in a highly sensitive antibody-sensitized sheep erythrocytes assay. In addition, they inhibit MAC deposition on complement-activating surfaces and prevent the cleavage of C5 to C5a and C5b as confirmed by ELISAs that directly detect generation of C5a and MAC. This series of inhibitors prevents the complement-mediated hemolysis of PNH erythrocytes (Type III) in a dose-dependent manner. More broadly, this series of molecules has been profiled by *in vitro* and *in vivo* ADME disposition studies and exhibits oral bioavailability (%F~30-50) in pre-clinical species.
Summary/Conclusions: The results presented here highlight, for the first time, the feasibility of an oral, potent small molecule inhibitor of C5. The development of an orally available complement C5 inhibitor has the potential to provide a new therapeutic modality to treat both rare and common conditions where terminal complement cascade inhibition is desired.

Quality of life, palliative care, ethics and health economics

S501

QUALITY OF LIFE WITH MELPHALAN/PREDNISONE PLUS EITHER THALIDOMIDE (MPT-T) OR LENALIDOMIDE (MPR-R) IN NON-TRANSPLANT ELIGIBLE NEWLY DIAGNOSED MULTIPLE MYELOMA; RESULTS OF THE HOVON87/NMSG18 STUDY

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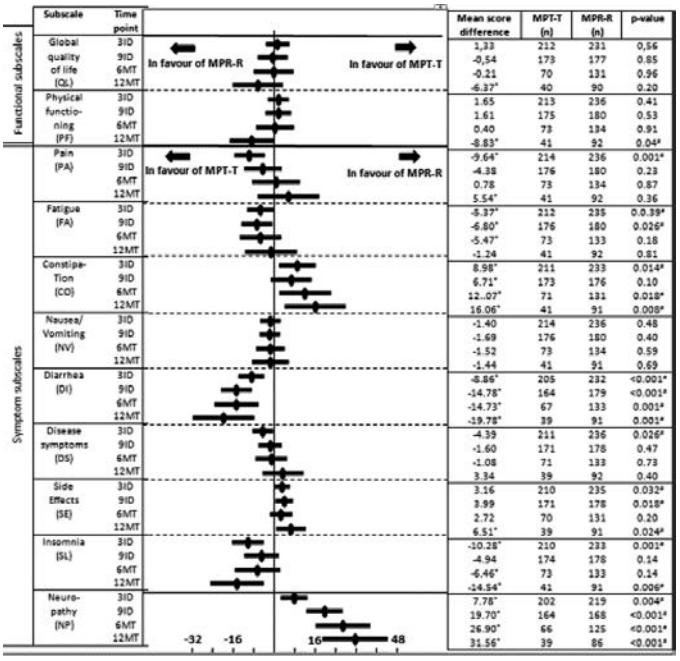
Background: We recently reported the results of the phase III randomized HOVON87/NMSG18 study showing comparable efficacy of treatment with melphalan, prednisolone and thalidomide following by thalidomide maintenance (MPT-T) *versus* melphalan, prednisolone and lenalidomide followed by lenalidomide maintenance (MPR-R) (Zweegman S *et al.* Blood 2016;127(9):1109-1116). As not only efficacy but also potential toxicity affecting quality of life (QoL) guides the choice of treatment, health-related (HR) QoL is important.
Aims: To evaluate the HRQoL results of the HOVON87/NMSG18 study.
Methods: Two validated HRQoL instruments (EORTC QLQ-C30 and MY20) were obtained at baseline, after 3 and 9 induction cycles (3ID and 9ID) and after 6 and 12 months of maintenance therapy (6MT and 12MT). The subscales global QoL, physical functioning, pain, fatigue, constipation, diarrhea, nausea/vomiting, insomnia, disease symptoms, side effects of treatment and neuropathy were analysed. Change in HRQoL score over time between treatment arms was assessed by linear mixed models. Independent sample t-tests were used to determine changes from baseline. Minimal important difference (MID) within arms was defined as a difference in score of ≥1 standard error of measurement (SEM) or, if a subscale consisted of one parameter only, MID-levels described in previous literature were used. To determine clinically relevant superiority of one arm, a difference in score of ≥5 was used and in addition significance level was calculated.
Results: From 553 (90.2%) of the 613 patients who participated in the HRQoL part of the study a baseline questionnaire was available. Forty (15%) of patients randomized to MPT-T *versus* 68 (24%) of patients randomized to MPR-R completed therapy until 12 months of maintenance therapy. *Change in HRQoL between arms over time:* in MPT-T improvement of HRQoL over time as compared to MPR-R was found for the subscales diarrhea and insomnia. In contrast, MPR-R showed improvement over time for the subscales pain, constipation, side effects of treatment and neuropathy, as compared to MPT-T. *Change in HRQoL per arm:* In MPT-T MID was reached for the following subscales; global QoL increased after 9ID until 12MT (MID range 7-13), pain decreased at every time point (MID range -21 to -23), disease symptoms deceased after 9ID (MID -12), fatigue decreased during MT (MID 12) and insomnia decreased at each time point (MID range -11 to -21). In MPR-R the MID was reached for the following subscales; global QoL increased after 9ID until 12MT (MID range 8-14), physical functioning increased at 12MT (MID 13), pain decreased at every time point (MID range -14 to -26) and insomnia decreased at 6MT (MID -10). *Difference between MPT-T and MPR-R:* In the MPT-T arm significantly ($p<0.05$) and/or clinically (mean score difference (MSD) ≥5 points) less pain and disease symptoms at 3ID, less fatigue at 3ID and 9ID, less diarrhea and less insomnia at all time points were observed. In contrast, patients on MPR-R reported better global QoL, better physical functioning and less pain at 12MT, in general less side effects of treatment, and less constipation and neuropathy separately, at all time points than patients treated with MPT-T.

Table 1.



Summary/Conclusions: Both treatment with MPT-T and MPR-R controlled pain and resulted in an improvement in global QoL as compared to baseline after 9ID and during maintenance. Treatment with thalidomide initially resulted in less pain and disease symptoms. At all treatment stages thalidomide caused less diarrhea, fatigue and insomnia as compared to treatment with lenalidomide. In contrast, therapy with lenalidomide resulted in less side effects of treatment, less constipation and less neuropathy as compared to thalidomide at all stages of treatment. In addition, long term maintenance therapy with lenalidomide resulted in better global QoL, better physical functioning and less pain.

S502

HEALTH-RELATED QUALITY OF LIFE RESULTS FROM THE PHASE III GALLIUM STUDY OF OBINUTUZUMAB-BASED AND RITUXIMAB-BASED THERAPY IN PATIENTS WITH PREVIOUSLY UNTREATED ADVANCED INDOLENT NON-HODGKIN LYMPHOMA

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Background: Maintenance of pretreatment health-related quality of life (HRQoL) and/or meaningful improvements in HRQoL are important for previously untreated indolent non-Hodgkin lymphoma (iNHL) patients (pts). GALLI-

UM (NCT01332968) is an open-label, randomized Phase III study of obinutuzumab (GA101; G) plus chemotherapy (chemo) followed by G maintenance (G-chemo) compared with rituximab (R) plus chemo followed by R maintenance (R-chemo) in pts with previously untreated iNHL. In GALLIUM, G-chemo produced a clinically meaningful improvement in investigator-assessed progression-free survival (PFS) among follicular lymphoma (FL) pts (34% reduction in risk of a PFS event relative to R-chemo). Grade 3–5 and serious adverse events were more common with G-chemo.
Aims: To compare changes in HRQoL in FL pts receiving G-chemo and R-chemo during GALLIUM.

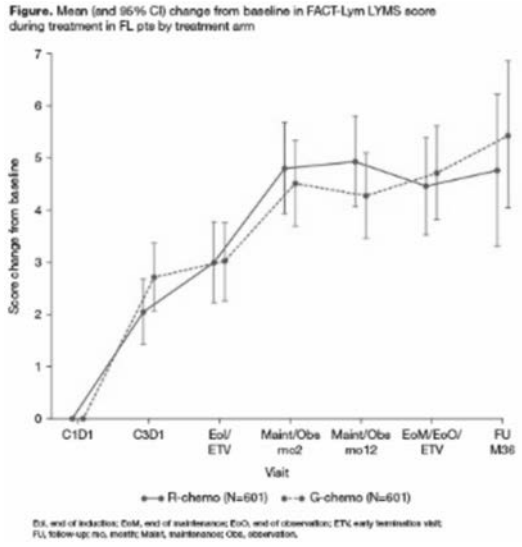

Figure 1.

Methods: Enrolled pts were aged ≥18 years with documented, previously untreated FL (grades 1-3a), advanced disease (stage III/IV or stage II with tumor diameter ≥7cm), ECOG performance status 0-2, and requiring treatment according to GELF criteria. Pts were randomized 1:1 to R 375mg/m^2 on day (D) 1 of each cycle (C) or G 1000mg on D1, 8, and 15 of C1 and D1 of C2-8, for 6 or 8 cycles depending on chemo (CHOP, CVP or bendamustine). Responders continued to receive R or G every 2 months (mo) for 2 years or until progression. The Functional Assessment of Cancer Treatment-Lymphoma (FACT-Lym) questionnaire (Webster *et al.* 2005) was used to assess overall HRQoL, physical and functional well-being, and disease- and treatment-related symptoms. FACT-Lym was administered on D1 of C1 and C3 during induction, at the end of induction, and at mo 2 and 12 during maintenance/follow-up. For each FACT-Lym scale, mean and 95% confidence interval (CI) were derived for recorded scores at each visit and changes from baseline. Minimally important differences (MIDs) were used to calculate the proportion of pts reporting improvement on the FACT-Lym lymphoma subscale (LYMS; ≥3 points), Trial Outcome Index (TOI; ≥6 points), and lymphoma total score (Lym-Total; ≥7 points). All pts gave informed consent.
Results: Of 1202 FL pts randomized (median age, 59 yrs; 53.2% female; median observation time, 34.5 mo [range 0-54.5]), 556/601 (92.5%; G-chemo) and 550/601 (91.5%; R-chemo) completed all FACT-Lym scales at baseline. Baseline demographics and disease characteristics were balanced between arms. At baseline, mean HRQoL scores were similar in the two treatment arms, with all pts having some impairment of physical function, functional wellbeing, emotional and social function. Over the course of treatment, mean HRQoL was similar in the two treatment arms. From end of induction onwards, pts in both arms experienced clinically meaningful improvements from baseline in LYMS scores (Figure), and the summary scales that included this subscale (TOI, Lym-Total). On each summary scale, ~50% of patients in each arm reported clinically meaningful improvements. There were no clear differences between arms in HRQoL scores over the course of therapy.
Summary/Conclusions: In previously untreated FL pts in GALLIUM, G-chemo and R-chemo produced similar improvements in HRQoL. These results suggest that lymphoma-related symptoms were reduced by both treatments and that the resulting improvements in well-being were not abrogated by treatment-related side effects. When viewed in the context of longer PFS, these results further support the relative benefit of G-chemo over R-chemo in GALLIUM.

S503

EFFECTIVE KEY WORKERS REDUCE THE NEED FOR CANCER SUPPORT GROUPS: RESULTS OF A POPULATION BASED SURVEY FROM GREATER MANCHESTER CANCER PATHWAY BOARD (GMCPB)

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Background: Cancer patient support groups appear to provide an important source of support to many patients and carers. In recent years there has been an increasing focus in the UK for services to provide cancer support groups, however it is unclear what proportion of patients believe access to these support groups would improve their experience of living with and beyond cancer.
Aims: A patient experience survey was undertaken by the Haematology-Oncology GMCPB across 10 NHS hospital trusts, where there are a number of cancer support groups.
Methods: The sample for the survey included all adult (aged >16) patients with a confirmed diagnosis of a haematological cancer who attended a haematological oncology outpatient appointment during a 4 month period (June-September 2016). The survey was available for competition on paper or online and was completed anonymously. A translation/interpretation facility was not provided for patients whose first language was not English (due to funding restraints).
Results: 277 responses were returned with 1 response excluded (non-haematological malignancy). Haematological diagnoses included acute leukaemia (n=40), chronic leukaemia (n=35), lymphoma (n=62), myeloma (n=102), MDS (n=15), MPD (n=12), other (n=2) and not specified (n=7). 257 (93.1%) patients had received anticancer therapy, 218 (79%) were receiving treatment at the time of survey and 54% had ongoing symptoms related to their treatment or cancer. 197 (71.4%) patients did not want access to a support group, 23 (19%) wanted access, 51 (8.3%) were not aware of the possibility and 6 (1.8%) did not respond. 51.8% of patients were aware of the existing support groups, 38.8% were not sure, 2.9% were not aware and 1.8% did not respond. The cohort of patients who did or did not want access to a support group was analysed further. 88% of patients had been given a key worker (eg clinical nurse specialist, research nurse, advanced nurse practitioner or nurse clinician); of the those 88% were satisfied and 1% were partly satisfied with the support they had received with 11% not responding. 93% (n=231) of patients were satisfied with the information they had received at diagnosis and 90% (n=224) felt their diagnosis had been given sensitively. Only 20% of patients currently on treatment wanted access to a support group and 24% not on treatment wanted access to a support group. Date of diagnosis was divided into three groups. Grp A: before 2005 (n=15), Grp B: after 2006 (n=229) and not stated (n=14). There was no difference in the three groups when asked if they wanted access to support group (13%, 22%, 7% respectively; p=0.3) or awareness that support group was available (40%, 57%, 50% respectively; p=0.6). There were additional comments from patients that support from family and online forums in addition to key workers was extremely valuable to them. On univariate analysis patients who were satisfied with their key worker support did not want access to a support group (p=0.04). There was no effect on wanting access to a support group and diagnosis (p=0.67), treating hospital (p=0.5), information given (p=0.6), need for in-patient treatment (p=0.3), quality of care (p=0.8) or satisfaction with overall care (p=0.8).
Summary/Conclusions: Our results suggest that a large majority of patients with a haematological malignancy do not want access to a cancer support group but providing satisfactory support through key workers and other health care professionals is likely to achieve better patient experiences.
Acknowledgements: We would like to acknowledge the members of the GMCPB and patients for their contribution to the survey.

S504

FRONT-LINE VASCULAR ACCESS DEVICES IN ACUTE LEUKEMIAS-PERIPHERALLY INSERTED CENTRAL CATHETER (PICC) VERSUS TRADITIONAL CENTRAL VENOUS CATHETER (CVC): A PHASE IV RANDOMIZED TRIAL (NCT02405728)

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Background: The use of PICC as an alternative to other CVC devices, particularly for prolonged infusions of cytotoxic agents, blood products and/or other supportive therapy, is becoming very frequent in cancer patients. PICCs are easier to insert, and associated to a lower rate of severe complications than traditional CVCs. However, there is limited information on the feasibility and safety of PICC as primary vascular access device in the setting of high-risk hematological patients.
Aims: Our Hematology Department is conducting a Phase IV randomized trial on this topic. We compare PICCs *versus* traditional CVCs as front-line venous access device in patients with acute leukemias undergoing intensive chemotherapy for remission induction (NCT02405728; ongoing). Primary endpoint is the occurrence of catheter-related bloodstream infections and/or thrombosis. Secondary endpoints are the occurrence of other complications, such as pneumothorax or catheter occlusion, and patients'quality of life. Questionnaire covering functional status, sleep and hygiene disturbance had been given to assess patients' quality of life.

Methods: From April 2015 to February 2017, 152 consecutive patients with acute leukemia planned for remission induction chemotherapy were randomly assigned (1:1) to PICC (Arm A) or traditional CVC (Arm B) (Table 1). Inclusion criteria were age >18 years, expected survival >4 weeks, and need of central venous access (long-term >4 weeks). Exclusion criteria were ongoing uncontrolled systemic infection, presence of significant thrombosis/stenosis in arm or central veins, and inability to communicate and/or to sign informed consent. All insertions were followed by ultrasonography assessments and chest X-ray.
Results: 152 patients (130 AML and 22 ALL) with a median age of 47 years (range, 13-82), were randomized in the two arms. In the Arm A, 76 PICCs (power injectable PICCs, in new generation polyurethane, open-ended) were inserted in 76 patients. Double lumen PICCs (5 Fr) were inserted in 70 patients, single lumen PICCs (4 Fr) were inserted in 5 patients, and triple lumen PICC (6 Fr) was inserted in 1 patient. 68 PICCs were inserted in the right basilica vein, 5 PICCs were inserted in the left basilica vein and 3 PICCs were inserted in the left brachial vein. In Arm B, 76 traditional CVCs (untunneled heparin-coated Vialon CVC, Becton-Dickinson) were inserted by the Seldinger technique in other 76 patients. 45 CVCs were inserted in subclavian vein and 31 CVCs were inserted in internal jugular vein. Overall, the median duration of *in situ* catheter placement was 5 months: 6 months (range, 3-12) in the arm A *vs.* 3 months (range, 1-10) in the arm B. In the arm A, catheter-related thrombosis occurred in 8 patients (6 basilica veins, 2 brachial veins) and catheter-related bloodstream infections in 4 patients (4 coagulase-negative *staphylococci*; of them, 2 meticillin-resistants). In the arm B, 20 cases of catheter-related thrombosis (7 subclavian veins, 13 internal jugular veins) and 15 cases of catheter-related bloodstream infections (10 *enterobacteriaceae;* 5 coagulase-negative *staphylococci,* and, of them, 3 meticillin-resistants) were observed. Thus, PICCs were significantly associated with fewer major complications than traditional CVCs (catheter-related thrombosis: 10.5% in the arm A *vs.* 26% in the arm B, p=0.01 by $\chi 2$ test; catheter-related bloodstream infections: 5% in the arm A *vs.* 19% in the arm B, p= 0.007 by $\chi 2$ test) (Figure 1). Questionnaire covering activities of daily living confirmed improvement of quality of life.

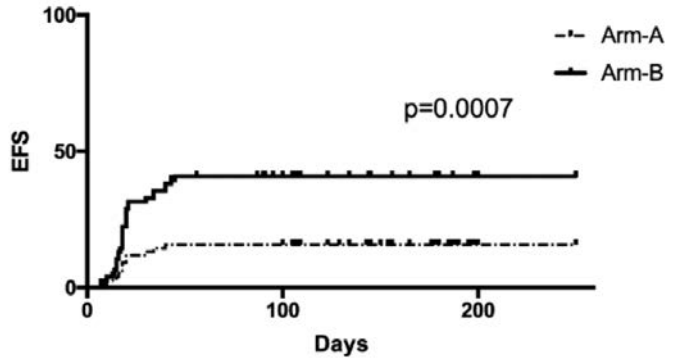

Figure 1.

Summary/Conclusions: The preliminary observations of this ongoing Phase IV randomized study, focusing on front-line use of central venous access device in a high risk hematological population, suggest that the use of PICC represents an advance in terms of decrease of complication rate and improvement of quality of life for patients with acute leukemia.

S505

THE SIMM STUDY: SURVEY OF INTEGRATIVE MEDICINE IN MYELOPROLIFERATIVE NEOPLASMS

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Background: Pharmacologic therapy stabilizes hematologic counts and splenomegaly in myeloproliferative neoplasms (MPN), however only partial symptom improvement typically occurs. Evidenced-based integrative care may address this need, however data is limited in patients with MPNs.
Aims: To investigate integrative therapy utilization association with symptom burden, quality of life, depression, and fatigue in MPN patients.
Methods: Patients were recruited via social media. Informed consent and online self-report surveys (Qualtrics) were completed capturing patient demographics, disease specific data, supportive care utilization, MPN symptom burden via MPN-SAF TSS, depression via Patient Health Questionnaire (PHQ)-9, fatigue via Brief Fatigue Inventory (BFI) Usual, and an overall quality of life (QOL) single question assessment. ANOVA, chi square tests, and Wilcoxon rank sum tests methods were applied.

Results: *Patients*: A total of 1087 patient surveys were consented. Of these, 858 had 10 or more responses. There were 338 essential thrombocytosis (ET), 188 myelofibrosis (MF), 315 polycythemia vera (PV), and 17 other. In MF: DIPSS risk categories included low (8%), Int-1 (19%), Int-2 (29%), high (12%), and unknown (32%). *Symptom association:* Overall, patients had lower MPN related symptoms when participating in aerobic activity (p<0.001), massage (p=<0.001), yoga (p=0.02), strength training (p<0.001), breathing exercises (p=<0.001), and support groups (0.001). Overall quality of life was higher with aerobic activity (p=<0.001), massage (p=0.02), yoga (p=0.02), strength training (p=<0.001), breathing exercises (p=0.01), and support groups (p=0.001). Depression (PHQ-9 total >3 category) was lower in aerobic activity group (p=0.001), yoga (p=0.001), strength training (p=0.001), and meditation (p=0.2). Fatigue was lower in aerobic activity (p=<0.001), massage (p=0.04), strength training (p=<0.001), breathing exercises (p=<0.001), and support groups (p=0.001). In subgroup analysis, ET and PV patients had lower symptom burden (MPN-SAF TSS) with aerobic activity (p=<0.001, <0.001), massage (p= 0.01, 0.02), and strength training (p=0.03,0.02). Support groups were found to be associated with lower symptoms in ET patients (p=0.03). In MF, breathing exercises (p=<0.001) and support groups (p=0.03) were associated with lower symptom burden. See Table #1.

Table 1.

	Overall N = 858				ET N=338	PV N=315	MF N=188
	MPN-SAF TSS	QOL	PHQ-2	BFI-USUAL	MPN-SAF TSS	MPN-SAF TSS	MPN-SAF TSS
Aerobic Activity (Overall) N=442	**<0.001**	**<0.001**	**0.001**	**<0.001**	**<0.001**	**<0.001**	0.06
Massage N=244	**<0.001**	**0.02**	0.94	**0.04**	**0.01**	**0.02**	0.14
Yoga N=220	**0.02**	**0.02**	**0.001**	0.07	0.35	0.14	0.34
Nutrition N=216	0.10	0.18	0.25	0.48	0.36	0.07	0.68
Strength training N=204	**<0.001**	**<0.001**	**0.001**	**<0.001**	**0.03**	**0.02**	0.14
Acupuncture N=166	0.18	0.08	0.18	0.63	0.20	0.90	0.14
Meditation N=163	0.16	0.45	**0.02**	0.16	0.16	0.99	0.25
Breathing exercise N=158	**<0.001**	**0.01**	0.12	**<0.001**	0.37	0.30	**<0.001**
Chiropractic N=139	0.87	0.81	0.15	0.60	0.39	0.55	0.94
Support groups N=124	**0.001**	**0.001**	0.15	**0.001**	**0.03**	0.08	**0.03**

Summary/Conclusions: Integrative therapies are associated with improved symptom burden, quality of life, depression, and fatigue in MPN patients. Interestingly, unique patterns were associated within MPN subtypes. Further studies are needed to understand the benefits of integrative therapies in MPN patients.

POSTER SESSIONS II

Acute lymphoblastic leukemia - Biology 2

P506

T CELL EXHAUSTION CHARACTERIZED BY COMPROMISED MHC CLASS I AND II RESTRICTED CYTOTOXIC ACTIVITY ASSOCIATES WITH ACUTE B LYMPHOBLASTIC LEUKEMIA RELAPSE AFTER ALLO-HSCT

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Background: B cell acute lymphoblastic leukemia (B-ALL) relapse contributes to the predominant mortality after allogeneic hematopoietic stem cell transplantation (allo-HSCT). However, the mechanism of B-ALL relapse after allo-HSCT remains unknown. Eradication of leukemia in allo-HSCT settings largely relies on graft-versus-leukemia (GVL) effects mediated by donor T cells. T cell exhaustion characterized by increased expression of inhibitory receptors including PD-1 and Tim-3 and impaired function may blunt the GVL effects and was reported in acute myeloid leukemia relapse after allo-HSCT. whether T cell exhaustion is involved in B-ALL relapse after allo-HSCT remains unknown.
Aims: To evaluate whether T cell exhaustion is involved in B-ALL relapse after allo-HSCT,and investigate the correlation of inhibitory ligands on leukemic cells, leukemic load and T cell exhaustion, as well as the impact of treatment outcome on T cell exhaustion.
Methods: Our study enrolled 18 B-ALL patients who underwent first hematological relapse after allo-HSCT and18 matched B-ALL patients in remission (without minimal residual disease MRD) and 14 healthy donors from April 2016 to November 2016 at the Peking University People's Hospital, Institute of Hematology. Transplant protocol and post-transplant time were matched in relapsed and non-relapsed patients. Post-transplant time were matched as follows: ±14days within 12 months ±1months from 12 to18months, ±3months from 18 to 36 months, ±12months over 3 years. Extra-medullary relapse were excluded in our study. All patients had achieved full donor chimerism before relapse or bone marrow collection. Peripheral blood (PB) were collected at the same day of bone marrow collection in relapsed patients. For patients who received re-induction therapy, we prospectively collected BM at least once after therapy. Sample collection was performed after patients was informed consent and approval by the institutional Human Ethics Review Committee of Peking University People's Hospital in accordance with the Declaration of Helsinki.phenotypic and functional studies of T cells in those patients were performed using multi-color flow cytometry.
Results: In the current study, we observed that increased co-expression of PD-1 and Tim-3 was observed in both $CD4^+$ and $CD8^+$ T cells in relapse settings. Moreover, both CD4+ and CD8+ T cells exhibited compromised proliferative capacity, cytokine production and cytotoxic potentials such as degranuation and granzyme B production (preferentially on $CD4^+$T cells) in relapsed patients. In addition, T cells at the tumor site are more easily exhausted than those in peripheral blood. Reversal of T cell exhaustion was associated with effective anti-leukemic response in relapsed patients who underwent re-induction therapy.
Summary/Conclusions: In conclusion,our study suggested that T cells experienced exhaustion with comprehensively functional impairments in B-ALL relapse setttings after allo-HSCT and reversal of T cell exhaustion was associated with effective anti-leukemic responses. These results also provide a foundation for the development of novel effective leukemia therapeutics, such as anti-PD-1 or PD-L1 therapy, by targeting T cell exhaustion

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RUXOLITINIB/NILOTINIB COTREATMENT BETTER INHIBITS LEUKEMIA-PROPAGATING CELLS IN PHILADELPHIA CHROMOSOME-POSITIVE ALL

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Background: Relapse remains the major cause of treatment failure in patients with Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph^+ALL), even in the modern era of tyrosine kinase inhibitors (TKIs). Relapse of Ph^+ALL may result from the persistence of leukemia-propagating cells (LPCs), which are defined by their ability to initiate human leukemia and self-renew in immunocompromised mice. Using an anti-CD122-conditioned NOD/SCID xenograft mouse assay, LPCs were reported to be enriched in the $CD34^+CD38^-CD58^-$ fraction in human Ph^+ALL (**YK,…,XJH*. *Leukemia*. 2014**). Furthermore, a cohort study demonstrated that Ph^+ALL patients with LPCs phenotype at diagnosis exhibited a significantly higher cumulative incidence of relapse than did the group with other phenotypes, even when receiving uniform front-line imatinib-based therapy pre- and post-allotransplant (**YK,…,XJH*. *BMT*. 2015**). Therefore, it is imperative to identify novel therapeutic strategies based on LPCs to improve the prognosis of Ph^+ALL patients.
Aims: To identify the potential molecular basis of LPC-mediated relapse, RNA sequencing(RNA-seq) and real-time reverse transcription-PCR (qRT-PCR) were performed to analyze the gene expression profiles of sorted LPCs and cells of other phenotypes from Ph^+ALL patients. To investigate whether selective BCR-ABL/JAK2 dual inhibition therapy could more effectively eliminate LPCs *in vitro* and in humanized Ph^+ALL mice.
Methods: RNA-seq and qRT-PCR were performed to analyze the gene expression profiles of sorted LPCs and cells of other phenotypes from patients with *de novo* Ph^+ALL. In order to assess the effects of the selective BCR-ABL and/or JAK2 inhibition therapy by the treatment with single agents or a combination of ruxolitinib and imatinib or nilotinib on Ph^+ALL LPCs, drug-induced apoptosis of LPCs was investigated in vitro, as well as *in vivo* using sublethally irradiated and anti-CD122-conditioned NOD/SCID xenograft mouse assay. Moreover, western blot analyses were performed on the BM cells harvested from the different groups of recipient mice.
Results: Using RNA-seq and qRT-PCR, we found that JAK2 was more highly expressed in the sorted LPCs than in the cells of other phenotypes in patients with *de novo* Ph^+ALL. *in vitro* study, cotreatment with nilotinib and ruxolitinib induced significantly higher levels of apoptosis in LPCs. In humanized Ph^+ALL mice model, treatment with the nilotinib and ruxolitinib combination, compared with either ruxolitinib or TKIs alone, led to the most significant reduction in human Ph^+ALL engraftment in the recipients. Further evidence that the most effective anti-LPCs effect occurred with the combination treatment was derived by the engraftment analysis of *BCR/ABL* expressing cells using a qRT-PCR assay and HE and IHC with anti-hCD19 staining. Moreover, the combination of nilotinib and ruxolitinib more effectively reduced the LPCs capacity through a deeper suppression of phospho-CrKL, JAK2 and STAT5 activities at the molecular level.
Summary/Conclusions: JAK2 was more highly expressed in the sorted LPCs than in other cell phenotypes in patients with *de novo* Ph^+ALL. Furthermore, selective BCR-ABL/JAK2 dual inhibition with nilotinib/ruxolitinib more effectively eliminated LPCs than either ruxolitinib or TKIs alone. Therefore, this pre-clinical study appears to provide scientific rationale for simultaneously targeting BCR-ABL and JAK2 activities, which represents a promising anti-LPCs therapeutic approach for patients with *de novo* Ph^+ALL.

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PREDICTING ANTI-LEUKEMIA ACTIVITY OF THE BCL-2-SELECTIVE INHIBITOR ABT-199 IN BCP-ALL BY FUNCTIONAL ASSESSMENT OF APOPTOSIS SIGNALING

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Background: Although survival rates of pediatric BCP-ALL patients have continuously improved during the past decades, therapy-related toxicity and relapse occurring in 10-20 % of patients are associated with poor outcome, clearly emphasizing the need of novel, targeted treatment strategies. Deregulated survival pathways and cell death resistance contribute to treatment failure and reoccurrence of the disease. ABT-199 (venetoclax) is a small molecule inhibitor of BCL-2 demonstrating anti-cancer activity among different malignancies. However, predictive biomarkers are required for up-front identification of patients who would benefit from BCL-2 directed therapies.
Aims: The aims of this study were to assess the efficacy of ABT-199 in BCP-ALL, to functionally evaluate factors mediating ABT-199 susceptibility or resistance and to identify markers indicative of successful anti-leukemia activity.
Methods: The activity of ABT-199 was assessed by cell viability assays in BCP-ALL cell lines (N=6) and patient-derived xenograft (pdx) samples (N=27), analyzing half maximal effective concentrations (EC50). Expression of apoptosis regulators was detected by western blot analysis. MCL-1 deficient cell lines were generated by CRISPR/Cas9 gene editing. BH3 profiling was used to measure the mitochondrial dependence of leukemia cells on anti-apoptotic BCL-2 family proteins. *In vivo* treatment of ABT-199 was performed in a set of three distinct ALL pdxs.
Results: Different sensitivities of ABT-199 were observed in a series of BCP-ALL pdxs and cell lines with heterogeneous anti-leukemia activities upon drug exposure. The majority of BCP-ALL samples showed sensitivity to ABT-199-induced cell death in the nanomolar range (EC50 <1µM) with four out of six cell lines and 20 of 27 pdxs, while ABT-199 insensitivities with EC50s of more than 1µM were identified in 26% of pdx leukemias. ABT-199 induces apoptosis by selectively inhibiting BCL-2 with a sub-nM binding-affinity thereby releasing pro-apoptotic molecules; however, the sequestration of drug-released BIM by anti-apoptotic MCL-1 might lead to resistance. Therefore, we investigated protein expression of both regulators and found the ratio (BCL-2/MCL-1) to be cor-

related with ABT-199 sensitivity (r_s=-0.71, p=0.008), highlighting the importance of simultaneous assessment of the direct target molecule and additional resistance mediating molecules. In line, MCL-1 knockout in two ABT-199-resistant cell lines led to sensitization towards ABT-199, however, resulted in different effects of sensitization, emphasizing that ABT-199 resistance is determined by the interplay of several apoptosis regulators. Therefore, we characterized the functional dependence of pdx leukemias on anti-apoptotic BCL-2 family members by BH3 profiling. Mitochondrial dependence on BCL-2 (mitochondrial priming by the BAD-peptide measuring BCL-2, BCL-XL and BCL-W, and subtracting the response to the HRK-peptide measuring BCL-XL) was found to be tightly correlated with ABT-199 sensitivity. In contrast, ABT-199-resistant samples were characterized by low BCL-2-dependence and addiction to other BCL-2 family members, including BCL-XL or MCL-1. Finally, we evaluated prediction of *in vivo* ABT-199 sensitivity in a pre-clinical ALL pdx mouse model by functional BH3 profiling. Strikingly, high mitochondrial BCL-2-dependency was clearly associated with prolonged leukemia-free survival upon ABT-199-therapy (two pdxs, log ranks p=0.0035 and <0.0001), in contrast to another leukemia with low BCL-2-dependence and *in vivo* ABT-199 resistance (log rank p=0.144).
Summary/Conclusions: BCP-ALL displays heterogenous ABT-199 sensitivities characterized by the level of the target molecule but also other interacting regulators. Functionally, mitochondrial BCL-2-dependence assessed by the BH3 profiling assay is clearly associated with ABT-199 sensitivity. Importantly, *in vivo* anti-leukemia activity of ABT-199 therapy in individual pdx leukemias is predicted by mitochondrial BCL-2 dependence, emphasizing the utility of identification of patients and guidance of future clinical application by functional assessment of apoptosis signaling.

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CD45RA- MEMORY T CELLS EXPRESSING AN NKG2D-CAR TARGET PEDIATRIC ACUTE LEUKEMIA

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Background: Lymphoid and myeloid acute leukemia are the most frequent type of cancer and the most frequent cause of cancer related death in children. Relapse and refractory disease are the main clinical problems that current therapies are still unable to solve. One of the main NK cell activating receptors is NK cell group 2D (NKG2D). NKG2D receptor recognizes human MICA, MICB and ULBP1-6 ligands. These NKG2D ligands (NKG2DL) are expressed in leukemia cells and constitute suitable targets for immunotherapy.
Aims: The aim of thi study was to analyze the NKG2DL expression on pediatric acute leukemia cells and determine their susceptibility to an NKG2D CAR cell based immunotherapy.
Methods: The expression of NKG2DL was analyzed in Peripheral Blood Mononuclear Cells (PBMCs) from patients suffering from acute leukemia, as well as in leukemia cell lines, by flow cytometry (FCM) using specific monoclonal antibodies directed against MICA, MICAB, ULBP-1, ULBP-2, ULBP-3 and ULBP-4, and by quantitative PCR using TaqMan probes. PBMC from healthy donors were labeled with CD45RA microbeads and depleted using AutoMACS device. The HL20i4r-MNDantiCD19bbz lentiviral vector was derived from the clinical vector CL20i4r-EF1a-hgcOPT27 but contained the extracellular domain of NKG2D, the hinge region of CD8a and the signaling domains of 4-1BB and CD3-z. The cassette was driven by MND promoter. Viral supernatant was produced by transient transfection of HEK293T cells with the vector genome plasmid and lentiviral packaging helper plasmids pCAGG-HIVgpco, pCAGG-VSVG and pCAG4-RTR2. Cytogenetic studies and array Comparative Genomic Hybridization were performed to analyze the genetic stability of lentiviral-transduced memory T cells. The *in vitro* cytotoxicity of CD45RA-NKG2DCAR+ memory T cells against leukemia cells, healthy PBMC and Mesenchymal Stem cells (MSC) was evaluated by performing conventional 4-hour europium-TDA release assays or by FCM using CFSE and 7AAD labeling of target cells.
Results: NKG2DL were heterogeneously expressed in leukemia primary cells and cell lines. For B cell ALL primary samples, we found expression of MICA/B, MICA and ULBP1 decreased in refractory disease compared to remission. Lentiviral transduction of NKG2D-4-1BB-CD3z increased NKG2D surface expression in CD45RA$^-$ memory T cells, which became consistently more cytotoxic than untransduced cells against leukemia cells. Additionally, no chromosomal aberrations nor cytotoxic activity against healthy PBMC or Mesenchymal Stem cells was observed in NKG2D CAR expressing T cells.
Summary/Conclusions: Our results show NKG2D-CAR redirected CD45RA$^-$ memory T cells target NKG2DL expressing leukemia cells *in vitro* and could be a promising and safe immunotherapeutic approach for pediatric acute leukemia patients.

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A BILINEAL ACUTE LYMPHOBLASTIC LEUKEMIA ORIGINATING AT A COMMON LYMPHOID PROGENITOR

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Background: Genetic mutations are crucial events during leukemogenesis and provide specific markers for backtracking the cellular origin of acute leukemias up to immature uni- or multi-potent progenitor cells in the hierarchy of the hematopoietic system.
Aims: To characterize the clonal architecture and cell of origin in a case of bilineal T- and B-ALL.
Methods: Bone marrow cells obtained at diagnosis were used for all studies. Immunophenotyping was done by flow cytometry. T- and B-leukemic cell purification was performed by immunomagnetics methods and DNA extracted afterward. TCR-gamma gene rearrangement was studied in T- and B-leukemic cells independently by PCR spectratyping. Somatic mutations in purified T- and B-leukemic cells were identified by deep-sequencing using a panel of 160 genes frequently mutated in cancer (Human comprehensive cancer panel, Qiagen). Mutations were validated by Sanger sequencing. Myeloid and erythroid clonogenic progenitors were isolated from methylcellulose cultures, DNA extracted, and assessed for the presence of the *H3F3A* p.K28N mutation by Sanger sequencing.
Results: The patient was a 10 years old boy. At diagnosis, the bone marrow was infiltrated by 60% leukemic cells, with 2 immunophenotypically different populations: a common B-ALL (54%) and a pro-T-ALL (6%). The patient showed a mediastinal mass in the chest X-ray image. A common TCR-gamma rearrangement was detected in purified (>95% pure) T-ALL and B-ALL cells, suggesting a common origin for both leukemic subpopulations. The B-ALL cells presented a c.35G>A p.G12D mutation in the *KRAS* gene, absent in the T-ALL. The T-ALL cells presented a c.35G>A (p.G12D) mutation in the *NRAS* gene, absent in the B-ALL. A c.1126_1127insTAGA (p.P376Lfs*10) mutation in the *WT1* gene was also detected only in the T-ALL. A c.84G>T (p.K28N) mutation in the *H3F3A* gene was detected in both the B-ALL and T-ALL subpopulations, confirming the involvement of a Common Lymphoid Progenitor in the process of leukemogenesis. The presence of the *H3F3A* p.K28N mutation in the myeloid compartment would point to a multipotent myeloid-lymphoid rather than a lymphoid-restricted progenitor as the cell origin of the leukemia. Therefore, we cultured myeloerythroid-committed progenitor cells in clonogenic cultures and sequenced the *H3F3A* gene. None of the 122 myeloid or erythroid clonogenic progenitors (41 CFU-GM, 73 BFU-E and 8 CFU-GEMM) presented the p.K28N mutation in the *H3F3A* gene.
Summary/Conclusions: Our results indicate the involvement of a Common Lymphoid Progenitor as the cell of origin in this case of bilineal ALL, as well as the crucial role of *H3F3A* and *RAS* family genes in the leukemogenesis process coupled with B and T differentiation.

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CYSTEINE AND GLYCINE-RICH PROTEIN 2 (CSPR2) TRANSCRIPT LEVELS CORRELATE WITH LEUKEMIA RELAPSE AND LEUKEMIA-FREE SURVIVAL IN ADULT B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA WITH NORMAL CYTOGENETICS

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Background: Outcome of adults with B-cell acute lymphoblastic leukemia (ALL) remains poor and relapse is the major cause of treatment-failure. Identifying new biomarkers in B-cell ALL especially in those with normal cytogenetics and studying their clinical significance and biological function will be helpful for risk-stratification, treatment decision and targeted therapy.CSRP2 (cysteine and glycine rich protein 2) maps to chromosome subband 12q21.1, which is frequently abnormal in diverse cancers. Increased CSRP2 transcript levels are associated with de-differentiation in hepatocellular carcinoma and CSRP2 is proved to be a new invadopodia actin bundling factor that critically promotes breast cancer cell invasion and metastasis. However, the clinical significance and biological function of CSRP2 in B-cell ALL remains unknown.

Aims: To identify novel biomarkers in B-cell ALL based on bioinformatics analyses; to examine the expression and clinical significance of CSRP2 in adults with B-ALL; to explore effects of CSRP2 on biological function of B-cell ALL.
Methods: We did bio-informatics analyses to identify mRNA transcripts aberrantly-expressed in B-cell ALL. RT-qPCR (real-time quantitative polymerase chain reaction) was used to examine CSRP2 transcript levels in bone marrow samples from 236 adults with B-cell ALL compared with samples from normal. A prognostic value was assessed in 168 subjects. CSRP2-knockdown and CSRP2-over-expression cell models were constructed to study the biological function of CSRP2 in B-cell ALL.
Results: We selected 9 candidate genes for validation 7 of which proved significantly-associated with B-cell ALL. CSRP2 was the most differentially expressed gene in our validation studies. CSRP2 was over-expressed in 228 out of 236 adults (97%) with newly-diagnosed B-cell ALL. In subjects with normal cytogenetics those with high CSRP2 transcript levels had a higher 5-year cumulative incidence of relapse (CIR) and worse relapse-free survival (RFS) compared with subjects with low transcript levels (56% [95% confidence interval 53-59%] *vs* 19% [18- 20%]; *P*=0.011 and 41% [17-65%] *vs* 80% [66-95%]; *P*=0.007). In multivariate analyses a high CSRP2 transcript level was independently-associated with CIR (HR=5.32 [1.64-17.28]; *P*=0.005) and RFS (HR=5.56 [1.87-16.53]; *P*=0.002). Functional analyses indicated CSRP2 promoted cell proliferation, cell-cycle progression, *in vitro* colony formation and migration. Abnormal CSRP2 expression was associated with resistance to chemotherapy; sensitivity was restored by down-regulating CSRP2 expression. CSRP2 activated ERK1/2 signaling pathway, regulated cell-cycle related protein and activated CREB signaling pathway, whose activation was associated with poor prognosis in adults with B-cell ALL.
Summary/Conclusions: CSRP2 was widely over-expressed in adults with B-cell ALL. Determination of CSPR2 transcript levels in subjects with normal cytogenetics might inform therapy-decisions. Consideration could be given to down-regulating CSPR2 expression as a way to reverse drug resistance.

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THERAPEUTIC TARGETING OF PRE-B CELL RECEPTOR SIGNALLING IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Acute lymphoblastic leukaemia (ALL) is the most common malignancy in children and adolescents and relapsed ALL remains one of the leading causes of cancer-related deaths in children. Components of the precursor-B cell receptor (Pre-BCR) signalling pathway are hijacked in ALL cells and this dependence may be therapeutically targeted. A number of tyrosine kinase inhibitors (TKIs) targeting effectors of this signalling pathway are showing great promise in the clinic and warrant preclinical evaluation in paediatric ALL. They include Dasatinib (BCR-ABL/SRC inhibitor), Fostamatinib R406 (SYK inhibitor), Ibrutinib (BTK inhibitor) and CAL-101 (PI3K-δ inhibitor).
Aims: To preclinically evaluate these candidate TKIs, as novel, targeted drugs for high risk and relapsed ALL.
Methods: ALL cell lines (Reh, Nalm-6, PreB 697 and its glucocorticoid resistant descendant, R3F9) and 36 primary-derived xenograft (PDX) cells from 16 ALL were used in the study. Cell viability was assessed by Resazurin. Pre-BCR expression (μHc, Vpreb and λ5) and functionality using a Calcium flux assay were detected by Flow cytometry. Intracellular phospho-flow cytometry was used to detect constitutive phosphorylation and activation in response to anti-Igμ antibody, as well as drug pharmacodynamic measures (p-BTK, p-SYK, p-AKT, p-ERK, p-PLC-Y2, p-BLNK). Apoptosis and cell cycle were analysed by flow cytometry using Annexin V and Propidium Iodide. RQ-PCR was used to measure *GILZ* expression. Bim induction, GR expression and phosphorylation were detected by western blotting.
Results: ALL cell lines were modestly sensitive to Dasatinib (mean GI50 5.33 μM, range 2.45 μM-12.5 μM) and R406 (mean GI50 4.32 μM, range 2.88 μM-5.83 μM). However, cells were resistant to Ibrutinib (mean GI50 15.9 μM, range 11.47 μM-18.3 μM) and CAL-101 (mean GI50 52.08 μM, range 25 μM-77.83 μM). Cell cycle arrest and significant apoptosis was seen with R406 and Ibrutinib treatment, while Dasatinib and CAL-101 were cytostatic, causing G1 arrest with no substantial cell death. Pharmacodynamic assays confirmed inhibition of the relevant drug targets. PDX cells showed greater sensitivity than the cell lines to Dasatinib (4 out of 16 patient samples <0.5μM), R406 (7 out of 16 patient samples <5μM), Ibrutinib (3 out of 15 patient samples <5μM) and CAL-101 (3 out of 15 patient samples <2μM). Pre-BCR positive ALL cell lines and PDX cells were sensitive to R406 and Dasatinib, with a Ph+ PDX confirming sensitivity to the latter. Combining TKIs with the glucocorticoid (GC), Dexamethasone showed synergism in ALL cell lines and was particularly notable for Dasatinib and R406 in PreB cell receptor positive lines. Synergism was associated with significantly enhanced apoptosis, an increase in expression of the GR target gene, *GILZ* and for Dasatinib, enhanced expression of the pro-apoptotic, Bim. Control REH cells (GC receptor negative) showed no synergism.
Summary/Conclusions: Significant sensitivity of TKIs targeting Pre-BCR signalling have been identified at clinically achievable concentrations. Dasatinib and R406 sensitivity was associated with Pre-BCR positive ALL and combination with Dexamethasone showed significant synergism in GC resistant cell lines and PDX samples. TKIs were also effective in some Pre-BCR negative ALL cells, however, predictive biomarkers need to be established. Confirmation of these data in preclinical models *in vivo* may define new therapies for high risk ALLs.

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BMP-4 LEVELS IN CHILDHOOD B-ALL OF LOW-/INTERMEDIATE-RISK GROUPS IDENTIFY CHILDREN WITH POOR OUTCOME

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Background: Leukemic relapses among children with acute lymphoblastic leukaemia (ALL) from low/intermediate-risk groups is a challenge for the cure of this disease. New biomarkers are needed for identifying children at high risk of relapses. Bone Morphogenetic Proteins (BMPs) are multifunctional secreted growth factors that belong to the TGF-β superfamily and are well-known for their indispensable roles in vertebrate development. In the cellular context, BMPs regulate fundamental processes such as cell proliferation, differentiation, migration and survival. In last years, important new information has been generated on the contribution of BMP family members, such as BMP4, in cancer pathogenesis.
Aims: Here we have evaluated the relevance of BMP4 signaling in ALL.
Methods: The expression levels of BMP-4 related genes (bmp-4, and bmp-receptors, signaling mediators, inhibitors and targets) in ALL blasts obtained at the time of diagnosis (n=56), and the BMP-4 levels in central system fluid samples (CSF), were quantitated by RT-qPCR or ELISA. The engrafting potential of primary ALL cells, exhibiting high or low BMP4 levels, were assessed in xenotransplantation experiments using unirradiated NSG mice.
Results: BMP4 was expressed at significantly higher levels in ALL blasts of children who later relapsed (178,78 *versus* 26,68, arbitrary units, AU, p<0,05). Relapses among children with high BMP-4 expression occured significantly later than those with low BMP-4 expression (845 days *versus* 282 days, p<0,05). The difference in the cumulative incidence of relapses (CIR) was quasi-significant between both groups (p=0,0831). The ratio Smad7:Smad1, suggesting inhibition of the Smad-dependent signaling pathway, was significantly higher in ALL blasts of children who later relapsed (14,33 *versus* 5,13, AU, p<0,05). CIR was significantly higher (p<0,05) in the group of children with the Smad-dependent pathway inhibited. All these differences were detected considering the whole population, as well as only the low/intermediate-risk groups. BMP4 levels were significantly higher in CSF samples of children with leukemic infiltration of the central nervous system (16pg/ml *versus* 3,4pg/ml, p<0,001), as well as in the group of children who relapsed (10,6 pg/ml *versus* 1,8 pg/ml, p<0,001). Hematopoietic engraftment (marrow, spleen and peripheral blood) and CNS leukaemia occured only in ALL samples with high BMP4 levels. Even more, no signs of disease were detected in mice transplanted with primary cells which expressed low levels of BMP4. In independent experiments, pharmacological blockade of the canonical BMP signaling pathway significantly decreased infiltration of CNS and consistently resulted in amelioration of clinical parameters including neurologic score.
Summary/Conclusions: These results indicate that high BMP4 levels are required for both bone marrow engraftment and CNS infiltration by B-ALL cells. BMP4 levels in leukemia cells could be a useful biomarker to identify children with poor outcome in the childhood B-ALL of low-/intermediate-risk groups. Furthermore, BMP4 could be a new therapeutic target to blockade leukemic CNS disease.

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TARGETING LOCALIZATION OF THE IL-7 RECEPTOR WITHIN LIPID RAFTS AS A THERAPEUTIC STRATEGY FOR T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: T-cell acute lymphoblastic leukemia (T-ALL) is a hematological malignancy characterized by immature T-cell excessive proliferation. To achieve remission, patients typically undergo 2 years of chemotherapy, associated with acute and chronic side effects. To enable reduced chemotherapy intensity and

treat relapsed patients, there is still a need to develop new and effective targeted therapies. Previous evidence has shown IL-7 as a fundamental cytokine for normal T-cell development and homeostasis, as well as an important determinant for the T-ALL cell viability and proliferation *in vitro* and *in vivo*. Several strategies have been explored for the treatment of T-ALL, and targeting Interleukin 7 receptor alpha-chain (IL-7Rα) and downstream IL-7 signaling offers a potentially effective therapeutic strategy for T-ALL. The IL-7Rα is recruited and concentrated into lipid rafts thereby amplifying its downstream signaling pathway. The IL-7/STAT5 signaling pathway mediates downregulation of pro-apoptotic signals on human T-cells such as BAD and BIM. Moreover, we have previously reported that a new compound named Pyrido[4,3-b]Quinoxaline (PyQ) has an anti-tumoral effect on Acute Myeloblastic Leukemia (AML). It strongly interacts with the plasma membrane of AML cells and affects the positioning of the protein tyrosine phosphatase CD45, which is usually organized in microdomains into lipid rafts floating on the cell surface, and is delocalized by PyQ.

Aims: The aim of this study was to assess the anti-tumoral effect of PyQ on T-ALL cells and to identify which signaling pathway is affected by the compound.

Methods: We have 2 models of human T-ALL which can be studied *in vitro* when cocultured with murine stromal MS5 cells and *in vivo* when transplanted into immunodeficient NOD/SCID/γc−/− (NSG) mice. We also work on primary T-ALL blasts isolated from 10 patients suffering of T-ALL and maintained frozen in a biobank.

Results: In this study, we have shown that PyQ delocalizes the IL-7Rα away from lipid rafts on the cell surface of human T-ALL cells. We have also proved that localization of the IL-7Rα among lipid rafts plays a crucial role in human T-ALL maintenance *in vitro*. Its delocalization leads to IL-7 signaling pathway inactivation, upregulation of BAD and BIM genes involved in apoptosis and T-ALL cells apoptosis. We furthermore assessed effect of PyQ on 10 samples of primary T-ALL blasts. All of them were sensitive to IL-7-dependent cell survival and revealed a marked response to PyQ treatment (Mean IC_{50}=5.7 ng/mL). For this work, T-ALL cells were co-cultured on murine stromal MS5 cells and PyQ has affected mainly T-ALL cell growth. No effect was observed on the stromal feeder cells, suggesting that injection of PyQ *in vivo* would not impact the stromal microenvironment in bone marrow. Finally, we provided evidence that PyQ delayed T-ALL progression *in vivo*, after treatment of immunodeficient mice xenografted with T-ALL cells.

Summary/Conclusions: The findings of this study highlight the importance of the IL-7Rα localization in maintenance of T-ALL cells and may lead to the design of a new generation of anti-cancer drugs able to modulate the protein positioning into lipid rafts.

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SYSTEMATIC MRI SCREENING IDENTIFIES EXTENSIVE ASYMPTOMATIC OSTEONECROTIC LESIONS IN ADOLESCENTS WITH ALL - FIRST INTERIM FINDINGS OF THE OPAL TRIAL

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Background: Cure rates for acute lymphoblastic leukemia (ALL) have increased to ~90% in the last decades, but come at a high cost as a substantial proportion of these children sustain toxic side-effects. Osteonecrosis (ON) is one of the most common and debilitating side effects, which severely impacts quality of life.

Aims: To analyze whether systematic magnetic resonance imaging (MRI) screening of adolescents can identify those with asymptomatic ON (stage I and II), who subsequently develop symptomatic ON.

Methods: Children diagnosed with ALL aged ≥10 years, who were enrolled in the ongoing prospective multicenter OPAL (osteonecrosis in pediatric patients with ALL or lymphoblastic lymphoma LBL) trial, were analyzed. Standardized MRI screening of the hips and knees was scheduled at diagnosis and 6, 9, 12, 15, 18 and 24 months into treatment. All patients were assessed according to a standardized case report form recording symptoms and activities of daily living and functional impairments of the hips and knees based on modified Harris Hip and Knee Society scores every 3 months from diagnosis to the end of antileukemic treatment.

Results: Between 03/2013-12/2016, 64 patients (pts) were enrolled, median age at ALL diagnosis was 15 years (range 10-17), median time under evaluation was 11 months (range 0-45). 31 (48.4%) pts were male, 33 (51.6%) female. 61 (95.3%) were diagnosed with ALL, 3 (4.7%) with LBL. 36 (56.2%) pts were treated according to the AIEOP-BFM 2009 trial, 25 (39.1%) pts to the CoALL-08-09 trial and 3 (4.7%) pts were enrolled in the NHL-BFM registry and treated accordingly. Until December 31st, 2016, 2 (3.1%) pts died treatment related, 4 (6.3%) underwent allogeneic stem cell transplantation, and 5 (7.8%) pts each relapsed while under treatment and dropped out for other reasons. Thus, so far, 166 MRIs comprising 664 joints could be evaluated. At initial diagnosis of the leukemia, MRI showed asymptomatic osteonecrotic lesions stage II or higher in 3 of 60 pts (5%), at 6 months in 7 of 34 pts (20.6%) osteonecrotic lesions, at 9 months in 14 of 23 pts (60.9%), at 12 months in 14 of 23 pts (60.9%), at 15 months in 3 of 11 pts (27.3%), at 18 months in 2 of 9 pts (22.2%), and at the end of treatment in 2 of 6 pts (33.3%). 11 (17.2%) pts developed symptomatic ON between 6 and 15 months from diagnosis (median 10 months). Of 23 pts, in whom screening MRI revealed ON stage II or higher, 11 pts (47.8%) subsequently developed symptomatic ON whereas in all adolescents developing symptomatic ON MRI had previously shown signs of ON. Median volumes of epiphyseal necrosis in pts with ON stage II remaining asymptomatic were 0.6 ml (range 0.1-7.2) and in pts developing symptomatic ON 12.5 ml (range 12.0-13.9) in the hips and 2 ml (range 0.4-20.5) and 30.5 ml (range 18.3-57) in the knees respectively. Epiphyseal involvement exceeded 30% in all symptomatic pts, but only in 2 pts remaining asymptomatic. With regard to the distribution pattern of ON, about twice as many knees as hips were affected by ON stage II or higher. MRI revealed ON stage III or higher in at least one joint in 12 pts (20%), predominantly in the knees. Radiological leukemic infiltration of bone detected by single screening MRI at diagnosis did not identify children at high risk of developing asymptomatic ON at six months into therapy or symptomatic ON anytime in the course of antileukemic treatment. These findings should be confirmed in larger patient numbers.

Summary/Conclusions: The first analysis of the OPAL trial shows that early MRI screening identifies extensive asymptomatic lesions in adolescents subsequently developing symptomatic ON.

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FINAL ANALYSIS OF A RANDOMIZED STUDY COMPARING PROPHYLACTIC AND MRD-TRIGGERED, PRE-EMPTIVE IMATINIB AFTER HSCT FOR PH+/BCR-ABL1 POSITIVE ALL: LONG-TERM PATIENT OUTCOME AND IMPLICATIONS OF MRD ANALYSIS

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Background: Front-line imatinib (IM) plus chemotherapy followed by allogeneic hematopoietic stem cell transplantation (HSCT) is standard therapy for patients (pts.) with Ph+ ALL. Relapse after HSCT remains a major cause of treatment failure, and pts. in whom *BCR-ABL* transcripts are detectable after HSCT are at particular risk. Posttransplant maintenance using tyrosine kinase inhibitors (TKIs) to reduce the relapse rate remains a subject of uncertainty, as data from prospective studies are limited.
Aims: To determine the impact of IM administration after HSCT on patient outcome and assess the predictive value of minimal residual disease (MRD) analysis by qRT-PCR of BCR-ABL1 transcripts.
Methods: In this prospective, multicentre trial by the GMALL study group, adult pts. (≥ 18 y) with Ph+ ALL in CR at HSCT were randomly assigned (1:1) to receive IM prophylactically after SCT or pre-emptively upon detection of MRD. Inclusion criteria included engraftment, sufficient hematopoietic and organ function, no uncontrolled GVHD or infections. Target dose of IM was 600mg/d, 400mg recommended as starting dose. Primary endpoint was molecular or hematologic relapse, secondary endpoints included survival, DFS, severe toxicity and transplant-related mortality. All pts. were followed by frequent serial MRD analysis after HSCT. An interim analysis was reported previously. We here provide results of the final analysis of this trial, with long-term follow-up of up to 11 years after HSCT.
Results: 74 pts. were evaluable, 36 received prophylactic and 38 pts. pre-emptive IM. Median age was 41 y (18-69) and 44 y (19-68), respectively. Disease status at HSCT was CR1 (n= 67), CR2 (n=5), CR3 (n=1), unknown (n=1). Most pts. received a PBSC graft (n=71) and myeloablative TBI-based conditioning (n=65), 8 pts. underwent RIC with 2Gy or 4Gy TBI (n=6) or non-TBI RIC (n=2). Median time from HSCT to starting IM was 48d and 77d, respectively. IM dose was 600mg/d in 22% of pts., remaining pts. received 400mg. Treatment was prematurely discontinued in 56% and 59% of pts., median time to discontinuation was 251d and 192d. Median follow-up of surviving pts. is 5.6 y (2.4-10.8) and 6.9 y (1.8-11). Relapse rate (14% *vs.* 18%), NRM (12% *vs.* 11%) and ongoing CR (69% *vs.* 71%) were not significantly different between arms. Probability of DFS and overall survival at 10 years was 64% *vs* 69% and 68% *vs* 71% with prophylactic and pre-emptive IM, respectively (p=ns). MRD levels were significantly predictive of relapse: BCR-ABL1/ABL1 (B/A) ratio $\geq 10^{-3}$ within 6 weeks prior to HSCT was associated with a higher cumulative incidence of relapse (CIR) at (47.5% *vs* 10.6%, p=0.006) and inferior DFS (45% *vs* 79%, p=0.027) at 10y. B/A ratio $\geq 10^{-4}$ within 100d after HSCT was likewise associated with a higher CIR (45% *vs* 13%, p=0.0046) and inferior DFS (55% *vs* 71%, p=0.054) at 8 y. An algorithm combining pre- and early (<100 days) post-transplant MRD levels (pre: $\geq 10^{-3}$; post: any positivity including below quantitative range) identified patients with a 60% *vs* 8.5% CIR at 10 y.
Summary/Conclusions: Post-HSCT intervention with prophylactic or pre-emptive IM is associated with a low relapse risk and excellent long-term survival and might be considered standard of care in Ph+ ALL pts. undergoing HSCT. BCR-ABL1 transcript levels prior to and early after SCT are predictive of outcome and identify a small subset of patients unlikely to benefit, emphasizing the need for rigorous MRD monitoring. The identified MRD thresholds should be validated in an independent dataset. Their applicability in the setting of RIC transplant or 2nd/3rd G TKI remain to be determined.

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ANALYSIS OF SAFETY DATA FROM 2 MULTICENTER TRIALS OF CTL019 IN PEDIATRIC AND YOUNG ADULT PATIENTS WITH RELAPSED/ REFRACTORY (R/R) B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (B-ALL)

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Background: The CD19-targeted chimeric antigen receptor (CAR) T-cell therapy CTL019, an investigational therapy that uses reprogrammed cytotoxic T cells to recognize and eliminate target cells, has shown high response rates in clinical trials for pediatric/young adult R/R B-ALL. The safety profile in this population has been limited to a single-center trial.
Aims: To identify any new safety issues with CTL019 emerging from use in multicenter trials.
Methods: Pooled data from 2 single-arm, multicenter phase 2 trials of CTL019 therapy in pediatric/young adult patients (pts) with R/R B-ALL (NCT02435849 and NCT02228096) were used to further characterize the safety of CTL019.

Table 1.

Table. Selected Adverse Events Post CTL019 Infusion

Adverse event, n (%)	Patients (N = 97)	
CRS	79 (81.4)	
Median time to onset (range), days	3 (1-22)	
Median duration (range), days	8 (1-36)	
Admitted to ICU	43 (44.3)	
Median duration of ICU stay (range), days	8 (1-34)	
Anticytokine therapy	33 (34.0)	
High-dose vasopressors	26 (26.8)	
Intubated	16 (16.5)	
Dialyzed	11 (11.3)	
	All grades	Grade 3/4
Grade 3/4 neutropenia with high fever	NA	59 (60.8)
Infections (>3%)	43 (44.3)	21 (21.6)
Staphylococcal infection	5 (5.2)	2 (2.1)
Clostridium difficile	4 (4.1)	3 (3.1)
Conjunctivitis	4 (4.1)	0
Rhinovirus	4 (4.1)	0
Candida	3 (3.1)	1 (1.0)
Clostridium difficile colitis	3 (3.1)	0
Pneumonia	3 (3.1)	2 (2.1)
Staphylococcal bacteraemia	3 (3.1)	3 (3.1)
Neuropsychiatric events (>3%)	39 (40.2)	11 (11.3)
Confusional state	12 (12.4)	0
Encephalopathy	9 (9.3)	4 (4.1)
Delirium	8 (8.2)	3 (3.1)
Agitation	6 (6.2)	0
Tremor	6 (6.2)	0
Irritability	5 (5.2)	0
Hallucination	4 (4.1)	0
Somnolence	4 (4.1)	1 (1.0)
Cognitive disorder	3 (3.1)	1 (1.0)
Lethargy	3 (3.1)	0
Seizure	3 (3.1)	1 (1.0)
Cytopenias not resolved by day 28	34 (35.1)	29 (29.9)
White blood cell count decreased	12 (12.4)	10 (10.3)
Platelet count decreased	11 (11.3)	8 (8.2)
Anemia	9 (9.3)	5 (5.2)
Neutrophil count decreased	9 (9.3)	7 (7.2)
Thrombocytopenia	9 (9.3)	8 (8.2)
Lymphocyte count decreased	6 (6.2)	4 (4.1)
Tumor lysis syndrome	3 (3.1)	3 (3.1)

Results: 123 pts were enrolled, 26 were not infused and not included in this analysis (10 deaths, 9 manufacturing failures, 3 adverse events [AEs], 4 pts pending infusion). 97 pts received a single infusion of transduced CTL019 cells (median dose, 3.2×10^6 [range, $0.2\text{-}5.4\times10^6$] cells/kg). Median age was 12y (range, 3-25). During the first 8 wk after infusion, 98% of pts experienced an AE of any grade (G), 82% experienced G3/4 AEs, and 72% experienced a serious AE (SAE). Common nonhematologic G3/4 AEs (≥10%) during the first 8 wk were cytokine release syndrome (CRS; 44%), hypotension (24%), decreased appetite (21%), increased AST (19%) and ALT (12%), hypoxia (16%), hypokalemia (13%), hypophosphatemia (11%), and pulmonary edema (10%). Rates of G3/4 AEs and SAEs decreased substantially >8 wk post infusion (41% and 24%, respectively). 21 pts died post infusion: 16 (76%) from B-ALL (n=2, ≤30 days after infusion; n=14, >30 days); cerebral hemorrhage (n=1) and embolic infectious stroke (n=1) (both ≤30 days); and infection (n=3, >30 days). Safety events were similar across pt subgroups based on age, sex, prior allogeneic stem cell transplant (alloSCT) (n=57), and Down syndrome (n=7). CRS, graded on the UPenn scale, occurred in 81% of pts (**Table 1.**). All CRS events occurred <8 wk post infusion. CRS was managed with supportive care, and 34% of pts were treated with anti-IL-6 agents. No deaths were attributed to CRS. Pts with ≥50% bone marrow (BM) blasts at enrollment (n=68) were

twice as likely to develop G3/4 CRS than pts with <50% BM blasts (n=29) (53% *vs* 24%). Earlier-onset fever correlated with severity of CRS. CRS grade correlated with serum IL-6 levels. CRS-associated coagulopathy with fibrinogen levels <1.0 g/L was observed in 10% of pts. Neuropsychiatric AEs occurred during or shortly after CRS resolution, were self-limiting, and were more likely in pts with severe CRS or history of CNS leukemia or other CNS diseases. No G4 neuropsychiatric events were observed. Other AEs of special interest within the first 8 wk included G3/4 neutropenia with high (>38.3˚C) fever (61%) and infections (G3/4, 22%). Prolonged G3/4 neutropenia (not resolved >28 days) occurred in 59 pts (61%). 36% of pts with prolonged G3/4 neutropenia had G3/4 infections after day 28. One pt with prior alloSCT was diagnosed with unconfirmed gut GVHD. Responding pts developed prolonged B-cell aplasia that was managed with immunoglobulin replacement. Tumor lysis syndrome was uncommon (3%).

Summary/Conclusions: This pooled analysis of global experience with CTL019 across 25 sites and 11 countries found no new safety issues. CRS and neuropsychiatric events, which are class effects of CAR T-cell therapy, were effectively managed. CTL019 appears similarly safe in pts with Down syndrome or prior alloSCT and across age groups. Prolonged follow-up will be required to determine the long-term safety of B-cell aplasia.

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UPDATED RESULTS OF A PHASE II STUDY OF HYPER-CVAD PLUS PONATINIB AS FRONTLINE THERAPY FOR ADULTS WITH PHILADELPHIA CHROMOSOME-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Ponatinib is a third-generation pan-BCR-ABL inhibitor that is effective in Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia (ALL) and overcomes the T315I gatekeeper mutation.
Aims: We designed a phase II trial to evaluate the efficacy and safety of hyper-CVAD plus ponatinib in adults with newly diagnosed Ph+ ALL.
Methods: Adults with newly diagnosed Ph+ ALL received 8 cycles of hyper-CVAD alternating with high dose MTX/Ara-C every 21-28 days, as permitted by peripheral count recovery. Patients (pts) who had received 1-2 courses of prior chemotherapy with or without other TKIs were still eligible. Ponatinib was given at 45mg daily on days 1-14 of cycle 1. Initially, pts then received ponatinib 45mg daily continuously beginning with cycle 2. Due to concern for potential cardiovascular toxicity with ponatinib, after 39 pts had been treated, the protocol was amended so that, beginning in cycle 2, pts in CR received ponatinib at a dose of 30mg daily and pts in complete molecular response (CMR) received 15mg daily. Rituximab was given during the first 4 cycles in pts with CD20 expression ≥20%; all pts received IT chemotherapy prophylaxis with the first 4 cycles. Pts in CR after 8 cycles of hyper-CVAD received maintenance with ponatinib, vincristine and prednisone for 2 years followed by indefinite daily ponatinib.
Results: To date, 64 pts have been treated; 10 pts had received prior treatment at the time of enrollment, 8 of whom were in CR and 2 of whom were not in CR. Median age was 48 years (range, 21-80 years). 23 pts (36%) were positive for CD20 expression. *BCR-ABL1* transcript was p190 in 45 pts (70%), p210 in 18 (28%) and unknown in 1 (2%). Of 56 pts not in CR at the time of study enrollment, 100% achieved CR; all but 1 pt achieved CR after the first cycle. The complete cytogenetic response rate was 98%, major molecular response (MMR) rate was 97% and CMR rate was 77%. The median time to MMR was 3 weeks (range, 2-14 weeks), and the median time to CMR was 10 weeks (range, 2-96 weeks). The median follow-up was 33 months (range, 2-62 months), and median number of cycles received was 6 (range, 2-8 cycles). 10 pts (16%) underwent allogeneic stem cell transplantation (ASCT) in first remission. Overall, 8 pts have relapsed (3 while still on ponatinib, 3 while on another TKI, 1 while not receiving a TKI, and 1 after ASCT). 14 pts have died, 8 of whom were in CR. Two deaths were attributed to ponatinib (both from MI while in CR). 38 pts are still receiving treatment (consolidation, n=7; maintenance, n=14; post-maintenance TKI, n=17). The 3-year continuous CR and OS rates were 79% and 76%, respectively. In a 4-month landmark analysis to assess the impact of ASCT, continued CR and OS did not differ according to whether ASCT was performed in first remission. Treatment was overall well-tolerated. Myelosuppression was as anticipated with hyper-CVAD-based therapy, with median times to platelet and ANC recovery in cycle 1 of 22 days (range, 17-35 days) and 18 days (range, 13-29 days), respectively. In cycles 2-8, the median times to platelet and ANC recovery were 22 days and 16 days, respectively. Grade ≥3 adverse events included pancreatitis in 12 pts (19%), hypertension in 8 (13%), thrombotic events in 4 (6%) and MI in 3 (5%). No grade ≥3 cardiovascular events have occurred after the protocol amendment with ponatinib dose reductions.
Summary/Conclusions: Hyper-CVAD plus ponatinib is effective in patients with newly diagnosed Ph+ ALL, resulting in a CMR rate of 77% and encouraging long-term survival.

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PROGNOSTIC IMPLICATIONS OF PRETREATMENT CYTOGENETICS IN ADULTS WITH RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA TREATED WITH INOTUZUMAB OZOGAMICIN

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Background: In the phase 3 INO-VATE study of relapsed/refractory acute lymphoblastic leukemia (R/R ALL) patients, inotuzumab ozogamicin (InO) showed improved complete remission or complete remission with incomplete hematologic recovery (CR/CRi) rates *versus* standard care (SC; 80.7% *vs* 29.4%; *P*<0.001) (NCT01564784; Kantarjian *NEJM* 2016 [data cutoff date: Oct 2, 2014]).
Aims: To assess the impact of baseline karyotype on response and toxicities in R/R ALL patients receiving InO from the INO-VATE study
Methods: Full study details have been previously published. At screening, karyotyping was performed locally; ≥20 metaphase count was recommended for cytogenetic analysis. Karyotypes were interpreted using the International System for Cytogenetic Nomenclature. CR/CRi and minimal residual disease (MRD) negativity rates (defined as <0.01% bone marrow blasts as assessed at a central laboratory) were compared using a chi-square test or Fisher exact test. Survival estimates were compared using a log-rank test. Data as of March 8, 2016, are presented. Informed consent was obtained from all patients. All analyses presented were not adjusted for multiple testing.
Results: Of 326 patients randomized, 284 had cytogenetic data at screening (InO: 144; SC: 140). Of 164 InO-treated patients, 21.3% had normal diploid karyotype (≥20 metaphases), 17.1% complex (≥5 abnormalities), 13.4% Philadelphia-chromosome positive (Ph+) disease, 6.7% diploid (<20 or unknown metaphases), 4.9% hyperdiploidy >50, 4.9% aberrations involving mixed lineage leukemia (MLL), 1.8% low hypodiploidy/near-triploidy, 1.2% Del (9p), 16.5% other chromosomal abnormalities, and 12.2% missing. Of 164 InO-treated patients, CR/CRi rate was 73% (95% confidence interval [CI] 66–80; **Table**) and MRD negativity rate was 59% (95% CI, 51–67). With InO, CR/CRi and MRD negativity rates were similar between the various cytogenetic subgroups. CR/CRi rates were significantly higher with InO *versus* SC in diploid (≥20 metaphases), complex, other, and missing cytogenetic subgroups (*P*≤0.015) and numerically higher in the other cytogenetic subgroups. With InO, more patients with diploid (≥20 metaphases) karyotype proceeded to stem cell transplant *versus* other cytogenetic subgroups. With InO, the duration of remission (DoR) was significantly different between cytogenetic subgroups (*P*<0.0001), with diploid (≥20 metaphases) and other subgroups having the longest median DoR numerically and MLL subgroup having the shortest median DoR numerically; no significant differences in DoR were seen between cytogenetic subgroups with SC (*P*=0.3783). Significant differences in PFS were seen between cytogenetic subgroups with InO (*P*=0.0063); no significant differences were seen between cytogenetic subgroups with SC (*P*=0.5427). Within the InO and SC arms, overall survival (OS) differences between cytogenetic subgroups were not significant (*P*=0.1629 and 0.3040, respectively); however, although not statistically significant based on 97.5% CI for hazard ratio (HR), OS was numerically longer (HR <1) with InO *versus* SC in diploid (≥20 metaphases), MLL, complex, other, and missing cytogenetic subgroups. Generally, adverse event profiles did not vary by cytogenetic subgroup.

Table 1.

	CR/CRi (95% CI)	PFS (95% CI)		OS (95% CI)	
	% Response	Median, mo	% 1 Year	Median, mo	% 1 Year
Dp with ≈20 metaphases (n=35)	83 (66–93)	6 (5–9)	25 (12–41)	9 (6–16)	36 (21–52)
Complex (n=28)	75 (55–89)	5 (3–6)	12 (3–28)	7 (4–12)	30 (14–47)
Ph+ (n=22)	73 (50–89)	4 (2–10)	26 (9–46)	9 (4–14)	41 (21–60)
Dp with <20 metaphases or unknown (n=11)	55 (23–83)	3 (1–5)	NA	5 (1–NA)	36 (11–63)
Hyperdiploid (n=8)	63 (25–92)	3 (1–9)	0 (NA)	6 (2–NA)	25 (4–56)
MLL (n=8)	63 (25–92)	2 (1–3)	0 (NA)	5 (2–13)	25 (4–56)
Ho-Tr (n=3)	67 (9–99)	4 (2–5)	0 (NA)	5 (2–6)	0 (NA)
Del (9p) (n=2)	100 (16–100)	NA	100 (100–100)	NA	100 (100–100)
Other abnormalities (n=27)	70 (50–86)	5 (3–9)	20 (7–38)	9 (5–NA)	41 (23–58)
Missing (n=20)	75 (51–91)	5 (2–6)	14 (3–34)	6 (4–10)	20 (6–39)
All (n=164)	73 (66–80)	5 (4–6)	17 (12–24)	8 (6–9)	34 (26–41)

CR/CRi=complete remission or complete remission with incomplete hematologic recovery; Dp=diploid; Ho-Tr=low hypodiploidy/near-triploidy; MLL=mixed lineage leukemia; NA=not available; Ph+=Philadelphia chromosome positive; PFS=progression-free survival; OS=overall survival.

Summary/Conclusions: In patients with diploid (≥20 metaphases), complex, other, and missing cytogenetic karyotypes, CR/CRi rates were significantly higher with InO *versus* SC. In the diploid (≥20 metaphases), MLL, complex, other, and missing cytogenetic subgroups, OS favored InO *versus* SC, though not statistically significant. Safety profiles generally were similar to the overall study population.

P520

A PHASE II STUDY WITH A SEQUENTIAL CLOFARABINE-CYCLOPHOSPHAMIDE COMBINATION SCHEDULE AS SALVAGE THERAPY FOR REFRACTORY AND RELAPSED ACUTE LYMPHOBLASTIC LEUKEMIA (R/R ALL) IN ADULT PATIENTS

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Background: Clofarabine (CLO) was demonstrated active in R/R ALL in combination with cyclophosphamide (CY) and/or other agents. An encouraging complete remission rate (CR 4/6, 67%) was obtained with a reference CLO-CY combination (Karp J et al, *Blood* 2007), however severe toxicity partly related to very high CY dosage forced stepwise reduction of CLO from 40 to 20-10mg/m^2/d.
Aims: A modified schedule combining full-dose CLO with attenuated CY (given after 2 hrs to inhibit CLO-damaged DNA repair) was piloted by GIMEMA. Here we report the activity and toxicity of the new CLO-CY regimen.
Methods: Study GIMEMA LAL1610 (EudraCT 2010-019742-12) had a two-stage Simon design, with an exploratory cohort of 10 patients and, provided a complete remissions (CR) was achieved in 3, an extension cohort of 17 patients. Primary study endpoint was CR rate, with 11 CRs (40%) required to declare CLO-CY worthy of further investigation. CRi was CR with incomplete recovery of neutrophils (<1.0) and/or platelets (<100). Eligible patients were adults with Philadelphia-negative (Ph-) R/R ALL, either primary refractory or in first isolated marrow relapse <24 months from first CR, including relapse after allogeneic stem cell transplant (SCT). CLO was administered IV at 40mg/m^2/d for 5 consecutive days, followed after 2 hrs by IV CY 400mg/m^2/d. Clinical and hematological response was assessed on day 28 or later, according to clinical course and patient condition. One/two courses were planned, followed by allogeneic SCT when possible.
Results: From October 2012 to December 2015, 35 patients were screened and 27 enrolled. Median patient age was 38.7 years (range 20.5-59.6), 15 were male, 4 with T- and 23 B-precursor ALL, 2 refractory and 25 relapsed (after a median of 5.9 months, range 1.9-23.4), 5/11 evaluable with high-risk cytogenetics [2 complex; 2 t(4;11), 1 MLL-rearranged]. Median white blood cells and marrow blast percentage were 5.67 (range 1-55) and 73 (range 8-100), respectively. All but one patient (treatment interruption due to traumatic fall) received CY-CLO as planned. Nine patients achieved CR and 7 CRi after course 1 (overall response 59.2%; 6/10 in study stage one), 2 had a partial response, 5 were refractory, 3 died early and one survived with pancytopenic marrow. In relapsed patients, only the length of prior remission affected the probability of CR, from 44.4% (n=8/18) to 100% (n=6/6) if ≤ or >12 months, respectively (P=0.02). In 10 evaluable patients, median post-induction MRD was 0.01% (<0.001% in 3). Six CR patients received a second course, which was curtailed in 2 due to toxicity, and 10 responders had an allogeneic SCT (62.2%). Concerning toxicity, 10 patients experienced grade ≥2 toxicity, but apart from expected myelosuppression/infections only 6 therapy-related grade >2 adverse events occurred: hepatic (n=4), renal (n=1) and neurological (n=1). After a median follow-up of 17.7 months (range 1.2-29.5), 5 patients are in continuous CR, 5 relapsed, and 6 died in CR (3 post-allograft; SCT-related mortality 30%). Median and 1-year overall and disease-free survival (OS, DFS) are 6.5 months and 28.6% (95% CI, 15.3-53.3%), and 4.5 months and 34.7% (95% CI, 17.3-69.7%), and apart from patient age (OS: HR 1.053, P=0.01) no other risk factor or length of prior CR affected DFS/OS probabilities.
Summary/Conclusions: Although the management of R/R Ph- ALL remains largely unsatisfactory, this regimen with full-dose CLO plus attenuated CY proved feasible and yielded an appreciable CR rate in adult patients suffering from isolated marrow relapse within 24 months from date of first CR.

P521

BLINATUMOMAB USE IN PEDIATRIC AND ADOLESCENT PATIENTS WITH RELAPSED/REFRACTORY B-PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA FROM AN OPEN-LABEL, MULTICENTER, EXPANDED ACCESS STUDY

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Background: Blinatumomab, a bispecific T-cell engager antibody construct, has shown antileukemia activity and tolerability in patients with relapsed/refractory B-precursor acute lymphoblastic leukemia (ALL).
Aims: We further evaluated safety and efficacy of blinatumomab in pediatric and adolescent patients with relapsed/refractory B-precursor ALL enrolled in an expanded access study (NCT02187354).
Methods: Eligible patients (aged >28 days to <18 years) had ≥5% blasts and relapsed/refractory B-precursor ALL (refractory to prior treatments, second or later relapse, or relapse after allogeneic hematopoietic stem cell transplantation [alloHSCT]). Blinatumomab was dosed by continuous intravenous infusion (4 weeks on/2 weeks off) for up to five cycles (≥5 to <25% blasts: 15µg/m^2/day; ≥25% blasts: 5µg/m^2/day on days 1-7 in cycle 1, then 15 µg/m^2/day). The primary endpoint was incidence of treatment-emergent (TE) and treatment-related (TR) adverse events (AEs). Key efficacy endpoints were complete response and minimal residual disease (MRD, measured by polymerase chain reaction or flow cytometry) response within the first two cycles, relapse-free survival, overall survival, and incidence of alloHSCT.
Results: Among the first 40 treated patients (median age, 9 [range, 1–17] years), 24 (60%) had experienced ≥2 relapses, 20 (50%) had relapsed after alloHSCT, and 5 (13%) were primary refractory; 18 (45%) had ≥50% blasts and 21 (53%) had prior alloHSCT. Safety and key efficacy outcomes are shown in the table. Twenty-five patients (63%) achieved a complete response within the first two cycles, 19 of whom had an MRD response. Eight patients relapsed and 20 died after treatment. Regardless of causality, the most frequent TEAEs were pyrexia (78%), cytokine release syndrome (CRS; 23%), vomiting (23%), and anemia (20%). All nine CRS events were grade 1 or 2, and one tumor lysis syndrome was grade 3. Ten (25%) patients interrupted treatment and two (5%) discontinued due to TRAEs; 13 (33%) patients had grade ≥3 TRAEs, including two of three neurologic events (depressed level of consciousness and headache; both grade 3). Two patients experienced fatal AEs, both of which were considered unrelated to blinatumomab.

Table 1.

	All Patients N=40
All TEAEs, n (%)	39 (98)
Grade 3	15 (38)
Grade 4	12 (30)
Fatal	2 (5)
Complete response[a], n (%)	25 (63)
<50% blasts	15 (68)
≥50% blasts	10 (56)
t(17;19)	2 (100)
	Responders N=25
MRD response among responders[a] <10^{-4}, n (%)	19 (76)
<50% blasts	12 (80)
≥50% blasts	7 (70)
t(17;19)	2 (100)
alloHSCT after complete response, n (%)	10 (40)

aWithin the first two cycles

Summary/Conclusions: Blinatumomab showed antileukemia activity in pediatric and adolescent patients with high-risk relapsed/refractory B-precursor ALL including t(17;19) and AEs were consistent with those previously reported for relapsed/refractory ALL.

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PRODUCT CHARACTERISTICS ASSOCIATED WITH IN VIVO EXPANSION OF ANTI-CD19 CAR T CELLS IN PATIENTS TREATED WITH AXICABTAGENE CILOLEUCEL (AXI-CEL)

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Background: The incidence of acute lymphoblastic leukemia (ALL) is increasing, with nearly 6600 new diagnoses expected in 2016, of which >40% will

affect adults aged >20 years (https://seer.cancer.gov). Adult patients (pts) with B cell ALL show high-risk disease biology, high rates of relapse, and poor survival (*J Clin Oncol* 2011;29:532; *Blood* 2012;119:34). Promising results have been observed with KTE-C19, an anti-CD19 chimeric antigen receptor (CAR) T cell therapy, in refractory, aggressive non-Hodgkin lymphoma (*Blood* 2016;128:LBA-6), and suggest an opportunity to improve outcomes in ALL. Here, we present updated results from the phase 1 portion of ZUMA-3, a multi-center study of KTE-C19 in pts with high tumor burden ALL.

Aims: The goal of this study is to assess safety and efficacy of KTE-C19 in adult pts with relapsed/refractory ALL who have high disease burden.

Methods: Eligible pts were ≥18 years of age with relapsed/refractory ALL (Ph+ pts eligible), ≥25% bone marrow lymphoblasts, adequate organ function, and Eastern Cooperative Oncology Group status 0-1. Pts received 1 or 2 × 10^6 CAR T cells/kg after conditioning with cyclophosphamide and fludarabine. The primary endpoint of phase 1 was incidence of dose-limiting toxicity (DLT). Secondary endpoints were efficacy outcomes of KTE-C19, including complete response (CR) rates and biomarker associations.

Results: As of Nov 1, 2016, 11 pts were enrolled, and 10 were treated with KTE-C19. One pt had a serious adverse event prior to dosing and was not treated. KTE-C19 was successfully manufactured in a centralized facility for all pts across a broad range of baseline absolute lymphocyte counts in 6 days, with a turnaround time of ~2 weeks. Pts were 60% men, with 1-4 prior lines of therapy and high disease burden (median, 81% bone marrow lymphoblasts). No pt (0/3) experienced a DLT at the 2 × 10^6dose, and phase 1 was then expanded to 6 pts at the 2 × 10^6dose. One pt experienced a grade 5 adverse event of multi-organ failure due to cytokine release syndrome (CRS), and subsequent pts (n=4) received 1 × 10^6 CAR T cells/kg. Across all pts, the most common grade ≥3 adverse events were cytopenias (80%), febrile neutropenia (50%), pyrexia (40%), and transaminitis (40%). Grade ≥3 CRS and neurologic events were reported in 20% and 40% of pts, respectively. Cerebral edema was not observed. All CRS events resolved (except the grade 5 event); neurologic events resolved in 5 of 6 pts (1 grade 3 neurologic event ongoing at cut-off). Anti-CD19 CAR T cells achieved peak expansion within two weeks of infusion. Of the 8 efficacy evaluable pts, 6 (75%) achieved remission (including CR and CR with either partial or incomplete hematopoietic recovery) by day 28 disease assessment or earlier. All remissions (100%) were minimal residual disease-negative. Of the 6 pts achieving minimal residual disease-negative CR, two eventually relapsed, one with CD19- disease and one with CD19+ disease. Safety and efficacy data were similar across KTE-C19 doses. Updated pt number, follow-up, and biomarker data will be presented.

Summary/Conclusions: No DLTs were observed with KTE-C19 in adult pts with high BM disease burden; one pt with high disease burden had grade 5 CRS after completion of the DLT cohort. Manufacturing was successful in all pts; most pts achieved a minimal residual disease-negative CR. These results demonstrate promising efficacy with a manageable safety profile. Based on these results, ZUMA-3 continues to enroll pts, adding measures to further enhance safety and with planned expansion to phase 2.

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KTE-C19 CHIMERIC ANTIGEN RECEPTOR (CAR) T CELL THERAPY IN ADULTS WITH HIGH-BURDEN RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA (R/R ALL): UPDATED RESULTS FROM PHASE 1/2 OF ZUMA-3

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Background: The incidence of acute lymphoblastic leukemia (ALL) is increasing, with nearly 6600 new diagnoses expected in 2016, of which >40% will affect adults aged >20 years (https://seer.cancer.gov). Adult patients (pts) with B cell ALL show high-risk disease biology, high rates of relapse, and poor survival (*J Clin Oncol* 2011;29:532; *Blood* 2012;119:34). Promising results have been observed with KTE-C19, an anti-CD19 chimeric antigen receptor (CAR) T cell therapy, in refractory, aggressive non-Hodgkin lymphoma (*Blood* 2016;128:LBA-6), and suggest an opportunity to improve outcomes in ALL. Here, we present updated results from the phase 1 portion of ZUMA-3, a multi-center study of KTE-C19 in pts with high tumor burden ALL.

Aims: The goal of this study is to assess safety and efficacy of KTE-C19 in adult pts with relapsed/refractory ALL who have high disease burden.

Methods: Eligible pts were ≥18 years of age with relapsed/refractory ALL (Ph+ pts eligible), ≥25% bone marrow lymphoblasts, adequate organ function, and Eastern Cooperative Oncology Group status 0-1. Pts received 1 or 2 × 10^6 CAR T cells/kg after conditioning with cyclophosphamide and fludarabine. The primary endpoint of phase 1 was incidence of dose-limiting toxicity (DLT). Secondary endpoints were efficacy outcomes of KTE-C19, including complete response (CR) rates and biomarker associations.

Results: As of Nov 1, 2016, 11 pts were enrolled, and 10 were treated with KTE-C19. One pt had a serious adverse event prior to dosing and was not treated. KTE-C19 was successfully manufactured in a centralized facility for all pts across a broad range of baseline absolute lymphocyte counts in 6 days, with a turnaround time of ~2 weeks. Pts were 60% men, with 1-4 prior lines of therapy and high disease burden (median, 81% bone marrow lymphoblasts). No pt (0/3) experienced a DLT at the 2 × 10^6dose, and phase 1 was then expanded to 6 pts at the 2 × 10^6dose. One pt experienced a grade 5 adverse event of multi-organ failure due to cytokine release syndrome (CRS), and subsequent pts (n=4) received 1 × 10^6 CAR T cells/kg. Across all pts, the most common grade ≥3 adverse events were cytopenias (80%), febrile neutropenia (50%), pyrexia (40%), and transaminitis (40%). Grade ≥3 CRS and neurologic events were reported in 20% and 40% of pts, respectively. Cerebral edema was not observed. All CRS events resolved (except the grade 5 event); neurologic events resolved in 5 of 6 pts (1 grade 3 neurologic event ongoing at cut-off). Anti-CD19 CAR T cells achieved peak expansion within two weeks of infusion. Of the 8 efficacy evaluable pts, 6 (75%) achieved remission (including CR and CR with either partial or incomplete hematopoietic recovery) by day 28 disease assessment or earlier. All remissions (100%) were minimal residual disease-negative. Of the 6 pts achieving minimal residual disease-negative CR, two eventually relapsed, one with CD19- disease and one with CD19+ disease. Safety and efficacy data were similar across KTE-C19 doses. Updated pt number, follow-up, and biomarker data will be presented.

Summary/Conclusions: No DLTs were observed with KTE-C19 in adult pts with high BM disease burden; one pt with high disease burden had grade 5 CRS after completion of the DLT cohort. Manufacturing was successful in all pts; most pts achieved a minimal residual disease-negative CR. These results demonstrate promising efficacy with a safety profile. Based on these results, ZUMA-3 continues to enroll pts, adding measures to further enhance safety and with planned expansion to phase 2.

P524

EXPOSURE-ADJUSTED ADVERSE EVENTS COMPARING BLINATUMOMAB WITH STANDARD OF CARE CHEMOTHERAPY IN ADULTS WITH RELAPSED/REFRACTORY B-PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA FROM A RANDOMIZED PHASE 3 STUDY

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Background: Blinatumomab, a bispecific T-cell engager antibody construct, has shown improved overall survival *vs* standard of care (SOC) chemotherapy in patients with Philadelphia chromosome−negative relapsed/refractory B-precursor acute lymphoblastic leukemia (ALL) in a randomized phase 3 study (*N Engl J Med* 2017;376:836-847).

Aims: We compared the incidence of adverse events (AEs) observed with blinatumomab *vs* SOC after adjusting for varying treatment exposure times for a more comprehensive evaluation of safety and tolerability.

Methods: Adults (aged ≥18 years) with relapsed/refractory B-precursor ALL (refractory to primary induction therapy or salvage therapy, first relapse <1 year, second or later relapse, or relapse after allogeneic hematopoietic stem cell transplantation) were randomized to receive either blinatumomab or SOC (1 of 4 predefined regimens). Blinatumomab was dosed by continuous intravenous infusion (4 weeks on/2 weeks off) for up to five induction cycles (9 µg/day on days 1-7 of cycle 1 and 28 µg/day thereafter). Up to four maintenance cycles (4 weeks on/8 weeks off) were allowed for up to 12 months. Exposure-adjusted event rates were calculated as the number of events x 100/total exposure time (shown in the table).

Results: Median (range) number of cycles were 1 (1-4) for SOC and 2 (1-9) for blinatumomab. The highest exposure-adjusted event rates (per 100 patient-years) were for pyrexia (507 SOC *vs* 376 blinatumomab), anemia (987 *vs* 229), thrombocytopenia (750 *vs* 126), and neutropenia (351 *vs* 121), all of which were lower for blinatumomab than for SOC. Febrile neutropenia (365 vs 93) and infections (1216 *vs* 436) were also lower for blinatumomab than for SOC (p<0.0001). Exposure-adjusted event rates for neurologic events were 743 for SOC *vs* 472 for blinatumomab, with median time (range) to onset of 7 (1−43) days and 7 (1−190) days, respectively, and grade ≥3 cytokine release syndrome (CRS) rates were 0 for SOC *vs* 10 for blinatumomab. The most frequently reported AEs in both cycles 1 and 2 were pyrexia, nausea, and anemia in both arms; CRS events in the blinatumomab arm decreased between cycle 1 and cycle 2 (14% *vs* 2%). The majority of fatal AEs were related to infection in both arms.

Table 1.

	Standard of Care N=109		Blinatumomab N=267	
Total exposure, years	14.8		89	
	Number of Events	Exposure-Adjusted Event Rate[a]	Number of Events	Exposure-Adjusted Event Rate[a]
All AEs	2037	13764	4108	4616
Grade 3	456	3081	707	794
Grade 4	195	1318	197	221
Fatal	19	128	51	57
Neurologic events	110	743	420	472
CRS	0	0	56	63
All serious AEs	95	642	311	349

aPer 100 patient-years

Summary/Conclusions: In this study, blinatumomab showed an AE profile consistent with that previously reported for relapsed/refractory ALL, including similar rates of manageable CRS and neurologic events. Exposure-adjusted event rates were generally higher in SOC *vs* blinatumomab, including for cytopenias and infections.

P525

FACTORS ASSOCIATED WITH STEM CELL TRANSPLANTATION OUTCOMES IN PATIENTS WITH RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA TREATED WITH INOTUZUMAB OZOGAMICIN *VERSUS* CONVENTIONAL CHEMOTHERAPY

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Background: Inotuzumab ozogamicin (InO) therapy in relapsed/refractory acute lymphoblastic leukemia (R/R ALL) resulted in superior complete remission (CR)/CR with incomplete hematologic recovery (CRi) rates *versus* (v) conventional chemotherapy (C) in the Phase 3 INO-VATE trial (NCT01564784; Kantarjian *NEJM* 2016 [data as of October 2, 2014). More InO v C patients (pts) proceeded to hematopoietic stem cell transplantation (HSCT; 41% [45/109] v 11% [12/109]; *P*<0.001).
Aims: To assess factors associated with outcomes after allogeneic HSCT in patients with R/R ALL who were previously treated with InO.
Methods: Full details have been published. Informed consent was obtained from all patients. Multivariate analyses (MVA) using Cox regression modeling were conducted to determine predictors of non-relapse mortality (NRM) and overall survival (OS).
Results: As of March 8, 2016, 108/326 pts underwent allogeneic HSCT (InO n=77; C n=31). Baseline characteristics were generally similar, except baseline platelet values were lower in InO v C pts. More InO v C pts achieved minimal residual disease negativity during study therapy (MRD^{neg} [best status]; 71% v 26%; *P*<0.0001). Less InO v C pts received additional therapy before HSCT (14% v 55%, *P*<0.0001). NRM rates were higher in InO v C pts at 1 year (yr; 36% [95% CI 26–47] v 20% [8–36]) and 2 yrs (39% [27–51] v 31% [13–51]), but relapse rates were lower (1 yr, 23% [15–33] v 29% [13–48]; 2 yrs, 33% [22–44] v 46% [24–65]). No significant difference in post-HSCT survival was detected in InO v C pts; however, visual inspection of the curve suggested the survival probability varied before and after 15 months post-HSCT (1 yr, 44% [95% CI 33–55] v 65% [44–79]; 2 yr, 39% [28–50] v 34% [15–54]). Fatal veno-occlusive disease (VOD) was observed in 5 InO pts (all during the first 100 days from the date of HSCT) and no C pts. MVA showed that conditioning regimens without dual alkylators and thiotepa were associated (2-sided; *P*<0.05) with lower risk of NRM and post-HSCT survival, respectively.
Summary/Conclusions: Compared with C, InO permitted more pts with R/R ALL to proceed to HSCT in CR/CRi with MRD^{neg} (best status). Despite increased NRM and fatal VOD, long-term survival was attainable in InO pts. In pts previously treated with InO, interventions to reduce NRM and improve OS after HSCT include avoiding dual alkylator conditioning regimens, especially those containing thiotepa.

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DESIGNING THE NEXT GENERATION CD33-TARGETING ADC: IMGN779, SELECTED FOR POTENCY, NOVEL MECHANISM AND PRECLINICAL TOLERABILITY, WITH HIGH ACTIVITY IN DISSEMINATED AML MODELS AND MULTI-DOSE REGIMENS

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Background: Antibody-drug conjugates (ADCs) targeting CD33 are promising therapeutics in AML, where challenges are achieving efficacy while maintaining tolerability. Here, we report the payload/ linker design and selection resulting in a high-Therapeutic Index (TI) ADC with favorable preclinical toxicity profile across multiple species and antitumor activity in disseminated AML models and in multi-dose regimens. IMGN779, the final ADC design, is comprised of an indolino-benzodiazepine mono-imine DNA-alkylating payload, DGN462, coupled by a cleavable *N*-succinimidyl 4-(2-pyridyldithio)-2-sulfobutanoate (s-SPDB) linker to a CD33-targeting antibody.
Aims: Select the best ADC out of multiple preclinical anti-CD33 ADC candidates, and assess its activity *in vitro* and *in vivo* in AML models.
Methods: Unconjugated payloads were evaluated *in vitro* for cytotoxicity on human AML cell lines. Payloads were compared, as CD33-targeting conjugates, *in vitro* for cytotoxicity on human AML cell lines and *in vivo* for tolerability in mice and TI against human AML xenografts. ADCs with cleavable and non-cleavable linkers were evaluated for cytotoxicity on MDR-positive and -negative AML cell lines, for tolerability in mice and TI in AML xenografts. IMGN779, the final ADC design, was evaluated *in vivo* for toxicity in rats and cynomologus monkeys. IMGN779's antitumor activity was evaluated in disseminated models and in fractionated- and multi-dose regimens in AML xenografts.
Results: First, we selected a high affinity antibody to CD33 with retained ADCC activity. Next, given concerns for long-term toxicity of DNA crosslinkers, we prepared DNA alkylating (single strand DNA damage) and DNA crosslinking (double strand DNA damage) versions of our novel IGN payload class. Both versions had comparable IC50s on human AML cell lines as free drugs (12-260 *vs*. 5-77 pM) and as CD33-targeting ADCs (0.7 *vs*. 0.5 pM). However, *in vivo*, the CD33-targeting DNA alkylating ADC had a 5-fold higher MTD (maximum tolerated dose) in mice and 5-fold larger TI in AML xenograft models (MTD 950 *vs*. 190 µg/kg, by payload; TI of 95 *vs*. 19). In addition, the DNA crosslinking version led to delayed systemic toxicity at MTD, not seen in the DNA alkylating version even at its 5-fold higher MTD. Thus we selected the DNA alkylating version for further development. To determine the optimal linker design, we created ADCs with three different linkers, one non-cleavable and two cleavable, and based on improved *in vitro* efficacy (IC50) and *in vivo* safety/efficacy (MTD, TI), the s-SPDB cleavable linker with the DNA alkylating payload was chosen as the lead clinical compound, and named IMGN779. In multiple species, IMGN779 had a consistent toxicity profile (mice, rats and monkeys), producing reversible cytopenias with no or minor changes in transaminases and without histologic evidence of hepatotoxicity. Importantly, IMGN779 was highly active at a single dose 10 µg/kg (payload) in an MV4-11 (FLT3-ITD+) disseminated AML xenograft model, producing a 90% increased life span, and was well-tolerated and highly active in repeat dosing regimens (10 or 30 µg/kg, qw x 3 and q3d x 3) in a HL60 AML xenograft model. Similarly, in a MV4-11 xenograft model, fractionated dosing (5 µg/kg, qw x 2 or q3d x 2) generated 33% more long-term tumor-free survivors compared to single-dose (10 µg/kg), demonstrating tolerability and enhanced efficacy in multi-dose and fractionated regimens.
Summary/Conclusions: IMGN779, designed as the next generation CD33-targeting ADC, utilizes a novel DNA alkylating DGN462 payload and a cleavable disulfide linker, selected to maximize anti-AML activity and preclinical safety. IMGN779 is highly active in multiple AML xenograft models, including models with poor prognostic factors, and is well-tolerated in preclinical repeat dosing regimens, where an additional benefit was achieved with a fractionating the dosing regimen over a single high dose. These results provide the foundation for the clinical evaluation of IMGN779 in AML.

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THE MIXED LINEAGE LEUKEMIA FUSION PARTNER ENL RECRUITS PAF1 TO CLEAR POLYCOMB-INDUCED TRANSCRIPTIONAL REPRESSION

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Background: In mixed lineage leukemia *ENL* is frequently found juxtaposed to *MLL* creating MLL-ENL fusion proteins that initiate leukemogenic transformation. Interestingly, mutations of *ENL* have also been found in Wilms' Tumor, a pediatric nephroblastoma. In its wild-type configuration ENL serves as scaffolding factor in protein complexes that stimulate transcriptional elongation but, paradoxically, it also co-purifies with polycomb repressive complex 1 (PRC1).

Aims: This work examines how ENL influences PRC1 repressive activity.
Methods: The effect of ENL on transcriptional activity of model promoters and endogenous transcriptional control elements was studied by biochemical and molecular biology methods.
Results: Here we demonstrate that ENL overcomes polycomb induced silencing through recruitment of polymerase associated factor 1 (PAF1) a chromatin reader linked to a histone ubiquitination complex. The ability to bind PAF1 controlled the ability of ENL to neutralize polycomb-mediated repression in an elongation reporter system and also during transformation of primary cells by MLL-ENL *in vivo*. Inactivation of polycomb by ENL was accompanied by ubiquitination of histone H2B, the hallmark activity of PAF1 allied enzymes. On a global level ChIP-Seq and nascent RNA-Seq demonstrated that MLL-ENL target genes stood out with a supraphysiological accumulation of H2BUb accompanied by hyper-accelerated transcription rates. Interestingly, examination of Wilms tumor specific ENL mutants allowed to elucidate the underlying mechanism of the MLL-fusion induced ENL hyperactivity. Introduction of Wilms-specific ENL mutants into primary hematopoietic cells induced aberrant transcription and H2BUb modification of *Hoxa9* and *Meis1,* two sentinel loci for polycomb action. This was dependent on the conserved YEATS domain of ENL that operated as "switch" binding either histone H3 or PAF1 thus effectively regulating ENL function as anti-repressor or elongation factor, respectively. Wilms-mutations aberrantly favored interaction with PAF1 and thus perturbed proper silencing. This effect was intensified in a MLL-ENL fusion where MLL itself provided a constitutive tether to PAF1 effectively creating a "super-transcription factor" that constitutively combined anti-repression with elongation capabilities.
Summary/Conclusions: In summary, targeting histone ubiquitination may be an additional Achilles heel for mixed lineage leukemia that merits further investigation of therapeutical utility.

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PKC EPSILON SUPPORTS ACUTE MYELOID LEUKEMIA BY MAINTAINING MITOCHONDRIAL REDOX HOMEOSTASIS

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Background: Although numerous genetic mutations contribute to the etiology and pathophysiology of acute myeloid leukemia (AML), the molecular machinery that is not mutated but supports AML biology remains largely unknown. Several studies have shown that AML cells, irrespective of genetic sub-type, display an oxidized intracellular redox environment compared to their healthy counterparts. The redox environment of AML cells is largely due to the elevated reactive oxygen species (ROS) levels, which are a class of free radical molecules. Though ROS are by-products of several cellular processes, in excess, they can damage DNA and destroy organelles, resulting in the acquisition of genetic mutations or cell death. As a result, ROS homeostasis is tightly regulated by an array of molecular pathways. Although ROS is elevated in AML cells, the role of ROS and the identity of its regulators remain largely unknown. Here we report that the serine/threonine kinase, PKCε regulates the ROS-neutralizing enzyme SOD2 to support mitochondrial redox homeostasis and AML progression.
Aims: The goal of this study was to identify and subsequently assess how targeting key ROS-regulatory pathways impacts AML biology.
Methods: Loss-of-function studies for PKCε and SOD2 were performed with recombinant lentiviruses expressing gene-targeting shRNAs. Recombinant retroviruses expressing either PKCε and SOD2/Catalase were used for gain-of-function assays. Cytoplasmic and mitochondrial superoxides and peroxides were measured using redox-sensitive GFP (roGFP) probes followed by flow cytometric analysis. Mitochondrial superoxides were also assessed by flow cytometric analysis of MitoSox stained cells. Proteomic analysis was achieved using nano LC-MS/MS. Annexin-V staining was analyzed by flow cytometry to measure cell death. *In vivo* and *in vitro* mouse studies were performed by FACS-based purification of shRNA-expressing cells followed either by: 1) growth in cytokine-enriched media or 2) transplantation into syngenic mice for survival analysis.
Results: We have discovered that inhibition of PKCε: 1) promoted the death of human and murine AML cell lines *in vitro*, 2) reduced AML progression driven by MLL-AF9 *in vivo* (p=0.0014) and 3) obstructed the growth of 5 out of 7 PD-AML samples *in vitro*. At the molecular level, we observed that PKCε inhibition led to a significant and dose-dependent increase in mitochondrial-produced superoxides–a specific type of ROS. Moreover, we found that enforced expression of PKCε can protect AML cells from lethal effects of superoxide-inducing agents 2-thenoyltrifluoroacteone and Antimycin A. To identify potential ROS-regulatory enzymes downstream of PKCε, we performed whole cell proteomics and found that the mitochondrial superoxide-neutralizing enzyme SOD2 is decreased in AML cells depleted of PKCε. Similar to PKCε inhibition, we also observed that genetic inhibition of SOD2 reduced the expansion of AML cell lines and PD-AMLs *in vitro* as well as significantly extended the onset of MLL-AF9-driven AML *in vivo* (p=0.0042). Finally, we also found that enforced expression of SOD2 in tandem with another anti-oxidant enzyme Catalase, reverses the anti-leukemia effects of PKCε inhibition confirming that PKCε supports AML pathophysiology by maintaining mitochondrial redox homeostasis.
Summary/Conclusions: Our results indicate that PKCε and SOD2 regulate mitochondrial redox homeostasis to support AML cell survival and disease progression and thus may represent a foundation for designing and developing novel therapeutic strategies.

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Abstract withdrawn.

P530

ROLE OF SHP2 IN A MOUSE MODEL OF AML CARRYING FLT3-ITD ALONG WITH LOSS OF TET2

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Background: SHP2, a protein tyrosine phosphatase coded by Ptpn11, is an essential protein that integrates signals from several different tyrosine kinase receptors with all the major intracellular signaling pathways such as ERK, PI3K and STAT pathways and regulates cell survival, proliferation and differentiaition. One of the SHP2 dependent cytokine receptor kinase, FLT3 when mutated to be constitutively activated co-operates with other genetic lesions like loss of Tet2 and Dnmt3a leading to transformation of myeloproliferative neoplasm (MPN) to acute myeloid leukemia (AML) in mouse models. Tet2 and Dnmt3a are involved in regulation hematopoietic stem cell (HSC) self-renewal and differentiation programs through regulation of DNA methylation. One their own each of them leads to MPN but when present in combination, leads to AML. These mouse models of AML have a more pronounced stem cell phenotype and are resistant to traditional chemotherapy or FLT3 targeted kinase inhibitor.
Aims: Inhibition of SHP2 catalytic activity by a small molecule allosteric inhibitor has been recently demonstrated to retard the growth of receptor tyrosine kinase driven malignancies. Therefore, we wanted to investigate the role of SHP2 in leukemogenesis driven by loss of an epigenetic regualtor (Tet2) and aberrant cytokine receptor tyrosine kinase (Flt3-ITD) signaling.
Methods: Mice were intercrossed to generate $Ptpn11^{F/F}Tet2^{-/-}Flt3^{ITD/+}Mx1Cre^{+}$ or $Ptpn11^{F/F}Tet2^{-/-}Flt3^{ITD/+}Mx1Cre^{-}$ mice. Deletion of Ptpn11 was induced at 8-10 week of age by injecting poly IC and changes in the hematopoietic compartment were analyzed by flow cytometry. Cell autonomous and non-autonomous effects of Ptpn11 on leukemogenesis were also evaluated in transplantation models.
Results: After ploy IC induced deletion of Ptpn11 there was a significant difference in the median survival between leukemic mice with with deletion of Ptpn11 *versus* non-deleted (n=8). Though the Ptpn11 deleted leukemic mice showed almost complete loss of long term HSC with concomitant increase in short term proliferating HSC in the bone marrow, they were still able to home and engraft in lethally irradiated recipient mice. These results indicate that loss of Ptpn11 does not impair the engraftment of leukemic stem cells though in normal mice deletion of Ptpn11 impairs the ability to stem cells to home to bone marrow niche and engraft. Deletion of Ptpn11 in both primary mice and secondary recipients was also associated with deregulation of myeloid and lymphoid cell distribution both in the periphery and bone marrow. Mice with deletion of Ptpn11 in the context of $Flt3^{ITD/+}$did not generate immature or mature B cells. The effects of Ptpn11 deletion were more severe in primary mice as compared to mice that received Ptpn11 deleted cells or when Ptpn11 was deleted after transplantation suggesting a role for SHP2 function in the bone marrow microenvironment in this model of leukemogenesis.
Summary/Conclusions: SHP2 has been recognized as a proto-oncogene on the basis of its ability to induce hematological malignancies when it is constitutively active and loss of SHP2 catalytic activity is associated with inhibition of tyrosine kinase driven malignancies. Our results demonstrate that the role of SHP2 in AML is dependent upon the presence of other genetic mutations. SHP2 regulates AML with loss of Tet2 with concomitant expression of Flt3-ITD through influence on both leukemic cells and the bone marrow microenvironment.

P531

CLUSTER REGULATION OF RUNX FAMILY BY "GENE SWITCH" TRIGGERS A PROFOUND TUMOR REGRESSION OF DIVERSE ORIGINS

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Background: Although Runt-related transcription factor 1 (RUNX1) has been generally considered to be a tumor suppressor, a growing body of evidence suggests its pro-oncogenic property in acute myeloid leukemia (AML).
Aims: Demonstrate the anti-tumor potential of cluster regulation of RUNX with a "gene-switch" in AML as well as in dismal-prognostic solid tumors arising from diverse origins *in vivo.*
Methods: To assess the effect of RUNX-inhibition in AML cells, we performed series of shRNA-mediated RUNX knockdown experiments. To achieve cluster regulations of RUNX, we have computationally designed an agent which could irreversibly block the RUNX cluster genes expression profiling through dismantling protein-DNA interactions sequence-specifically (CRoX-1).
Results: Firstly, shRNA-mediated silencing of *RUNX1* stimulated cell cycle arrest at G_0/G_1 phase and induced apoptosis in AML cells bearing wild-type *p53*. Besides, RUNX1 depletion induced remarkable induction of p53 as well as its target gene products and additive knockdown of p53 in these cell lines reverted the phenotype of RUNX1-depletion, indicating that RUNX1 is functionally dependent on proficient p53 pathway. In addition, cycloheximide chase assay revealed that RUNX1 negatively regulates the protein stability of p53 in AML cells. In silico data analysis and ChIP-seq experiments together with series of knockdown and restore experiments identified BCL11A and TRIM24 as critical mediators of p53 pathway activation in *RUNX1*-inhibited AML cells.
Though *RUNX1*-depleted AML cells exhibited drastically slowed proliferation rate, a small sub-population of leukemia cells retained the proliferation potential even after the silencing of *RUNX1*. Analysis of these residual AML cells revealed the reciprocal up-regulation of *RUNX2* and *RUNX3* expressions, suggesting that *RUNX2* and *RUNX3* might compensate for the loss of *RUNX1* functions. As expected, additional knockdown of *RUNX2* and *RUNX3* in *RUNX*1-depleted AML cells effectively suppressed their proliferations. Thus the simultaneous targeting of all RUNX family members as a cluster provides more stringent control of leukemia cells. Finally, we examined the antitumor potency of CRoX-1-mediated cluster regulations of RUNX. CRoX-1 treatment was indeed highly effective against leukemia as well as dismal-prognostic solid tumors arising from diverse origins *in vitro*. Moreover, this reagent was exceptionally well-tolerated in mice and exerted excellent efficacy against xenograft mice models of AML, acute lymphoblastic leukemia, lung and gastric cancers, extending their overall survival periods *in vivo*. Since RUNX families take part in diverse physiologic functions not only in AML cells but also in normal hematopoietic cells and in various other vital organ tissues, we might expect criticisms in targeting whole RUNX family that it could trigger undesirable side-effects *in vivo*. Intriguingly, however, our drug was well-tolerated *in vivo* and through these experiments, we have coincidentally found that the amount of total RUNX expressions was consistently higher in malignant tissues compared to their normal counterpart ones, and we believe that this gap offers pharmacological window to be targeted in anti-tumor strategies. Furthermore, we found that higher expressions of estimated total RUNX amount demarcate significantly poorer-prognostic patient cohorts in a wide variety of cancers, underpinning the rationality of RUNX-inhibition strategies in cancer treatment.
Summary/Conclusions: This work identified the crucial role of RUNX cluster in the maintenance and the progression of cancer cells, and the indicated gene switch technology-dependent its modulation would be a novel strategy to control malignancies.

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PHOSPHOPROTEOMICS AND MASS CYTOMETRY SIGNATURES OF PRIMARY AML CELL DIFFERENTIATION ARE ASSOCIATED WITH SENSITIVITY TO KINASE INHIBITORS

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Background: Kinase signalling is frequently deregulated in cancer cells. In the case of AML, the high recurrence of activating mutations in kinases and other kinase signalling regulators including FLT3 and RAS has stimulated the investigation of treatments based on kinase inhibitors. The success of kinase inhibitors depends of an accurate stratification of patients into response groups. The impact of genetic mutations on the sensitivity of primary AML to kinase inhibitors remains poorly defined and these have not been translated into effective therapies. The activity of a kinase can be affected by factors other than gene mutations and the sensitivity of leukemic cells to kinase inhibition depends not only on the activity of the targeted kinase. Thus, the integrative analysis of different biochemical features could improve the implementation of precision medicine therapies based on kinase inhibitors.
Aims: By the integration of multiple omics approaches, we aimed to generate molecular signatures, which can rationalize why some primary AML cells are resistant to treatment with different kinase inhibitors while others are sensitive to the same treatments.

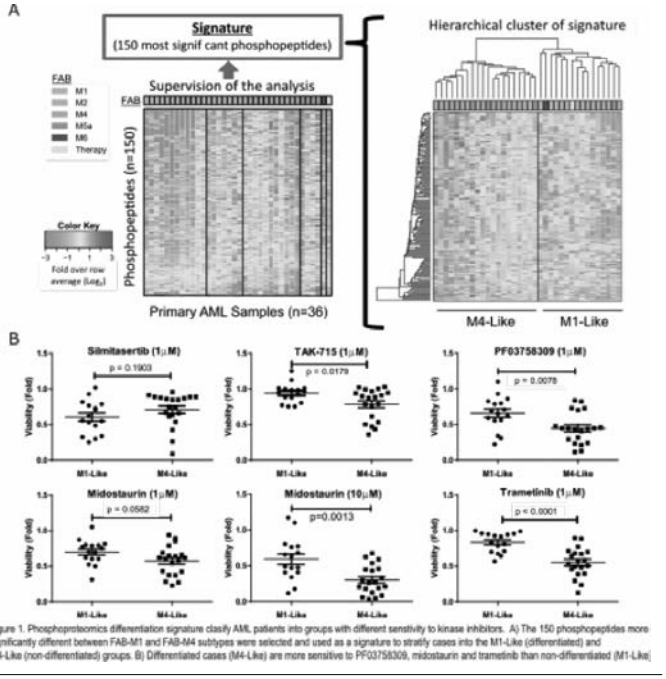

Figure 1. Phosphoproteomics differentiation signature classify AML patients into groups with different sensitivity to kinase inhibitors. A) The 150 phosphopeptides more significantly different between FAB-M1 and FAB-M4 subtypes were selected and used as a signature to stratify cases into the M1-Like (differentiated) and M4-Like (non-differentiated) groups. B) Differentiated cases (M4-Like) are more sensitive to PF03758309, midostaurin and trametinib than non-differentiated (M1-Like)

Figure 1.

Methods: In this investigation, we used a multiomics approach to stratify 36 AML biopsies as a function of their cellular sensitivity to "ex vivo" treatment with TAK-715, silmitasertib, PF03758309, midostaurin and trametinib, which target P38, CK2, PAK, FLT3/PKC and MEK, respectively. The same samples were analysed using different omics platforms: (i) mass spectrometry for phosphoproteomics, proteomics and kinomic profiling, (ii) mass cytometry for immunophenotyping and (iii) next generation sequencing for mutational profiling.
Results: Our integrative analysis identified two independent signatures that stratified our cohort of patients in sets of differentiated and undifferentiated cases. The phosphoproteomics signature divided our set of AML cases in the M1-like and M4-like groups (Figure 1A). The mass cytometry signature, which represented myelomonocytic markers that were co-expressed at the cell surface, split our cohort of patient in the CDs+ and CDs- groups. Remarkably, the M4-Like and CDs+ groups representing the differentiated cases, as well as the M1-Like and CDs- groups representing the non-differentiated cases, showed a high degree of overlap. Differentiated groups over-phosphorylated 3 times as many proteins as the non-differentiated groups, including kinases at sites linked to their activity. Mutations in genes involved in kinase signalling were also more frequent in differentiated cases. Kinase activity analysis using KSEA estimated that differentiated groups presented an enriched activity for PAK, MEK, ERK or PKC. Ontology analysis showed that non-differentiated cells over-phosphorylated nuclear proteins with DNA binding properties, while the differentiated cells increased the phosphorylation of membrane and cytoplasmic proteins linked to the small GTPase signalling. More interestingly, cases in differentiated groups were more sensitive to PF03758309, trametinib and midostaurin than those in the non-differentiated sets (Figure 1B for groups defined by the phosphoproteomics signature). Finally, differentiated cases as defined by the mass cytometry signature in our cohort of patients, or by a CD marker mRNA expression signature in the ATCG database, presented with significantly reduced survival when compared to the groups of non-differentiated cases.
Summary/Conclusions: Our data indicate that differentiated cells activate pro-survival kinases like PAK, PKCD or MEK which make them more sensitive to the inhibitors PF03758309, midostaurin or the FDA-approved drug trametinib. Since patients with differentiated cells present a reduced overall survival, treatment with these compounds may benefit patients in this higher risk group.

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CLINICAL IMPACT OF TET2 MUTATIONS IN ACUTE MYELOID LEUKEMIA PATIENTS HARBORING CEBPA MUTATIONS: A STUDY OF THE AML STUDY GROUP (AMLSG)

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Background: Based on the prognostic significance, as well as the association with certain biological and clinical features, acute myeloid leukemia (AML) with biallelic mutations in the *CCAAT/enhancer-binding protein-alpha* (*CEBPA*^{bi}) gene has been included as a distinct entity into the 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *CEBPA* mutations (*CEBPA*^{mut}) are mainly found in AML with normal cytogenetics, and approximately 60% of the mutated patients (pts) carry biallelic mutations. Several studies showed that *CEBPA*^{bi} occur almost mutually exclusive with regard to other AML associated gene mutations such as *NPM1* or *FLT3*-ITD mutations. Recently, mutations in the *tet oncogene family member 2* (*TET2*^{mut}) gene were described as a frequent concurrent mutation of *CEBPA*^{bi}. Both genes are involved in the control of proliferation (*CEBPA*, *TET2*) and differentiation (*CEBPA*) of myeloid progenitors. Preliminary data suggest that pts harboring the *CEBPA*^{bi}/*TET2*^{mut} genotype have a significantly worse overall survival (OS).
Aims: To evaluate the frequency and the clinical impact of *TET2*^{mut} within a large cohort of *CEBPA*^{mut} AML pts.
Methods: In total 200 AML pts (age 18 to 78 years) with *CEBPA*^{bi} (n=113) or *CEBPA* single mutations (*CEBPA*^{sm}) (n=87) were analysed for the presence of *TET2*^{mut}. All pts were enrolled in one of 6 AMLSG treatment trials applying intensive therapy [AMLHD93 n=14; AMLHD98A (NCT00146120) n=53; AMLHD98B n=12; AMLSG 07-04 (NCT00151242) n=74; AMLSG 06-04 (NCT00151255) n=25 and AMLSG 12-09 (NCT01180322) n=22]. *TET2* mutation screening was performed using a DNA-based PCR-assay covering exons 3 to 9 followed by Sanger sequencing.
Results: In total we detected 52 *TET2*^{mut}in 39 of the 200 pts (19.5%); in 16 pts *TET2*^{mut} co-occurred with *CEBPA*^{bi} (16/113, 14.2%), 23 pts had concurrent *CEBPA*^{sm} (23/87, 26.4%). All *TET2*^{mut} were heterozygous, with 13 pts having two mutations. The median follow-up of the 200 pts was 76.3 months (95%>CI: 68.5–84.2). *TET2*^{mut} were restricted to the cytogenetic intermediate-risk group (100%), and pts with *TET2*^{mut} were significantly older than pts with *TET2* wild-type (*TET2*^{wt}) (64y *vs* 49y, *P* <.0001). In addition, *TET2*^{mut} were more frequent in secondary/therapy-related AML (*P* =.04), and there was a significant association with *SRSF2* gene mutations (*P* =.01). With regard to outcome, pts with *TET2*^{mut} had a significantly shorter event-free (EFS), relapse-free (RFS), and OS compared to *TET2*^{wt}pts (*P* <.0001, *P* <.0001 and *P* =.0001, respectively). Evaluating the impact of *TET2*^{mut} within the subgroup of *CEBPA*^{bi} pts (n=113), we found a significant association of *TET2*^{mut} with older age (49y *vs* 46y, *P* =.05), and an inferior EFS (*P* =.001), RFS (*P* =.0003), and OS (*P* =.007). Finally, we analysed the impact of *TET2*^{mut} within the subgroup of *CEBPA*^{sm} pts (n=87). Also, in this subgroup *TET2*^{mut} were found to be significantly associated with older age (66y *vs* 51y, *P* <.0001), and with *SRSF2* mutations (*P* =.02). Clinically, pts with *TET2*^{mut} had a shorter RFS (*P* =.02) and OS (*P* =.05), and in trend a shorter EFS (*P* =.09).
Summary/Conclusions: In our study on a large cohort of *CEBPA*^{mut}AML pts we could confirm the high incidence of concomitant *TET2* mutations (19.5%). Pts with concurrent *TET2*^{mut} were significantly older and had an inferior outcome.

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GFI1B–A NOVEL ONCOSUPPRESSOR WHICH RESTRICTS NUMBER OF LEUKEMIC STEM CELLS

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Background: Myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) are hematopoetic disorders, which affect the myeloid lineages of hematopoiesis. Both are characterized by an accumulation of blast cells in the bone marrow (BM) that have the lost the ability to differentiate to mature cells. The proper differentiation of hematopoietic stem cells (HSCs) is regulated by transcription factors. Growth factor independence 1b (Gfi1b) is a repressing transcription factor regulating quiescence of HSCs and the proper emergence and maturation of erythrocytes and platelets.
Aims: Aim of the study was to identify I) do different level of Gfi1b influence onset and development of MDS and AML in human patients II) how does Gfi1b act in MDS/AML development on a molecular level.
Methods: We correlated Gfi1b expression level in blast cells of patients with MDS and AML with the overall disease course. To get a better insight how does different Gfi1b level influence MDS/AML development, we used three different murine models of human AML with expression of different oncogenes (NUP98/HOXD13, MLL-AF9 and expression of a mutated K-Ras). In these models we either downregulated or conditionally knocked out Gfi1b expression. Finally, we performed ChIP Seq analysis as well as whole genome gene expression arrays to study the molecular functions of Gfi1b in AML development.
Results: Low expression or absence of Gfi1b expression was associated with an inferior outcome with regard to overall-survival as well as event-free survival of MDS/AML patients. Using the above murine models of MDS/AML, loss or low expression of Gfi1b accelerated AML development. Additionally we could show that loss of Gfi1b significantly enhanced number of functional leukemic stem cells. It is well known that Gfi1b has a function to recruit histone modifying enzymes to induce among other deacetylation of H3K9. ChIP seq data of Gfi1b deficient leukemic cells revealed that loss of Gfi1b led to a higher H3K9 acetylation of a number of target genes, among them a number of oncogenes. Among these target genes, we found MAPK as well as Reactive oxygen species (ROS) signalling, as one of the top hit in our data. Previously it was reported that loss of Gfi1b enhanced the ROS level in HSCs. In our case we also see an increased expression of ROS in Gfi1b deficient leukemic cells, a higher activity of the FOXO pathway as well as reduced p38 activity. The combination of these foundings contributes to the higher number of leukemic stem cells in Gfi1b deficient leukemic cells. To reduce the high level of ROS in leukemic stem cells we use with N-Acetylcystein (NAC). Use of NAC impeded growth of Gfi1b deficient cells in-vitro.
Summary/Conclusions: Gfi1b act as a tumorsuppressor by restricting number of leukemic stem cells and treatment with NAC opens a potential targeted therapy for AML patients with low/absent expression of Gfi1b.

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VARIANT ALLELE FREQUENCY KINETICS OF TYROSINE KINASE GENE MUTATIONS IN CORE-BINDING FACTOR ACUTE MYELOID LEUKEMIA (CBF-AML) UNDER TREATMENT WITH AND WITHOUT DASATINIB

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Background: Background: Recent next-generation sequencing (NGS) studies have improved our understanding of the genomic landscape of CBF-AML (Faber *et al. Nat Genet* 2016; Duployez *et al. Blood* 2016). While these studies have mainly focused on the genetic differences between inv(16)- and t(8;21)-AML at the time of diagnosis, the clonal architecture of relapsed disease is not well defined. Mutations affecting signaling genes, such as *KIT* and *NRAS* are known to be among the most common oncogenic drivers in CBF-AML, however their impact at relapse remains unclear.
Aims: To characterize clonal evolution in paired samples obtained at diagnosis, during remission and at relapse under treatment with dasatinib *vs* conventional chemotherapy, and ii) to conduct gene set enrichment analyses.
Methods: Whole-exome-sequencing (WES) was performed in paired diagnosis, remission and relapse samples of 36 patients with CBF-AML [inv(16), n=24; t(8;21), n=12] using paired-end sequencing (read length 100 bp) on an Illumina HiSeq platform. Library preparation was done with the Nextera® Rapid Capture Exome kit following manufacturer's instructions. All patients were treated within one of five trials of the German-Austrian Study Group (AMLSG). In two of the trials (AMLSG 11-08, NCT00850382; AMLSG 21-13, NCT02013648) patients received intensive chemotherapy in combination with the multi-kinase inhibitor dasatinib.
Results: The mean WES coverage was 133x. Mutations and indels were called with a threshold >10% variant allele frequency (VAF) after filtering for SNPs and sequencing artefacts. In sum, we identified 587 variants in 430 genes. At

diagnosis, 8.9 variants per patient were found as compared to 5.7 at relapse. 52% variants were present at diagnosis, 26% at relapse only, and 22% were present at both, diagnosis and relapse. With regard to the most commonly altered signaling genes *KIT* and *NRAS* we found the following pattern: The median VAF at diagnosis was 23% and 26% for *KIT* and *NRAS*, respectively. Of note, the initial *KIT* and *NRAS* clone was lost (VAF <5%) in 71% (exon 17, n= 9; exon 8, n=2; exon 11, n=1) and 100% of cases (exon 2, n=5; exon 3, n=3). Comparing the VAF kinetics between patients treated with and without dasatinib, baseline *KIT* mutations became subclonal (VAF <5%) in all patients receiving dasatinib (n=8), whereas they were still detectable in 4/6 (67%) patients who were intensively treated without the addition of dasatinib. *NRAS* became subclonal (n=8) irrespective of the treatment regimen. In one *KIT* mutated patient treated with dasatinib the baseline KIT^{D816V} mutation (exon 17) was lost at the time of relapse, but a KIT^{D419Y} mutation (exon 8) was acquired instead. Gene set enrichment analyses revealed different mutation signatures at diagnosis and relapse: At diagnosis, there was a significant enrichment for genes associated with MYC overexpression. Variants that were recurrently present at diagnosis and relapse showed enrichment for genes affected in KRAS overexpression models. Relapse samples were additionally enriched for gene mutations involved in the mitotic spindle assembly.

Summary/Conclusions: Differences in the allelic composition were found between diagnosis and relapse regardless of the CBF-AML subtype. Our data suggest that the *KIT* clone might be successfully eradicated under dasatinib treatment whereas persistence of *KIT* mutant clones was more commonly seen under conventional chemotherapy. The frequent loss of *KIT* and *NRAS* mutations during therapy suggests that relapse is triggered by alternative genetic lesions. Relapsed disease may represent a distinct biology which is characterized by mutations that cluster in different pathways. Further analyses are ongoing including study cohort expansion, as well as inclusion of RNA sequencing results.

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P38B MAPK INTERACTS WITH SET REGULATING ITS INHIBITORY EFFECT ON PP2A ACTIVITY IN ACUTE MYELOID LEUKEMIA

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Background: Despite improvements in our understanding of the molecular evolution of acute myeloid leukemia (AML), the overall cure rates remain low, and most patients die from the disease despite achieving initial remission upon treatment. It is therefore necessary to open new therapeutic perspectives aimed at molecular targets. PP2A phosphatase inactivation is a recurrent event in hematological tumors. Our group has reported that SET, an endogenous inhibitor of PP2A, is overexpressed in 28% of patients with AML. Furthermore, the anticancer activity of PP2A activating drugs (PADs) depends on the interaction/sequestration of SET, pointing out the significance of this oncogene in AML. Drug inhibition of several MAPKs in AML cell lines showed that only p38 inhibitors activate PP2A and decrease SET protein.

Aims: Therefore, we hypothesized that p38 could regulate SET at posttranslational level, leading to PP2A inactivation.

Methods: AML cell lines and primary human samples were analyzed by western blot, immunoprecipitation, immunofluorescence, treatment with pharmacological inhibitors and siRNAs. Phosphorylation assays by *in vitro* kinase assay with recombinant proteins were performed.

Results: Knockdown of the two major isoforms of p38-MAPK, p38α and p38β, demonstrated that only p38β was able to reduce SET protein levels and increase PP2A activity. To decipher this mechanism of action, we performed protein immunoprecipitation and immunofluorescence in the AML cell lines HL-60 and MOLM-13. p38β co-localized and bound to SET mostly in the cytoplasm stabilizing it, since treatment with ciclohexymide in the absence of p38β induced SET degradation. The stabilization role was in coordination with SETBP1, which co-localized with both SET and p38β. Interestingly, 12 out of 14 AML cell lines tested showed high expression of p38β protein levels but not of p38α, as well as 5 out of 7 AML primary patient samples. Furthermore, expression analysis in a large series of adult *de novo* AML cases previously reported (*Cancer Genome Atlas Research Network*, 2013) showed a positive correlation between *p38β* (*MAPK11*) and *SET* (R^2=0.416, p<0.001), but not between *p38α* and *SET.* We and others have shown that PADs retain SET in the nucleus. Our results showed that p38 phosphorylates SET not directly, but through the activation of casein kinase 2 (CK2), leading to the retention of SET in the nucleus and, therefore, contributing to the inactivation of PP2A in AML cells. Of note, CK2 is overexpressed in both AML cell lines and patient samples.

Summary/Conclusions: p38 is able to activate CK2 which phosphorylates SET and, as consequence, facilities its trafficking to the cytoplasm, contributing to PP2A inactivation in AML cells. Moreover, p38β binds to SET in the cytoplasm, contributing to its stability and leading to PP2A inactivation. In this regard, we have preliminary evidences that combination therapy with PADs and the CK2 inhibitor CX4945 reduces significantly the viability of AML cells, supporting that novel treatment modalities that can target multiple components of the same pathway may help to achieve a more sustained therapeutic benefit.

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GENETIC LANDSCAPE OF ACUTE ERYTHROID LEUKEMIA

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Background: Acute erythroid leukemia (AEL) is a unique subtype of acute myeloid leukemia (AML) characterized by the predominance of erythroid components with increased ring sideroblasts as well as frequent myelodysplasia. However, due to its rarity, the molecular pathogenesis of AEL has not fully been elucidated, except for frequent *TP53* mutations.
Aims: This study was designed to clarify the mutation profile of AEL distinct from other types of AML and myelodysplastic syndromes (MDS).
Methods: We performed a comprehensive genetic study, in which paired tumor/normal DNA from 22 AEL cases were analyzed using whole exome sequencing (WES). Whole-exome sequencing data from 3 AELs generated by The Cancer Genome Atlas (TCGA) was also included in the analysis. Subsequently, a total of 84 AEL cases were screened for mutations in 67 driver genes associated with myeloid malignancies using targeted-capture sequencing, in which RNA baits were also designed for a total of 1158 single nucleotide polymorphism sites to allow for genome wide copy number abnormalities and other allelic imbalances.
Results: Median age at diagnosis was 58.5 (21-87) years old. Among the 77 patients with clinical information available, 62 patients were diagnosed with *de novo* AML, 13 with secondary AML, and 2 with treatment-related AML. On average, 18.4 and 3.4 mutations were detected per sample in whole-exome and targeted-capture sequencing in AEL, as compared to 12.2 and 2.9 mutations (P<0.001 and 0.05) in other AML, respectively. Both platforms being combined, most frequently observed was *TP53* mutations (n=26, 31%) with complex karyotype being accompanied in most cases (25 cases), which were associated with a significantly shorter overall survival (P<0.001). Other frequently mutated genes were those encoding major components of the cohesin complex, including *STAG2* (24%), *SMC1A* (4.8%) and *RAD21* (2.4%), which were mutated in as high as 30% of the cases. The splicing machinery (18%) and epigenetic regulators (45%) were also common targets of mutations, including *SRSF2* (12%), *U2AF1* (4.8%), *WT1* (15%), *TET2* (19%) and *IDH1/2* (12%). *TP53* mutations were mutually exclusive with cohesin mutations (p<0.01) and those in epigenetic regulators (p=0.014). Compared to other types of AML cases reported from TCGA, AEL showed a significantly higher frequency of mutations in *TP53*, *STAG2*, *SRSF2*, *ASXL1*, *BCOR*, *WT1*, and *TET2*. These mutations have previously been shown to be enriched in secondary AML and MDS relative to *de novo* AML, which were also confirmed in our analysis involving 2,250 MDS cases. By contrast, *FLT3* (12%) and *DNMT3A* (12%) mutations and *SF3B1* mutations (0%) were significantly less frequent in AEL, compared to *de novo* AML and MDS. The frequency of these mutations was not statistically different between *de novo* AEL and secondary AEL.
Summary/Conclusions: WES and follow-up targeted-capture sequencing revealed a landscape of mutations in AEL. Frequent mutations in *TP53*, splicing factors, the cohesin complex, and epigenetic regulators were characteristic of AEL and thought to be involved in its pathophysiology. Mutations in *TP53* defined a subgroup with distinct genetic and prognostic features. Our result indicated a similarity between AEL and high-risk MDS/secondary AML, supporting the recent revision of the WHO classifications, in which AEL was reclassified into MDS and AML not otherwise unspecified.

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THE MOLECULAR LANDSCAPE OF MLL-PTD AML: SPECIFIC CONCURRENT MUTATIONS, CLINICAL OUTCOME AND GENE EXPRESSION SIGNATURES

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Background: Partial tandem duplications (PTDs) in the Mixed Lineage Leukemia (*MLL*) gene, currently known as Lysine Methyltransferase 2A (*KMT2A*) are acquired in-frame internal duplications present in 5–11% of acute myeloid leukemia (AML). *MLL*-PTDs are predominantly present in cytogenetic normal AML and occasionally in AML with trisomy of chromosome 11. *MLL*-PTDs are generally considered as a poor prognostic marker in AML.
Aims: Evaluate the mutational landscape, prognostic value and gene expression signatures of *MLL*-PTD AMLs in comparison to a well-characterized AML cohort without *MLL*-PTD.
Methods: cDNA of 2310 AML patients enrolled in the adult HOVON-SAKK clinical trials (from 1995 to 2013) were analyzed for the presence of an *MLL*-PTD. Mutational screening based on next generation sequencing (NGS) was performed using the Illumina Trusight Myeloid panel on the Illumina HiSeq2500. An independent cohort of 632 *de novo* AML patients without *MLL*-PTD served as control. The gene expression profiling was assessed of all AML cases using Affymetrix HGU133 Plus2.0 GeneChips as previously described (Verhaak *et al.*, 2009).
Results: *MLL*-PTD was detected in 118 (5.1%) out of 2310 AML patients. *MLL*-PTDs were significantly associated with trisomy 11: 7% *vs* 1% (p=0.0037), normal karyotype: 65% *vs* 53% (p=0.0102) and complex karyotype: 1% *vs* 14% (p=0.0004). None of the *MLL*-PTD AML patients had any of the core binding leukemia fusion genes. The targeted NGS was performed on 87 out of 118 patients due to availability of gDNA. The number of mutation detected in *MLL*-PTD AMLs ranged from 0–6 mutations with an average of 3 mutations per patient. The most frequently mutated genes in *MLL*-PTD AMLs were *DNMT3A*, *FLT3*-ITD, *IDH2*, *RUNX1*, *IDH1*, and *TET2*. In the context of the 632 AMLs without *MLL*-PTD mutations in several genes appeared to be significantly associated with the *MLL*-PTD, i.e., *FLT3*-ITD: 34% *vs* 13% (p<0.0001), *IDH1*: 16% *vs* 9% (p=0.0133), *U2AF1*: 9% *vs* 3% (p=0.0158) and *IDH2*: 23% *vs* 12% (p=0.0181) or inversely associated, i.e., *NPM1*: 3% *vs* 32% (p<0.0001), *NRAS*: 5% *vs* 22% (p=0.0002) and *TP53*: 3% *vs* 10% (p=0.0487). Overall, the numbers of mutations in the spliceosome (p=0.03), tumor suppressor (p=0.0388), and transcription factor genes (p=0.0408) were significantly higher in *MLL*-PTD AMLs compared to *MLL* wild-type AMLs. As expected, the *MLL*-PTD appeared to be significantly associated with inferior outcome (*MLL*-PTD (n=74) and without *MLL*-PTD (n=1764); OS: p=0.05). Association of the presence of an *MLL*-PTD with EFS was only borderline significant (p=0.07). Within *MLL*-PTD AML, *DNMT3A* mutations are associated with inferior overall survival (HR: 2.06; 95%CI: 1.19-3.58; p=0.010). Although low numbers, *MLL*-PTD AML patients that harbor *NRAS* mutations do even worse (HR: 6.54; 95%CI: 2.45-17.49; p<0.001. In multivariate analysis both markers remain significant when combined with WBC counts and cytogenetics. Multiple homeobox-related gene family members were overexpressed in MLL-PTD AML. The top-35 differentially expressed genes included *HOXB5, HOXB6, HOXB7, HOXB8*, *HOXB9* and *NKX2.3.* In an association model, which takes all other known subsets of AML into account, other *HOX*-related genes, such as *HOXA7, HOXA9* and *NKX2.5*, also appeared significantly differentially expressed. In both analyses *MLL* itself seemed to be most consistently overexpressed in *MLL*-PTD AML. In contrast, these specific gene expression changes were absent in AML with translocations involving *MLL* on 11q23. Additional analyses in AML subsets based on the concurrent mutations will be carried out to investigate whether these are limited to certain *MLL*-PTD AML molecular subsets.
Summary/Conclusions: *MLL*-PTD AML carries specific gene expression signatures and specific subsets of concurrent mutations with clinical value.

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EXPLORING THE IMPACT OF LOSS OF FUNCTION STAG2 MUTATIONS ON CHROMATIN ARCHITETCURE IN MDS/AML

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Background: The Cohesin complex is an evolutionarily conserved multimeric protein complex consisting of SMC1, SMC3, RAD21, STAG1 & STAG2. The complex plays pivotal roles within mitosis and sister chromatid cohesion however, substantial data exists elucidating roles for the complex within the DNA damage response, homologous recombination and long-range interaction between *cis* regulatory elements of the genome. Within hematological malignancies upwards of 20% of patients diagnosed with either Acute Myeloid Leukemia (AML), Myelodysplastic syndrome (MDS) or Myeloproliferative neoplasm (MPN) have been shown to harbour mutations within the Cohesin complex, with many more showing significantly reduced expression of core complex members.
Aims: To explore the impact of a loss of function *STAG2* mutation on the chromatin architecture within a isogenic cell based model.
Methods: Using an CRISPR generated isogenic model we have investigated the impact loss of *STAG2* has on the chromatin architecture of a hematopoietic environment. Genome wide binding profiles for STAG1, STAG2 and CTCF were generated using ChIP-Seq to elucidate areas of differential between STAG members. In addition, binding profiles for H3K27ac, H3K27me3 and H3K4me1 were generated using ChIP-Seq to provide genome wide identification of active and repressed enhancer regions, with the regions ranked to identify both normal and super-enhancer regions. These samples were matched to ATAC-Seq profiling of open and closed chromatin regions as well as RNA-seq samples to provide information on gene activity in relation to chromatin state in the absence of STAG2.
Results: Our results indicate that STAG1 bindng profiles alter following loss of function of STAG2, with an increase in binding peaks from ~17,000 to 25,000, however several sites identified by ChIP-Seq are not compensated for. Histone mark profiling identified wide spread expansion of the H3K27ac mark and a decrease in regions of H3K27me3 consistent with loss of boundaries within topologically associated domains. This spread of an activator mark correlates with altered gene expression and the changes observed in ATAC-seq profiling of altered chromatin accessibility. The open chromatin regions identified through the use of ATAC-seq peak motif analysis yielded information regarding transcription factor occupancy. An enrichment for transcription factors relevant for myeloid transcriptional programmes was observed. This increase in enrichment aids in the suggestion that the impact of mutated/altered cohesin complex function relates directly to the specific cell type and maturation state at which it occurs.
Summary/Conclusions: This research into the aberrant and non-canonical nature of the cohesin complex provides evidence of the impact of mutant STAG2 on the spatio-temporal genomic architecture in hematological malignancies and begins to yield insight into the clinical implications of mutations within the cohesin complex.

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NEXT GENERATION SEQUENCING TECHNIQUES REVEAL MOLECULAR MECHANISMS OF MYB REGULATION AND FUNCTION IN MLL-AF9 LEUKEMIA

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Background: Mutations involving the *MLL* gene at 11q23 are found in 10% of adult and 18% of childhood acute myeloid leukaemia (AML) cases. The most frequently occurring *MLL* mutations are chromosome translocations that fuse the *MLL* gene in-frame with a second partner gene, creating novel fusion proteins (MLL-FPs). MLL-AF9 is the most common MLL-FP in AML. Despite much progress in the overall management of AML, patients carrying *MLL*-rearrangements still have a poor survival prognosis and limited response to existing therapy. This is in part due to the low therapeutic indices and narrow therapeutic windows of current chemotherapeutic agents, therefore underscoring the need to develop improved, targeted therapies. *MYB* is a direct downstream target of MLL-AF9. Recent studies indicate that MLL-AF9 leukemia cells are more affected by *MYB* knockdown compared to normal hematopoietic stem progenitor cells. This is despite the fact that *MYB* is known to be essential for the establishment of definitive hematopoiesis. This suggests that a therapeutic window may be achieved through targeting *MYB.* Therefore, by understanding more about the role of *MYB* in MLL-AF9 leukemia and the network it regulates, we maybe able to exploit this knowledge to target *MYB* directly by interfering with its function or indirectly via its downstream targets.
Aims: To understand the molecular function of *MYB* in MLL-AF9 leukaemia.
Methods: We performed genome-wide MYB, MLL-AF9, H3K27ac, H3K4me3 and H3K4me1 chromatin immunoprecipitation sequencing (ChIP-seq) and Assay for Transposase-Accessible Chromatin with high throughput sequencing (ATAC-seq) in two MLL-AF9 leukemia models to identify putative regulatory regions of *MYB* and those of a direct MYB gene target, *BCL2*. The chromatin conformation capture technique, Capture-C (one *vs* all) was used to further characterize interactions from the MYB promoter. We then performed siRNA knockdown of *MYB* and assessed the effect of MYB loss on its downstream druggable target *BCL2*, using RT qPCR, Western blotting and ChIP qPCR.
Results: We identified MLL-AF9 binding to novel putative enhancers of *MYB* as defined by regions co-bound by H3K27ac, H3K4me1 and marked by open chromatin on ATAC-seq. Furthermore, Capture-C from the MYB promoter identified novel putative enhancer-promoter interacting domains 100-200kb apart that are co-bound by MYB but not MLL-AF9. This suggests long-range autoregulation of MYB. Next, siRNA knockdown of MYB results in loss of MYB binding at the BCL2 promoter and its downstream enhancer by ChIP qPCR. There is a corresponding loss of *BCL2* mRNA and protein expression in MYB knocked-down cells compared with control, confirming that BCL2 is directly regulated by MYB.
Summary/Conclusions: We have identified for the first time, regulation of *MYB* by MLL-AF9 via putative enhancers, and also an autoregulatory role of MYB involving long-range cis-interactions. Furthermore, we confirm that *BCL2* is directly regulated by MYB in MLL-AF9 leukemia, suggesting a molecular rational for using BCL2 inhibitors in MLL-AF9 leukemia therapy.

P541

CD123-SPECIFIC CHIMERIC ANTIGEN RECEPTOR T-CELL THERAPY IN ACUTE MYELOID LEUKAEMIA

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Background: Acute myeloid leukaemia (AML) is a heterogeneous disease characterized by clonal evolution of myeloid precursors in bone marrow and peripheral blood resulting in accumulation of leukemic blasts and severe impairment of normal haematopoiesis. Despite advances in our understanding of AML biology, development of novel therapies has been limited with 43% relapse rate and 18% of patients never attaining clinical remission (CR) with frontline induction treatment. Chimeric antigen receptor (CARs) T cells specific for tumour-associated antigens are emerging to be an effective form of immunotherapy for AML. A small number of *in vitro* and *in vivo* studies have evaluated the efficacy and specificity of CAR T cell immunotherapy in AML by targeting interleukin three receptor alpha (IL3RA; CD123), a molecule over expressed on AML blasts and leukaemia stem cells (LSC) compared to normal haematopoietic stem cells (HSCs).
Aims: In this study, we investigated the efficacy of a second generation CAR expressing six single-chain variable fragments (scFv) with different affinities for CD123 and evaluated the cytotoxic effect of different co-stimulatory domains (CD28 *versus* 41BB) using a co-culture assay. Furthermore, we also evaluated the cytotoxic effects of a dual targeting CAR (against CD123 and CD33) using the same assay conditions.
Methods: Six lentiviral constructs (two high, two moderate & two low affinity) were transduced (MOI 1:5) into peripheral blood mononuclear cells (PBMCs) from healthy donors and their cytotoxicty was examined by flowcytometry on leukaemic cell lines; KG1 (CD123$^+$, CD34$^+$, CD33$^+$) [Fig:1a], Kasumi-1 (CD123$^+$, CD34$^+$, CD33$^+$), U937 (CD123$^+$, CD34$^-$, CD33$^+$), K562 (CD123$^-$, CD34$^-$, CD33$^+$) and AML mononucelar cells (MNCs).
Results: Flowcytometric analysis confirmed the expansion of T cells from PBMCs and the cytotoxicity of the six CARCD123 constructs against CD123^{+ve} cells. The high affinity CARCD123 (4nM kD & 4nM kD K136Q) T cells demonstrated enhanced cytotoxicity compared to moderate (56nM kD, 56nM kD A105G) and low affinity (101nM kD, 101nM kD V24G) CARCD123 in both leukaemic cell lines and also in allogenic AML MNCs. Both the highest affinity CARCD123 constructs were also tested in cell lines using increasing effector: target ratios (1:2, 1:4 & 1:10) displaying consistent cytotoxicity and were also effective against autologous AML MNCs (target cells) and PBMCs (effector cells) from two patients. T cell activation was confirmed by ELISA and showed increased IFN-γ (500-2000 fold) and TNF-α (150-200 fold) levels. Previous studies have confirmed the distinction in CAR efficiency using CD28 *versus* 41BB co-stimulatory domains; CD28 co-stimulation augmented, whereas 4-1BB co-stimulation reduced T cell exhaustion induced by continuous CAR signaling. To confirm persistence of the CAR cytoxicity, we constructed a high affinity CAR substituting CD28 with a 4-1BB co-stimulatory domain and obtained similar cytotoxicity results on K562 and U937 cell lines. Furthermore, a novel a dual targeting CAR in which the activation domain (CD3ζ) is directed against CD33 and the costimulatory domain (CD28) directed against CD123 enhanced the specificity of the CAR towards leukaemic cells; reducing "on-target but off-organ effects". Results obtained in co-culture assay against KG1 [Fig:1b] and K562 cell lines [Fig:1c] with varying effector: target ratios were demonstrated results similar to the high affinity single targeting CAR.

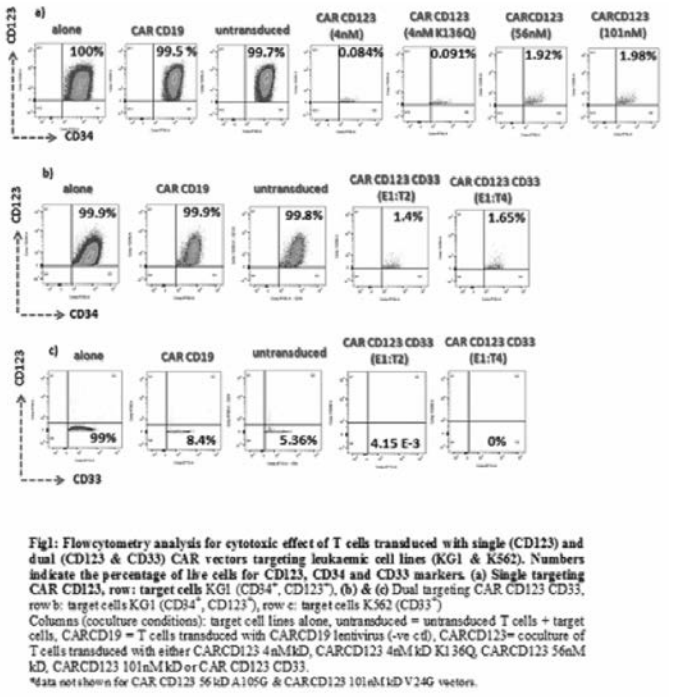

Fig1: Flowcytometry analysis for cytotoxic effect of T cells transduced with single (CD123) and dual (CD123 & CD33) CAR vectors targeting leukaemic cell lines (KG1 & K562). Numbers indicate the percentage of live cells for CD123, CD34 and CD33 markers. (a) Single targeting CAR CD123, row: target cells KG1 (CD34$^+$, CD123$^+$), **(b) & (c)** Dual targeting CAR CD123 CD33, row b: target cells KG1 (CD34$^+$, CD123$^+$), row c: target cells K562 (CD33$^+$)
Columns (coculture conditions): target cell lines alone, untransduced = untransduced T cells + target cells, CARCD19 = T cells transduced with CARCD19 lentivirus (-ve ctl), CARCD123= coculture of T cells transduced with either CARCD123 4nMkD, CARCD123 4nM kD K136Q, CARCD123 56nM kD, CARCD123 101nM kD or CAR CD123 CD33.
*data not shown for CAR CD123 56 kD A105G & CARCD123 101nM kD V24G vectors.

Figure 1.

Summary/Conclusions: In conclusion, we demonstrate the importance of the scFv on CAR T cell cytoxicity and have constructed and validated the efficacy of a dual targeting CAR vector in the context of AML.

P542

TARGETED COMBINATION THERAPY WITH CDK4/6 INHIBITOR PALBOCICLIB IN AML

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Background: Acute myeloid leukemia (AML) is a clonal hematologic disorder marked by clinical and biological heterogeneity. AML remains incurable for a significant proportion of adult patients while no therapeutic option exists for patients with relapsed and refractory AML. Mutations of the fms-like tyrosine kinase 3 (*FLT3*) gene are among the most frequent events in AML and usually involves internal tandem duplication (ITD) of the juxtamembrane domain coding region or point mutations of the tyrosine kinase domain. There have been considerable efforts to develop FLT3 tyrosine kinase inhibitors (TKI). The clinical impact of FLT3-TKI has been limited as resistant clones have emerged rapidly.

We have recently discovered that *FLT3*-ITD$^+$ AML cells are highly sensitive to the FDA-&EMA-approved CDK4/6 kinase inhibitor palbociclib (IBRANCE by Pfizer). The effect is ascribed to the transcriptional activity of CDK6 on *FLT3* and *PIM1*- a feature not shared by CDK4.
Aims: FLT3-TKI treatment provides short-term disease control but relapse invariably occurs within months. Acquired resistance on *FLT3*-D835Y tyrosine kinase domain is an emerging clinical problem. The focus of our study is to investigate the potential of palbociclib treatment in *FLT3*-D835Y$^+$ cells and to identify critical downstream effectors of CDK6 to open a novel, clinically applicable therapeutic window.
Methods: Ba/F3 cells transformed with *FLT3*-D835Y were exposed to single agents and drug combinations. Viability was measured by the CellTiter-Glo ATP-based assay and FACS stainings after 3 days of treatment. Validation was performed by *in vivo* xenograft models and by studies with primary human *FLT3*-D835Y$^+$ AML biopsies.
Results: Palbociclib impaired the viability of murine Ba/F3 cells with *FLT3*-D835Y at low concentrations. The effect extended to primary *FLT3*-D835Y$^+$ patient samples and to xenograft models, where palbociclib treatment effectively repressed *FLT3*-D835Y driven tumor formation *in vivo* at clinically relevant concentrations. Besides *FLT3* itself, which is regulated by CDK6, transcriptional targets of CDK6 in AML included Aurora kinases (AURK) and AKT. Thus CDK6 inhibition lead to decreased expression of AURK and AKT in mutant Ba/F3 cells, two signalling nodes critical for survival of tumor cells. Dual targeting with palbociclib and AURK or AKT inhibitors resulted in synergistic cytotoxicity.
Summary/Conclusions: Palbociclib represents a viable therapeutic option for use in treatment of resistant clones in *FLT3*-D835Y$^+$ AML. Inhibitory effects are caused by cell cycle inhibition as well as by transcriptional activity of CDK6 on important signalling pathways including Aurora kinases and AKT. Our findings provide the basis for the design of synergistic combination therapies with a CDK4/6 inhibitor which could be readily translated to patients with AML.

P543

CANNABINOIDS DERIVATIVES MODIFY THE PATTERN OF SPHINGOLIPIDS IN ACUTE MYELOID LEUKEMIA CELLS AND PRODUCE A POTENT ANTI-LEUKEMIC EFFECT

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Background: Endocannabinoid system is a set of ligands, receptors and endogenous enzymes which modulate a variety of physiological effects. There are two well-characterized cannabinoid receptors, CB1 (mainly expressed in Central Nervous System) and CB2 (mainly in hematopoietic cells). Here, we tested the effect of the cannabinoid WIN-55 212-2 in acute myeloid leukemia (AML) *in vitro* and *in vivo* and studied the molecular signaling pathways involved in this effect, specially the role of sphingolipids. Moreover, we synthesized a new family of twelve cannabinoids that are specific to CB2 receptor.
Aims: - Development of new compounds derived from cannabinoids with CB2 selectivity and evaluation of their anti-tumor effect in AML *in vitro* and in vivo. To deepen in the knowledge of lipid metabolism in AML.
Methods: For the design and synthesis of new cannabinoids, computational techniques of docking, analytical and spectroscopic techniques such as mass spectrometry (MS) were used. To assess the anti-leukemia effect of the different cannabinoids, we analyzed cell viability by MTT and flow cytometry using six human AML cell lines, primary cells from healthy donors (hematopoietic progenitor cells (HPC) and lymphocytes) and blasts from AML patients. Mitochondrial damage was analyzed by flow cytometry using TMRE and by MitoSOXTM Red. In addition, we performed western blot and immunocytochemistry assays to determine the expression of different proteins to elucidate the molecular signaling pathways involved in the effect of these drugs.Moreover, we analyzed the ceramide levels, a membrane lipid associated with death/survival cell processes, and other sphingolipids by LC-MS/MS and immunohistochemistry. Finally, NOD/scid/IL-2R gammae null (NSG) mice were xenotransplanted with HL60 cell line. We confirmed disease infiltration in bone marrow (BM) by BM aspirates and flow cytometry assays. Once the presence of leukemic cells was confirmed, treatment with vehicle, WIN-55 cannabinoid at a dose of 5mg/kg/day or citarabine (ARA-C) at 50mg/kg during 5 days was administered. Also we tested the effect of these compounds on normal hematopoiesis by treating healthy BALB-C mice and the levels of sphingolipids in cell and plasma were studied in mice models.
Results: Cannabinoids induced a potent proapoptotic effect on AML cell lines and on primary leukemic cells, which was not observed in normal HPC and lymphocytes from healthy donors. Fragmentation of PARP and activation of caspases 2, 3, 8 and 9 were confirmed by Western Blot. The proapoptotic effect of cannabinoids on AML cells was abolished upon co-culture with either CB2 receptor antagonists or with pancaspase inhibitors. Other proteins involved in the effect of cannabinoids were p-AKT, p-ERK 1/2, p-38 and p- JNK. Also studies on p-PERK, p-IRE1 and CHOP confirmed an increased endoplasmic reticulum stress upon exposure to cannabinoids. Also we confirmed a very early mitochondrial damage in leukemic cells which was not observed in normal hematopoietic progenitor cells. Remarkably, we observed significant differences in the amounts of certain sphingolipids in untreated *versus* treated leukemic cells. More specifically in ceramide C16:0, C18:0 and C18:1. Also we observed a significantly increased survival among mice treated with WIN-55 cannabinoid as compared to both the control group and the group treated with ARA-C and we confirmed that cannabinoids did not affect the viability of the different populations of hematopoietic progenitors and, moreover, an increased platelet count was observed in treated mice.

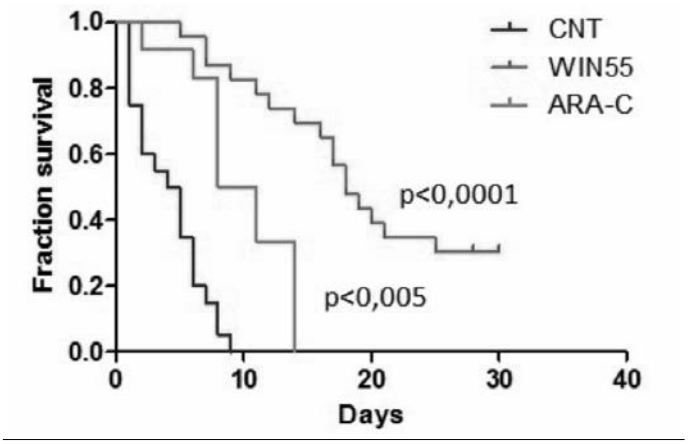

Figure 1.

Summary/Conclusions: Our findings indicate that cannabinoids display a highly selective proapoptotic effect against leukemic cells. Several pathways are involved in this effect, the modification in the sphingolipids pattern playing a main role.

P544

PROFILING THE MUTATIONAL LANDSCAPE OF ACUTE MYELOID LEUKEMIA AT RELAPSE AFTER CHEMOTHERAPY AND ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION.

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Background: Acute Myeloid Leukemia (AML) is a clinically and biologically heterogeneous disease that is known to dynamically evolve over time. Unraveling its genetic profile may provide relevant insights into the inception, propagation, and recurrence of the disease, and deliver new rationales for precision medicine approaches: still, whereas a comprehensive description of AML mutations at disease presentation is now available thanks to large-scale studies, a satisfying genomic characterization of AML at relapse, particularly after allogeneic stem-cell transplantation (allo-HSCT), is still needed.
Aims: To characterize the genetic profile of relapsed AML, highlighting the evolutionary trajectories in the two different settings of relapse after chemotherapy (CT) and after allo-HSCT
Methods: For our custom-designed targeted Next Generation Sequencing panel we took advantage of the HaloPlex High Sensitivity (HS) technology, allowing a more precise definition of mutations and clonal architecture through a molecular barcoding system. We included in our panel 192 genes and miRNAs known to be involved in the pathogenesis of myeloid malignancies (n=112), in the DNA damage response (n=50), or in immune-related processes (n=30). Sequencing was performed on an Illumina HiSeq2500n instrument. Variant calling was performed using a pipeline based on the FreeBayes algorithm, and FLT3-ITD status was inferred using Pindel.
Results: We sequenced a total of 138 AML samples, including 79 diagnoses, 36 relapses after CT and 23 relapses after allo-HSCT. Sequencing yielded uniform and consistent coverage of all target amplicons and a 612x mean depth-of-sequencing, resulting on average in 117 unique barcodes for each region. Among the 79 diagnosis samples we identified 293 mutations (204 of which definable as oncogenic), with a median of 3 oncogenic mutations per patient (range 0-8), and mutation frequencies in line with the largest published dataset (Papaemmanuil, N Engl J Med, 2016; r^2=0.83). In relapses after CT and after allo-HSCT the median number of oncogenic mutations per patient was 3 (range 0-4) and 2 (range 0-6), respectively. Comparing mutation frequencies at relapse

with the Papaemmanuil dataset, we observed a weaker correlation for relapses after CT (r^2=0.69) and an even more marked deviation for post-transplant relapses (r^2=0.45). This difference was mainly explained by the enrichment in both relapse cohorts for FLT3-ITD (25% in diagnoses *vs* 55% and 48% at relapses after CT and allo-HSCT, p <0.01 for both comparisons) and WT1 mutations (5% *vs* 25% and 22%, p <0.01 for both comparisons). For 24 cases it was possible to longitudinally compare the mutational profile of AML at diagnosis and relapse in the same patient: we observed higher stability in relapses after CT, with 50% of cases carrying the same pattern of mutations present at diagnosis, whereas at relapses after allo-HSCT changes were more frequent, with 70% of patients displaying new gains or losses.

Summary/Conclusions: Taken together, our data evidence that the genomic landscape of AML at relapse can be significantly different from the one documented at diagnosis, suggesting that the selective pressure mediated not only by intensive chemotherapy, but also by the graft-versus-leukemia effect, can be potent drivers of clonal evolution. From the practical standpoint, the pattern of emergence of novel mutations that we documented should be taken into account not only for targeted salvage approaches, but also for the design of post-remission strategies aiming to prevent relapse.

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Abstract withdrawn.

Acute myeloid leukemia - Clinical 4

P546

AML PATIENTS AGED ≥75 YEARS ENROLLED INTO AMLCG TRIALS: DO GENETIC ALTERATIONS IMPACT CLINICAL OUTCOME IN VERY OLD, INTENSIVELY TREATED PATIENTS?

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Background: Acute myeloid leukemia (AML) is a disease of the elderly (median age at diagnosis ~68 years). The prognosis of elderly patients (pts) is poor. Advanced age often leads to the judgement that pts are unfit for induction chemotherapy, although several trials have revealed a positive impact of intensive induction therapy in terms of sustained remissions and long-term survival in a subset of elderly pts.

Aims: We sought to validate existing risk classification systems and identify genetic factors associated with clinical outcomes in very old AML pts who received induction chemotherapy.

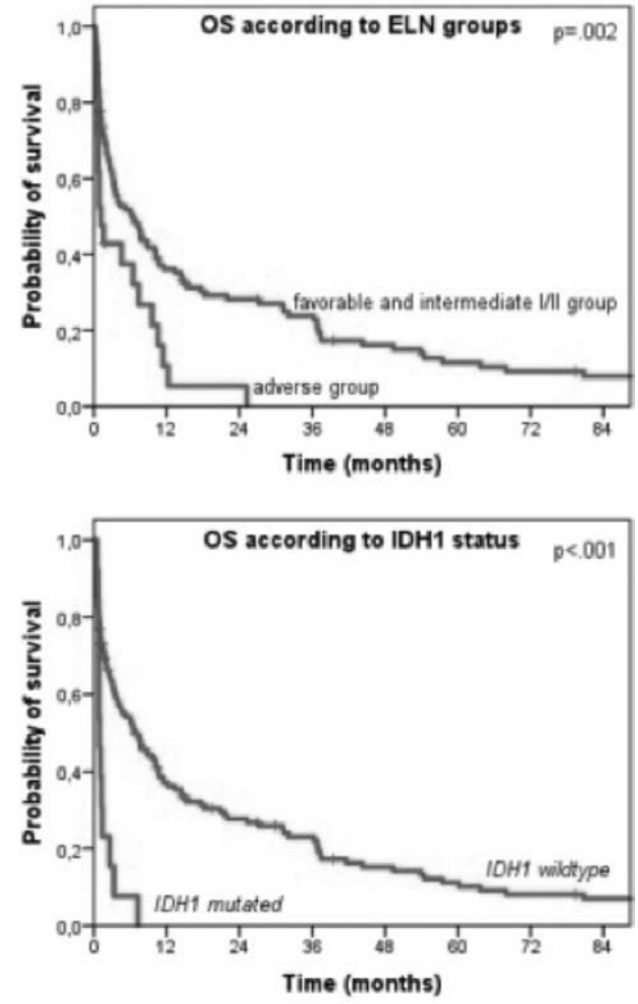

Figure 1.

Methods: We identified 151 AML pts aged ≥75 years who received intensive induction therapy in the AMLCG-1999 trial with suitable material for genetic

analyses. 81% of pts had *de novo* AML, 15% secondary AML, 3% therapy-related AML and 2% high-risk MDS. Recurrent gene mutations in AML were studied from bone marrow aspirates or peripheral blood using a targeted leukemia genotyping assay covering 68 genes. We analyzed known mutational hotspots or the entire coding sequence of the genes by multiplexed amplicon sequencing (Agilent Technologies, mean target coverage of 460 x). We studied associations between cytogenetics, gene mutations and other potential prognostic factors which might influence the clinical outcome.

Results: The median age in the total cohort was 76 years (y) (range: 75-86 y). 44% of pts reached complete remission (CR) and 4% CR with incomplete blood count recovery (CRi). The median overall survival (OS) was 6 months with a 3-year OS of 21%. According to the ELN 2010 classification, 20% of pts were in the favorable, 39% and 25% in the intermediate I or II group, respectively, and 15% in the adverse group (ELN 2017 data will be presented at the meeting). Pts in the favorable and intermediate I/II groups had significantly longer OS compared to the adverse group (median OS 6.3 *vs* 1.2 months, p=.002, Figure). Likewise, pts in the favorable and intermediate MRC cytogenetic risk categories had longer OS than those in the adverse category (median OS 6.5 *vs* 1.2 months, p=.001). By targeted sequencing, we detected 622 leukemia-associated mutations in 66 genes. The median number of mutated genes per patient was four. The most commonly mutated genes were *TET2* (42%), *DNMT3A* (35%), *NPM1* (32%), *SRSF2* (25%) and *ASXL1* (21%). Both *NPM1* or *EZH2* (5%) mutated pts showed a non-significant trend towards longer OS (*NPM1*: p=.09; *EZH2*: p=.065). *FLT3*-ITD mutations were identified in 29 pts (19%), but had no impact on OS (p=.297). The *NPM1*^{mutated}/*FLT3*-ITD^{negative} genotype also did not associate with OS. Notably, none of the 13 *IDH1* mutated pts (9%; all within the ELN favorable/intermediate groups) reached CR, and consequently the OS in this group was significantly shorter than for *IDH1* wild-type pts (p<.001; Figure). The positive impact of mutated *NPM1* on OS was reversed when it co-occurred with *IDH1* mutations (p=.014).

Summary/Conclusions: Among very old (≥75 y), intensively treated AML pts, adverse-risk cytogenetics predict inferior survival. On the other hand, 3-year OS was 24% for MRC/ELN favorable and intermediate-risk pts, suggesting that even in this age group, selected pts without medical contraindications benefit from intensive induction chemotherapy. The spectrum of driver gene mutations in elderly pts differs from that in younger pts. While *NPM1* and *FLT3*-ITD mutations had no significant impact on OS in intensively treated pts aged ≥75 y, our data imply *IDH1* mutations as a novel marker for chemorefractory disease and inferior prognosis in this age group.

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GMI-1271, A POTENT E-SELECTIN ANTAGONIST, IN COMBINATION WITH CHEMOTHERAPY IN RELAPSED/REFRACTORY AML: A NOVEL, WELL-TOLERATED REGIMEN WITH A HIGH REMISSION RATE

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Background: Expression of the adhesion molecule E-selectin (E-sel) in the vasculature of the bone marrow is associated with infiltrative disease, relapse, and poor survival in AML. GMI-1271 is a novel antagonist of E-sel that down-regulates cell survival pathways and enhances chemotherapy response with improved survival compared to chemotherapy alone (Becker ASH 2013; Winkler ASH 2014). Protection from common toxicities (neutropenia and mucositis) has been observed in preclinical models, also affording survival benefit (Winkler ASH 2013).

Aims: We assessed GMI-1271 plus salvage chemotherapy with mitoxantrone, etoposide, and cytarabine (MEC) for the treatment of patients (pts) with relapsed/refractory (R/R) AML.

Methods: A Phase (Ph) 1 trial in pts with R/R AML escalated GMI-1271 across pharmacologically active doses from 5-20mg/kg combined with MEC to evaluate safety, tolerability and anti-leukemia activity. MEC consisted of mitoxantrone 10mg/m^2/d, etoposide 100mg/m^2/d, and cytarabine 1000mg/m^2/d IV for 5 days; supportive care was given as per institutional guidelines. Dose limiting toxicity (DLT) was defined as either persistent neutropenia and/or thrombocytopenia beyond day 42 in the absence of disease or any Grade 3 (Gr3) non-hematologic toxicity that did not resolve to Gr2 by day 42. After confirming safety and tolerability, a Ph 2 study of GMI-1271 at 10mg/kg plus MEC salvage chemotherapy was initiated; responders to salvage could receive consolidation with GMI-1271 plus reduced dose MEC (4 days). GMI-1271 was given 24 hrs prior, then every 12 hrs during and for 48 hrs post induction/consolidation. Eligible pts had an ECOG score 0-2, received ≤2 prior inductions, had WBC <20K (<40K after 2 dose levels), no active CNS disease, and adequate renal/hepatic function. Baseline E-sel ligand expression on leukemic blasts in the bone marrow (CD45/SSC by flow) was assessed, as were plasma levels of soluble E-sel (sE-sel).

Results: To date, 47 pts have enrolled (Ph 1=19; Ph 2=28 of planned 47). The recommended Ph 2 dose is 10mg/kg based on drug exposure, time over IC50 for E-sel binding, evidence of on-target effect (reduction in sE-sel), lack of DLT at any dose level, and clinical outcomes. Ph1/Ph2 combined median age was 55yrs (range 26-84) with 70% male pts. Prior AML history included 26% primary refractory, 36% CR1<6 mos; 17% prior SCT; 52% unfavorable cytogenetics (by SWOG). Common Gr 3/4 AEs were febrile neutropenia (36%), sepsis (26%), bacteremia (13%), hypoxia (13%). Oral mucositis (≥Gr 2) developed in 12% of pts. The 30 and 60 day mortality rates were 0 and 7%, respectively; induction mortality was 0%. The remission rate (CR/CRi) was 45% (19/42) with an ORR (CR/CRi/MLFS/PR) of 50% (21/42). Observed/expected remission (CR/CRi) ratio was >2.75 (Estey, Blood 1996). With a median follow-up of 11 mos, the Ph 1 median Disease Free Survival was not reached and Overall Survival was 7.6 mos. The median E-sel ligand expression at baseline was 35% (range, 1-75%) of blasts in the bone marrow, and was higher in those achieving remission.

Summary/Conclusions: The addition of GMI-1271, a novel E-sel antagonist, to MEC chemotherapy is well tolerated; oral mucositis, commonly severe with MEC, is observed at low severity in this study. Clinical outcomes include a high ORR and remission rate (CR/CRi), low induction mortality, and promising survival outcomes in pts with R/R AML. Furthermore, the baseline expression of E-sel ligand in this population suggests relevance of the target and is predictive of response.

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BST 236, A NOVEL CYTARABINE PRO-DRUG ALLOW, FOR THE FIRST TIME, THE DELIVERY OF HIGH CYTARABINE DOSES FOR OLDER OR UNFIT PATIENTS WITH ACUTE LEUKEMIA. RESULTS OF AN ONGOING PHASE I/IIA STUDY

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Background: Acute myeloid leukemia (AML) is associated with poor outcome in older patients and in patients unfit for standard induction therapy. Therapy of AML has not changed significantly since the 1970s and still relies on cytarabine as the first-line treatment. However, cytarabine therapy is associated with severe side effects, such as cerebellar toxicity, bone marrow suppression, and infections, leading to high treatment-related mortality rates. Hence, while the incidence of AML increases with age, advanced age and comorbidities may preclude the administration of intensive therapy altogether.

Aims: BST-236 (Astarabine) is a new compound of cytarabine covalently bound to asparagine. It acts as a pro-drug of cytarabine, enabling delivery of high cytarabine doses to target cells with lower systemic exposure to the free drug and relative sparing of normal tissues. As such, BST-236 may serve as an ideal therapy for leukemia, particularly for delivering high doses of cytarabine to medically unfit or older adults. The aim of this study was to evaluate the safety and optimal dose of BST-236 in refractory/relapsed or newly-diagnosed AML patients unfit for standard induction therapy.

Methods: A Phase I/IIa prospective open label study enrolled adult relapsed/refractory or newly-diagnosed acute leukemia patients unfit for standard therapy. Patients are enrolled into 6 BST-236 escalating-dose cohorts (0.3-6 g/m^2/d), each composed of 3-6 patients. Treatment was administered as 1-hour daily infusion for 6 days.

Results: To date, treatment of cohorts 1-5 is completed, with 18 patients treated with up to 4.5 g/m^2/day, median age 77 (27-90): 6 relapsed/refractory AML patients, median age 64 (27-81), and 12 newly-diagnosed patients, unfit for standard chemotherapy (7 secondary AML; 5 *de novo* AML/ALL), median age 79 (70-90). BST-236 treatment was well-tolerated. Only 6 SAEs in 4 cases were assessed by the treating physician as possibly/probably related to BST-236, all "on-target" hematological toxicity events or bacterial infections derived from it. No neurological or grade >2 typical cytarabine events such as gastrointestinal, mucositis, or alopecia were reported during BST-236 treatment or within 30 days of follow up. Response to the treatment was observed in 6 of the 12 newly-diagnosed patients, 4 of whom had a continuous complete remission (CR) and 2 had a partial remission (PR). The median overall survival (OS) of the responding (CR+PR) and of the CR patients was 7 and 10 months, respectively. The median OS of the newly-diagnosed non-responders was 2.5 months. No remission was reached in the 6 patients suffering from relapse or refractory AML and their median OS was 2.3 months.

Summary/Conclusions: BST-236 is safe and very well tolerated, enabling delivery of high dose cytarabine to older and unfit patients, resulting in overall response and CR rates of 50% and 33%, respectively, and a 3-fold increase in median OS of the responding compared to the non-responding newly-diagnosed patients. Notably, 67% of the responding patients had secondary AML,

refractory to hypomethylating agents. To the best of our knowledge, this is the only experimental drug permitting high-dose cytarabine, considered a cornerstone of leukemia therapy, to be given to a population of patients that currently do not have this option. A phase II study is planned to confirm these encouraging results.

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FEASIBILITY AND BENEFIT OF TARGETED RNA SEQUENCING FOR THE DETECTION OF RECURRENT FUSION TRANSCRIPTS AND THE IDENTIFICATION OF NOVEL FUSION TRANSCRIPTS IN MYELOID MALIGNANCIES

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Background: Gene fusions are frequent genetic abnormalities in myeloid malignancies. The impact of the detection of such gene fusions is rising due to an increasing number of drugs targeting them as has been impressively shown for *e.g. BCR-ABL1* and *PML-RARA*. Further, they can be used as biomarkers for disease monitoring.
Aims: Evaluation of targeted RNA sequencing for the detection of recurrent and novel fusion transcripts.
Methods: 102 cases with myeloid malignancies harboring 105 translocations identified by chromosome banding analysis were selected. Recurrent fusion genes had been confirmed by FISH and/or RT-PCR. In cases with suspected novel fusions the rearrangement of one partner gene had been confirmed by FISH. The following recurrent rearrangements identified by standard diagnostic procedures were present: *PML-RARA* (n=11), *RUNX1-RUNX1T1* (n=7), *CBFB-MYH11* (n=3), *KMT2A-ELL* (n=4), *KMT2A-MLLT1* (n=4), *KMT2A-MLLT10* (n=3), *KMT2A-MLLT3* (n=3), *KMT2A-MLLT4* (n=2), *BCR-ABL1* (n=3), *NUP98-NSD1* (n=3), *DEK-NUP214* (n=1), and *KAT6A-CREBBP* (n=1). Further, cases harboring rearrangements involving *KMT2A* (n=14), *RUNX1* (n=21), *ETV6* (n=10), *PDGFRB* (n=10), *RARA* (n=2), *NPM*1 (n=2) and *NUP98* (n=1) were included. Targeted RNA sequencing was performed using the TruSight RNA Fusion panel (Illumina, San Diego, CA) consisting of 7690 probes covering 507 genes known to be involved in gene fusions. Library was prepared according to manufacturer's protocol with ~50ng RNA extracted from fresh/frozen samples. Sequencing was performed on the NextSeq instrument (Illumina) and analysis with the RNA-Seq Alignment App (BaseSpace Sequence Hub) using Star for Alignment and Manta for gene fusion calling with default parameters (Illumina).
Results: In 42/45 (93%) cases with a recurrent rearrangement identified by standard diagnostics, RNA sequencing detected the respective fusion transcript. In addition, RNA sequencing was able to identify known and novel fusions in the remaining 57 cases. For *KMT2A* these were the following partner genes: *MLLT1* (n=5), *ELL* (n=3), *ITPR2*, *FLNC*, *ASXL2, DCP1B, MAML1* and *ARHGEF12*. Seven different partner genes were identified in *RUNX1* translocated cases: *PLAG1* (n=2), *PRDM16*, *MECOM*, *ZFPM2*, *MAN1A2*, *N6AMT2*, and *KIAA1549L*. Five different partner genes were identified in *ETV6* rearranged cases: *ABL1*, *CCDC126*, *ERG*, *FOXO1* and *CFLAR-AS1*. Most strikingly was the identification of the *ETV6-ABL1* fusion, which could not be suspected by cytogenetics as the 5' *ETV6* FISH signal was located on chromosome 17. In 7/10 *PDGFRB* rearranged cases the partner genes were identified. These were *WDR48, CCDC88C, MPRIP, TNIP1, TPR, NFIA* and *ZBTB11*. Further the following fusions were found: *NPM1-RPP30, NPM1-SETBP1, NUP98-ING3, IRF2BP1-RARA,* and *ZBTB16-RARA*. Thus, RNA sequencing identified 39 fusions in 57 (68%) cases in which standard diagnostics had identified only one of the partner genes. Failure to detect gene fusions should initiate improvements in calling algorithms and may also have biological reasons. It was reported that genomic rearrangements of *RUNX1* occur, which do not lead to *RUNX1* in frame fusion transcripts but to termination of transcription.
Summary/Conclusions: 1) RNA sequencing was able to detect recurrent gene fusions with high accuracy and to characterize rare gene fusions providing the basis for the design of RT-PCR based assays for monitoring MRD. 2) Targeted RNA sequencing may be a valuable tool in routine diagnostics for patients with rearrangements unresolved by standard techniques. 3) These findings may have consequences for targeted treatment approaches.

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COMPREHENSIVE MOLECULAR ANALYSIS OF ADULT MIXED PHENOTYPE ACUTE LEUKEMIA (MPAL)

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Background: Mixed phenotype acute leukemia (MPAL) is a rare subgroup of acute leukemia characterized by blasts that show immunophenotypes of both myeloid and lymphoid lineages and therefore not traceable to single lineage of origin. Diagnosis of MPAL is challenging due to the possible discrepancy between immunophenotype and morphology. Clinically, MPAL has poor prognosis and poses therapeutic challenges. Genetic basis of MPAL is not well understood.
Aims: To clarify the underlying pathogenesis of MPAL and provide clue on future personalized therapy in MPAL, we performed comprehensive molecular characterization of adult MPAL.
Methods: We studied 31 patients with adult MPAL (median age 53) that met 2008 WHO classification criteria. Pre-treatment bone marrow samples were studied by targeted capture exome sequencing of 295 genes that are recurrently mutated in hematologic malignancies (median 393x coverage, N=31), RNA sequencing (N=24), and Infinium methylation EPIC array (Illumina, N=31). Mutational landscape was compared to that of 194 AML, 71 B-ALL, and 6 T-ALL cases of which pre-treatment samples were sequenced internally with the same platform. Promoter CpG methylation pattern was compared to the data from 194 AML (data derived from The Cancer Genome Atlast Project), 505 B-ALL and 101 T-ALL cases (data shared by Nordlund *et al.* Genome Biology. 2013). Copy number variation was inferred from methylation array data.
Results: Among 31 MPAL cases, 18 (58%) had myeloid-T and 13 (42%) had myeloid-B phenotype. Four cases had Philadelphia chromosome, 1 had 11q23 abnormality, and 8 had complex karyotype. MPAL had similar numbers of mutations (median 2 [range: 0-6]) with AML (median 3 [range: 0-7], P=0.79) or T-ALL (median 3 [range: 1-4], P=0.92) but had significantly higher number of mutations than B-ALL (median 0 [range: 0-4], P<0.001). Consistent with the mixed immunophenotypic features, MPAL had both AML-type and ALL-type mutations. However, *NPM1* mutation was specific to AML and was not found in MPAL cases. Myeloid-T and myeloid-B showed distinct patterns of somatic mutations. Genes in which mutations were enriched in myeloid-T than in myeloid-B included *DNMT3A* (33% *vs* 8%), *IDH2* (33% *vs* 8%), *NOTCH1* (39% *vs* 0%), *IL7R* (17% *vs* 0%), and *FBXW7* (6% *vs* 0%). Genes in which mutations were less frequently observed in myeloid-T than in myeloid-B included *RUNX1* (6% *vs* 46%), *ASXL1* (0% *vs* 23%), *TET2* (0% *vs* 15%), *SRSF2* (6% *vs* 23%), and *FLT3* (11% *vs* 23%). Myeloid-T and myeloid-B showed distinct patterns of promoter CpG methylation. Overall, myeloid-T had more hypermethylated CpG loci than myeloid-B in all different CpG locations (island, shore, shelf, and others). Genes that are essential in T-cell receptor (TCR) signaling (*CD3D*, *CD7*, *CD247*, *LCK*, *PRKCQ, CCR9, and TCL1A*) were differentially methylated and consequently differentially expressed between myeloid-T and myeloid-B. Copy number analysis showed that *DLL1*, one of the essential NOTCH pathway genes, was amplified and was overexpressed. RNA sequencing revealed several known translocations such as *NSD1-NUP98* and *KMT2A-MLLT4,* in addition to the novel translocations such as *FOXP1-DNAJC15*, *FLI1-IFT46*, and *ITPR2-ARID5B*. Unsupervised hierarchical clustering of all MPAL, AML, B-ALL and T-ALL by promoter CpG methylation pattern revealed that myeloid-T consistently showed similar methylation pattern with T-ALL, while myeloid-B showed random similarity with either B-ALL or AML.
Summary/Conclusions: MPAL is genetically heterogeneous disease and myeloid-T and myeloid-B shows distinct patterns of mutation landscapes, methylation and gene expressions. Therapy for MPAL may need to be personalized based on genomic profiles.

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THE EFFECTS OF EARLY INTENSIFIED INDUCTION CHEMOTHERAPY IN ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA COMPARED TO STANDARD ANTHRACYCLINE PLUS CYTARABINE 3+7 CHEMOTHERAPY

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Background: Standard remission induction chemotherapy for adult acute myeloid leukemia (AML) which consists of anthracycline for 3 days plus cytarabine for 7 days was first introduced in 1970's and has been used for a long time. Several modification or intensification for this conventional regimen did not prove the effect for higher complete remission (CR) rate or lower relapse rate which led to superior overall survival (OS) rate.
Aims: We tried to find out possible benefit of early intensification of standard induction chemotherapy in adult AML patients.
Methods: This single center retrospective study enrolled 1195 adult AML patients from 2002 to 2013. All patients were initially treated with idarubicin (12mg/m^2) plus cytarabine (100mg/m^2) or BHAC (300mg/m^2) induction chemotherapy (3+7), and among them, 731 (61.2%) patients received additional early augmentation using cytarabine 3 days (3+10, n=363) or anthracycline 2 days plus cytarabine 3 days (5+10, n=368). The decision for augmentation was based on the follow-up BM blast counts on the 7th day of 3+7 chemotherapy; totally 3+10 for blast counts 5~20% and 5+10 for blast counts >20% (early intensified group). The rest 464 with blast counts < 5% finished with 3+7 regi-

men (standard group). Re-induction and consolidation therapy was performed according to a consistent strategy and post-remission therapy was mainly based on hematopoietic cell transplantation.

Results: Early intensified group was consisted of younger patients (median age, 37 years old [range 17-69] *vs* 45 years in 3+7 *vs* 43 years in 3+10 subgroup) and larger proportion of t(8;21) (n=102 [27.7%] *vs* 3+7 [n=33, 7.1%] *vs* 3+10 [n=47, 12.9%], *P*<0.001). Also, initial BM blast counts were higher in two intensified groups (73.3% in 5+10 and 70.1% in 3+10) compared to 3+7 subgroup (66.8%, *P*<0.001). Early death rate at 8 weeks was higher in patients older than 55 years (10.8% *vs* 3.7%, *P*<0.001) especially when they were treated with intensified chemotherapy (21.7% in 5+10 and 15.7% in 3+10 *vs* 6.3% in 3+7, *P*=0.038). CR rate after induction was higher in young patients especially in 3+10 subgroup (79.8%, *P*<0.001) and we also found that patients with favorable to intermediate-risk karyotype might benefit with intensified chemotherapy in the context of CR rate (79.7% *vs* 68.3% *P*<0.001), although final CR rates became similar after re-induction. Next, we found that pre-HCT relapse rate was lower in patients younger than 55 years (9.4% *vs* 18.0%, *P*=0.002) and favorable to intermediate-risk group (8.9% *vs* 20.2%, *P*<0.001) after intensified induction. In young patients with favorable to intermediate-risk karyotype, intensified group showed superior 5-year OS (55.0% *vs* 45.5%, *P*=0.010) and lower long-term relapse rate (32.2% *vs* 39.0%, *P*=0.084), but multivariate analysis revealed no effects for both OS and CIR. In patients older than 55 years, intensified group showed inferior 5-year OS (19.2% *vs* 22.8%, *P*=0.014) with higher early death rate (17.6% *vs* 6.3%, *P*=0.015), and multivariate analysis also showed intensified induction was related inferior OS (HR=1.89, 95%CI; 1.14-3.15, *P*=0.013).

Table 1.

Table 2. Treatment outcomes according to the induction treatments.

Total n=1195	IDA/ARA 3/7 (n=464)	IDA/ARA 3/10 (n=363)	IDA/ARA 5/10 (n=368)	P
Post-induction early death† (n=57, 4.8%)	**21 (4.5%)**	**15 (4.1%)**	**21 (5.7%)**	0.578
≤ 55 years	14/352 (4.0%)	7/312 (2.2%)	16/345 (4.6%)	0.246
> 55 years	7/112 (6.3%)	8/51 (15.7%)	5/23 (21.7%)	0.038*
Favorable-risk	4/75 (5.3%)	2/74 (2.7%)	5/108 (4.6%)	0.710
Intermediate-risk	10/266 (3.8%)	7/204 (3.4%)	8/172 (4.7%)	0.822
Adverse-risk	6/112 (5.4%)	6/81 (7.4%)	8/85 (9.4%)	0.549
CR after induction CTx. (n=849, 71.0%)	299 (64.4%)	278 (76.6%)	272 (73.9%)	< 0.001*
≤ 55 years	**229/352 (65.1%)**	**249/312 (79.8%)**	**250/345 (75.1%)**	**< 0.001***
> 55 years	70/112 (62.5%)	29/51 (56.9%)	13/23 (56.5%)	0.736
Favorable-risk	64/75 (85.3%)	70/74 (94.6%)	100/108 (92.6%)	0.107
Intermediate-risk	169/266 (63.5%)	154/204 (75.5%)	121/172 (70.3%)	0.019*
Adverse-risk	60/112 (53.6%)	52/81 (64.2%)	51/85 (60.0%)	0.319
CR within two cycles of CTx. (n=950, 79.5%)	**356 (76.7%)**	**299 (82.4%)**	**295 (80.2%)**	0.235
≤ 55 years	277/352 (78.7%)	266/312 (85.3%)	280/345 (81.2%)	0.144
> 55 years	79/112 (70.5%)	33/51 (64.7%)	15/23 (65.2%)	0.536
Favorable-risk	70/75 (93.3%)	71/74 (95.9%)	102/108 (94.4%)	0.780
Intermediate-risk	201/266 (75.6%)	167/204 (81.9%)	134/172 (77.9%)	0.534
Adverse-risk	79/112 (70.5%)	58/81 (71.6%)	59/85 (68.2%)	0.206
Relapse after CR (before HCT) (n=148, 15.6%)	79/356 (22.2%)	36/299 (12.0%)	34/295 (11.5%)	<0.001*
≤ 55 years	**51/277 (18.4%)**	**29/266 (10.9%)**	**31/280 (11.1%)**	**0.009***
> 55 years	28/79 (35.4%)	7/33 (21.2%)	3/15 (20.0%)	0.218
Favorable-risk	11/70 (15.7%)	1/71 (1.4%)	5/102 (4.9%)	0.003*
Intermediate-risk	41/201 (20.4%)	20/167 (12.0%)	16/134 (11.9%)	0.037*
Adverse-risk	23/79 (29.1%)	14/58 (24.1%)	13/68 (22.4%)	0.502

† Early death due to any cause (with or without aplasia) within 56 days after chemotherapy
†† Overall response rate (ORR, complete remission (CR)+ CR with incomplete recovery (CRi))
CR - PB, ANC > 1,000 (10^9/L), platelet > 100,000 (10^9/L), BM, blast < 5%. No extramedullary disease.
CRi - incomplete CR with persistence of cytopenia
1,2,3 Different numbers indicate significant difference between groups based on Tukey's multiple comparison test.
Abbreviation: Allo, allogeneic; Auto, autologous; PB, Peripheral blood; BM, Bone marrow; CTx ,chemotherapy; HCT, hematopoietic cell transplantation; NA, Not assessed

Summary/Conclusions: Our data revealed that intensified induction chemotherapy was not influential for poor-risk karyotype, while higher post-induction CR rate and low pre-HCT relapse was shown in young patients with favorable to intermediate-risk karyotype although it was not influential for final OS and CIR rate. In elderly patients, intensified induction chemotherapy was related with higher early death rate which finally showed poor OS.

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VARIANT FLT3 MUTATIONS CAN BE ERADICATED BY CYTARABINE/ANTHRACYCLINE/CRENOLANIB INDUCTION IN ADULT PATIENTS WITH NEWLY DIAGNOSED FLT3 (ITD/TKD) MUTANT AML

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Background: Patients (pts) with FLT3-internal tandem duplication (ITD) and FLT3-D835 mutant AML have a high relapse rate. These relapses are typically due to outgrowth of mutant FLT clones. Previously available PCR-based tests only checked for presence of FLT3-ITD and FLT3-D835/I836 mutations. Whole genome sequencing of 799 pediatric AML samples from COG trials have shown novel FLT3 variants in not only the tyrosine kinase domain but also the juxtamembrane (JM) and transmembrane domains in 7.6% of these samples (Tarlock *et al.* ASH 2015). Some of these mutations result in autophosphorylation of FLT3 and therefore may be oncogenic.

Aims: Identify novel FLT3 mutations in pts with FLT3 mutant AML and further investigate whether these novel clones are sensitive to induction chemotherapy plus a potent pan-FLT3 inhibitor, crenolanib.

Methods: Pts with newly diagnosed FLT3 mutant AML were enrolled and treated with cytarabine/anthracycline/crenolanib induction followed by high dose cytarabine (HiDAC) consolidation. Crenolanib 100mg TID was started on day 9 of induction and continued till next chemotherapy. Crenolanib was given following consolidation and allogeneic stem cell transplantation. Bone marrow samples were collected at baseline and at the time of remission assessment. Sequencing of the entire FLT3 gene was performed through FoundationOne Heme panel (n=18) and MSKCC multigene panel (n=5). Sequencing of exons 14,15,16, and 20 was performed through the Rapid Heme Panel at Dana-Farber Cancer Institution in additional 6 pts.

Results: Out of 29 newly diagnosed FLT3 mutant AML patients with full/partial FLT3 gene sequencing performed, 4 pts were found to have novel variant FLT3 mutations consisting of V491L, V592L, D593H, A680V, and N841I/T/K (Table 1). The majority of these novel mutations were located at the JM, kinase domain 1 and the activation loop (kinase domain 2). The allele fractions of these FLT3 variants ranged as high as 29% (higher than that of FLT3-ITD in pt3), suggesting that some of these clones may have been potentially driving clinical leukemia progression in some pts. All 4 pts had NPM1 mutations, and two also had DNMT3A mutations. All 4 pts achieved CR with full count recovery (3/4 pts achieved CR after just one cycle of cytarabine/anthracycline/crenolanib induction). The pt with FLT3-D835Yand N841T achieved a CR after cytarabine/anthracycline/crenolanib induction and one cycle of HiDAC consolidation. All pts became FLT3-ve and have remained FLT3-ve. 3 out of 4 pts received 1-4 cycles of HiDAC consolidation followed by crenolanib maintenance. Only one pt underwent allo SCT. With a median follow up of 13 months, one pt relapsed (at 8.4-month following treatment). This 61F pt was found to have FLT3-ITD, D593H and I836del FLT3 abnormalities at the time of diagnosis. A full FoundationOne gene panel done at the time of relapse, showed no residual FLT3 mutant clones.

Table 1.

Table 1: Novel FLT3 mutations in adult AML patients

Pt	Age/ Sex	FLT3 Mutations	Variant FLT3 Mutations	Other Mutations	Response after Induction 1	FLT3 Status after Treatment	Current Status
1	54/F	ITD	V491L (3%), V592A (1%), I836del (18%), N841I (8%)	NPM1, IDH2, SRSF2	CR	Negative	alive, in remission for ~12 months
2	61/F	ITD 9% (3 clones)*	D593H (9%), I836 del (3%)	NPM1, WT1	CR	Negative	alive, relapsed with non-FLT3 clones (*N-RAS* and *JAK3*)
3	54/F	ITD 13.5%	A680V (29%), N841K (16.2%)	NPM1, DNMT3A, CEBPA	CR	Negative (ITD)	alive, in remission for ~10.5 months
4	36/F	D835Y^	N841T (in trans with D835Y)	NPM1, DNMT3A, PTPN11	PR	Negative	alive, in remission for ~7.7 months

*99 bp insertion: 0.05, 60bp insertion: 0.03, 30 bp insertion: 0.01
^TKD 49%

Summary/Conclusions: This abstract reports multiple novel variant FLT3 mutations in adult pts with newly diagnosed FLT3-ITD or FLT3-D835 mutant AML. The allelic burden of these FLT3 variant mutations can sometime be higher than that of FLT3-ITD. Detailed FLT3 analyses in this subset of pts suggests that crenolanib in combination with standard induction chemotherapy has the ability to eradicate variant FLT3 clones. All 4 pts treated with chemotherapy followed by crenolanib showed clearance of FLT3-ITD, TKD, as well as other novel variants. To achieve maximal clinical benefit, a potent pan-FLT3 inhibitor with the ability to inhibit ITD, D835, as well as other activating mutations maybe beneficial.

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PATIENTS WITH ACUTE MYELOID LEUKEMIA WHO HAVE MUTATIONS IN IDH1 OR IDH2 RESPOND WELL TO INDUCTION CHEMOTHERAPY WITH "7+3" DESPITE THE PRESENCE OF COMPLEX KARYOTYPE OR FLT3-ITD

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Background: Mutations in isocitrate dehydrogenase isoforms 1 and 2 (IDH1/IDH2) occur in 8-12% of patients with acute myeloid leukemia (AML). Mutant IDH enzymes catalyze the conversion of alpha ketoglutarate to beta hydroxyglutarate. Increased concentrations of intracellular 2-HG lead to histone hypermethylation and a block in cellular differentiation and may also lead to suppression of homologous recombination. Previous studies of outcomes in patients given induction chemotherapy are limited by the heterogeneity of induction regimens. In this study, we investigated the outcomes of patients given induction chemotherapy with daunorubicin and cytarabine (7+3), the most common regimen used in the United States.

Aims: To delineate the complete remission rate in AML patients with IDH1 or IDH2 mutations who receive standard 7+3 induction chemotherapy.
Methods: After receipt of IRB approval, an institutional database of genomic abnormalities in all patients with AML was queried for patients with IDH1 or IDH2 mutations between the years of 2010 and 2016. Pathology records of patients identified as having an IDH1/IDH2 mutation were reviewed to confirm the presence of an IDH mutation. After confirmation of IDH mutational status, all patients who received standard induction chemotherapy with 7+3 were included in this retrospective chart review.
Results: Between 2010 and 2016, 82 patients with *IDH1/IDH2* mutations who had been treated with "7+3" induction chemotherapy were seen at MSKCC. Of these, 33 (40.2%)had *IDH1* mutations and 49 (59.8%) had *IDH2* mutations. Of those with IDH2 mutations *IDH2 R140Q* mutations were present in 34 (69.3%)and *IDH2 R172K* mutations were present in 15 (30.6%). The median age of all patients treated was 63. 56 patients (68%) had *de novo* AML, 16 (20%) had AML with myelodysplasia related changes, 5(6%) had a a known prior history of MDS and 5 (6%) had therapy related AML. Nearly half of the patients (49%) had karyotypic abnormalities. Of the 82 patients who received induction chemotherapy with "7+3", 51 achieved a complete remission (CR) after 1 cycle and 16 after 2 cycles for a CR rate of 82%. The strongest predictor of response to induction chemotherapy was the presence of an NPM1 mutation. There was a trend towards decreased response to induction chemotherapy in patients with a complex karyotype (p=.079) that did not reach statistical significance. The presence of an IDH2 R172K mutation was predictive of non-response to one cycle of (7+3) of 7+3 but when two cycles of induction chemotherapy were given, response rates were equivalent to patients with R140Q mutations. Co-occurring mutations in FLT3 (ITD or TKD), DNMT3A or NRAS were not predictive of responses to induction chemotherapy.
Summary/Conclusions: Induction chemotherapy with 7+3 leads to a robust CR rate of 82% in patients with AML that harbor and IDH1 or IDH2 mutation. CR is not affected by IDH2 isoform (R172 or R140), although more patients with R172 mutations required two cycles of chemotherapy to achieve a remission. Karyotypic abnormalities did not influence the response to induction chemotherapy, nor did the presence of co-occurring FLT3-ITD, FLT3-TKD or NRAS mutations. For AML patients with IDH mutations who are eligible for induction chemotherapy "7+3" is a reasonable induction regimen regardless of the presence of FLT3 mutations, or karyotypic abnormalities.

Acute myeloid leukemia - Clinical 5

P554

VALIDATION OF PRECISION MEDICINE TEST FOR ACUTE MYELOID LEUKEMIA IN AN OBSERVATIONAL CLINICAL TRIAL

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Background: Treatment of Acute Myeloid Leukemia (AML) is limited to few different treatments in each clinical trial group guideline, but integrating current and previous guidelines, and clinical trial publications, there are up to 45 drug combination treatments among approved chemotherapy drugs in Europe and USA. There is a need for Precision Medicine (PM) tests to identify which of these different treatments maybe optimal for each individual patient, independently of where he/she lives.
Aims: To provide actionable data to improve disease management with existing treatments with a PM test to guide the hematologist among all possible treatments to achieve a CR.
Methods: AML bone marrow (BM) samples from adult patients were received at the laboratory within 24 hours from extraction and incubated for 48h in 96-well plates containing single drugs or combinations representing up to 45 different treatments that are currently given in the clinical practice. The analysis is performed in the automated flow cytometry PharmaFlow platform. 72 hours after the extraction of the sample, an encrypted report is sent to the hematologist before the patient begins treatment. Pharmacological responses were calculated using pharmacokinetic population models. Induction response was assessed according to the Cheson criteria (2003). Patients attaining a CR/CRi were classified as responders and the remaining as resistant, excluding early deaths. Final scores and treatments ranking is based on a therapeutic algorithm that integrates *ex vivo* activity; monotherapy dose responses quantified by the area under the curve (AUC) with limits such as Cmax values, and synergism calculated measuring 8 concentration ratios requiring consistency in their results in a 3D surface (so called alpha factor synergism). The PM Test attempts to identify at least one treatment, among all evaluated alternatives, predicted sensitive for each patient; conversely, if sensitive treatments can be identified the PM Test can provide the hematologist with valuable guidelines for individualized treatment.
Results: (Figure 1) The scoring method was tested using *ex vivo* results from samples obtained in an observational clinical trial with Spain´s PETHEMA group from a cohort of 123 samples from *de novo* diagnosed AML patients, treated with the standard PETHEMA 1st line guideline 3+7 with CYT+IDA. The score predicts sensitive patients with 90% accuracy. This accuracy can be compared with an independently derived 92% accuracy in identifying sensitive patients in a statistically significant clinical correlation study (EHA Poster 2016 Montesinos *et al.*). The score is a simplified version of such correlation algorithm. Both methods identify a similar % of all clinically sensitive patients (67% *vs* 71%).

However, the correlation is only valid for CYT-IDA while the PM Test is applied to up to 45 treatments. Any such treatment identified as sensitive means the PM Test can provides a valuable guideline to hematologists. This means the PM Test can suggest sensitive treatments for the vast majority of patients.

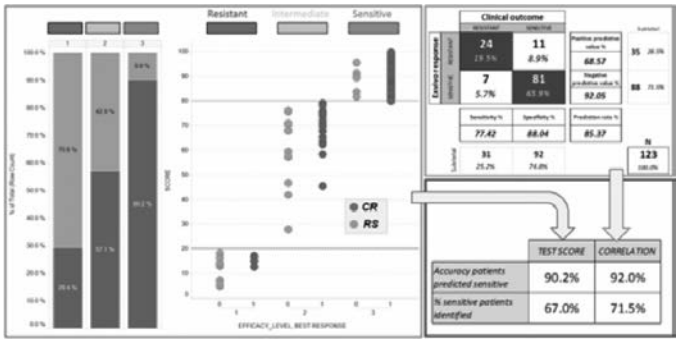

Figure 1.

Summary/Conclusions: We have developed a novel *ex vivo* PM test for induction treatment in AML patients to guide hematologists selecting the right treatment to achieve CR in individual patients leveraging up to 45 different validated chemotherapeutic regimes. Assuming a similar response rate for all these treatments, our test could estimate a net prediction for sensibility to AML treatment higher than 80% in 1st line. This PM Test will be evaluated in an interventional clinical trial on relapse/refractory patients that is expected to begin in the next few months in collaboration with the PETHEMA group from Spain.

P555

RESPONSE-ADAPTED AZACITIDINE AND INDUCTION CHEMOTHERAPY IN PATIENTS >60 YEARS OLD WITH NEWLY DIAGNOSED AML ELIGIBLE FOR CHEMOTHERAPY: RESULTS OF THE DRKS00004519 STUDY OF THE EAST GERMAN STUDY GROUP

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Background: AML treatment in elderly patients (pts) >60 years (y) with intensive chemotherapy (IC) or azacitidine (AZA) are not necessarily mutually exclusive. **Aims:** Results of the multicenter DRKS00004519 (RAS-AZIC) study of the East German Study Group (OSHO) which evaluated first-line treatment with AZA followed by response-based AZA or IC in pts >60y with AML are presented.

Methods: pts >60y with newly diagnosed AML (n=112) were included. Recruitment was completed in May, 2016. In the phase I part, safety of upfront AZA (75mg/m^2/day s.c) for 7 days followed by IC (mitoxantrone 10mg/m^2/day on day (d) 1-3 and cytarabine 1g/m^2/BID on d 1, 3, 5, 7) on d17 was established through a 3+3 design. In the multicenter phase II part (figure), upfront AZA was sequentially followed by AZA or IC based on d15 bone marrow (BM) blasts (<45 *vs* ≥45%) and CR/CRi on d56 which were both previously identified as early predictors for long-term response to AZA in AML (Al-Ali *et al. Leuk Lymph* 2012). The primary endpoint was response (OR) [CR/CRi, and PR] at d90 according to the International Working Group criteria. Based on the optimal two-stage design (Simon. *Control Clin Trials* 1989), protocol treatment was non-inferior to standard IC if, on an intention-to-treat basis, an OR of 61% was reached. Adverse events (AEs) were reported according to the NCI CTCAE 4.03. All pts gave written informed consent.

Results: Median age was 70y (52% males). *de novo* AML was present in 65% of pts. Median BM blasts and WBC were 50% and 4.4x10^9/L respectively. Genetic risk was high in 30%, intermediate in 55%, and favorable in 15%. *FLT3* and *NPM1* were mutated in 12% and 22% respectively. All pts received first-line AZA. Only lower baseline blasts correlated with blasts <45% on d15 (*p<0.0005*). Yet, 40% of pts with baseline blasts >50% reached this goal. Protocol assigned treatment on d15 was applied to 101 (90.2%) pts (54.5% continued with AZA; 46.5% received IC). Of 152 AZA cycles given till d56, 33.6% were applied in an outpatient setting. Until d90, one IC cycle was needed in 77 (68.8%) pts. In the intention-to-treat cohort (n=112), OR and mortality at d90 were 62.5% [CR/CRi (n=43%/15%); PR (4.5%)] and 8.9% respectively. The probabilities of achieving CR/CRi with AZA alone, two AZA cycles + one IC, and one AZA cycle + one IC were 28.3%; 53.3%, and 58.3% respectively. Age, WBC, and type of AML had no impact on response in the three treatment scenarios. Similarly, response was not influenced by baseline BM blasts. CR/CRi was lower in high risk genetics (48%) compared to other risk categories (78%) (*p=0.007*). This negative association was particularly marked in pts with high-risk genetics and d15 BM blasts >45% [CR/CRi 38.5% *vs* 84% in other genetic categories (p=0.009)]. Interestingly, the impact of genetics on OR was not seen in the two AZA cycles + one IC cohort (p=1.0). CR with AZA alone was remarkably high (70%) in pts with favorable genetics including those with *NPM1mut*/*FLT3wt* (*p=0.003*). Protocol therapy was generally well tolerated. Constipation grade 1+2 was the most frequently reported AE under AZA (48%). The most frequent grade 3+4 non-hematologic AE was infection [IC (47%); AZA (20%)].

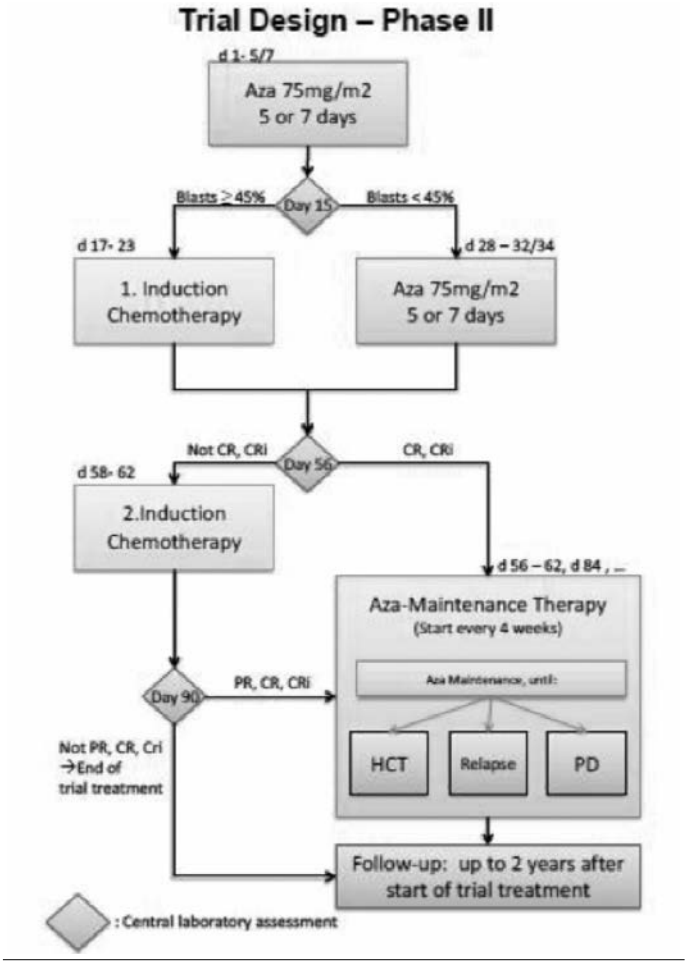

Figure 1.

Summary/Conclusions: Sequential response-based epigenetic and chemotherapy in elderly pts with AML is safe, associated with low mortality, and yields non-inferior responses compared to repeated cycles of IC. Marrow blasts d15 after the first AZA cycle and genetics could guide treatment-decision. The follow-up of this trial will scrutinize the impact of this approach on survival.

P556

OVERALL SURVIVAL WITH CPX-351 *VERSUS* 7+3 IN OLDER ADULTS WITH NEWLY DIAGNOSED, THERAPY-RELATED ACUTE MYELOID LEUKEMIA: SUBGROUP ANALYSIS OF A PHASE 3 STUDY

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Background: Therapy-related acute myeloid leukemia (tAML) may occur as

a late complication of cytotoxic or radiation therapy and is associated with a poor prognosis. CPX-351 is a liposomal formulation that delivers a synergistic 5:1 molar ratio of cytarabine and daunorubicin. In a randomized, open-label, controlled phase 3 trial in patients aged 60 to 75 years with newly diagnosed, secondary AML (eg, tAML or AML after myelodysplastic syndrome), CPX-351 significantly improved overall survival (OS) *versus* cytarabine/daunorubicin (7+3).

Aims: The current analysis of this phase 3 study evaluated outcomes in the subgroup of patients with tAML.

Methods: Enrolled patients were randomized 1:1 to receive induction with 1 to 2 cycles of CPX-351 (100 units/m^2 [cytarabine 100mg/m^2 + daunorubicin 44mg/m^2] on Days 1, 3, and 5 [2nd induction: Days 1 and 3 only]) or 7+3 (cytarabine 100mg/m^2/day x 7 days [2nd induction: x 5 days] + daunorubicin 60mg/m^2 on Days 1, 2, and 3 [2nd induction: Days 1 and 2 only]). Patients who achieved complete remission (CR) or CR with incomplete platelet or neutrophil recovery (CRi) could receive up to 2 cycles of consolidation therapy. Note, the study was not powered for this subgroup analysis.

Results: A total of 304 patients were enrolled and received study treatment, including 62 (20%) patients with tAML (CPX-351 arm, n=30; 7+3 arm, n=32). Characteristics of tAML patients were similar between the CPX-351 and 7+3 arms: median age was 69.0 *versus* 67.5 years, and 47% *versus* 53% were male. Prior treatment in patients with tAML included prior non-anthracycline chemotherapy alone (26%), radiation alone (26%), non-anthracycline chemotherapy + radiation (32%), non-anthracycline + anthracycline chemotherapy (5%), and non-anthracycline + anthracycline chemotherapy + radiation (11%). CPX-351 was associated with a significant OS benefit *versus* 7+3 in older tAML patients and numerically longer event-free survival and remission duration (Figure). Additionally, a greater proportion of tAML patients in the CPX-351 arm *versus* the 7+3 arm achieved CR+CRi (47% *vs* 36%, respectively; odds ratio=1.33 [95% CI: 0.47, 3.81]) and proceeded to stem cell transplantation (37% *vs* 27%; odds ratio=1.54 [95% CI: 0.53, 4.49). Serious treatment-emergent adverse events (TEAEs) were reported for 18/30 (60%) of tAML patients in the CPX-351 arm and 12/32 (38%) of tAML patients in the 7+3 arm; the observed difference in serious TEAEs in this subpopulation appeared to primarily be due to the incidence of febrile neutropenia (n=6/30 [20%] *vs* n=0/32 [0%]). Three (10%) patients in the CPX-351 arm and 5 (16%) patients in the 7+3 arm experienced a TEAE that resulted in death during the treatment period; there was no pattern in the individual TEAEs that led to death.

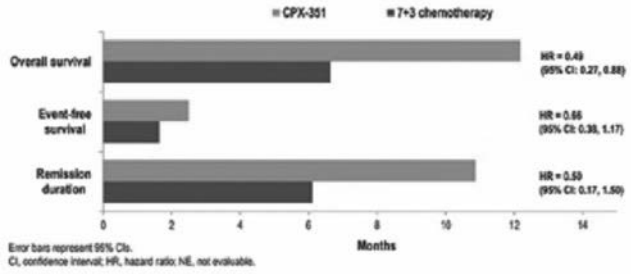

Figure 1.

Summary/Conclusions: CPX-351 is associated with improved efficacy and a safety profile comparable to 7+3 in older patients with newly diagnosed tAML. Outcomes in the tAML subgroup mirrored the overall study population, indicating CPX-351 may represent a new therapeutic option for this difficult to treat population.

P557

HYPERFERRITINEMIA IS AN INDEPENDENT POOR PROGNOSTIC FACTOR IN ACUTE MYELOID LEUKEMIA

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Background: The prognostic impact of ferritinemia has been studied in myelodysplastic syndromes and acute myeloid leukemia (AML) patients undergoing allogeneic stem cell transplantation (SCT). In this context, high levels of serum ferritin have been correlated to a shorter overall survival (OS) and an increased relapse risk.. We have previously shown that hyperferritinemia at diagnosis has a strong prognostic impact in a cohort of 162 AML patients with intermediate cytogenetic risk and younger than 60.

Aims: We now extend the analysis to all age and cytogenetic risk, in order to confirm the impact of hyperferritinemia in AML.

Methods: This study included 525 adult AML patients (excluding acute promyelocytic leukemia) treated by intensive chemotherapy in Toulouse and Lyon University Hospitals between January 1st, 2005 and December 31st, 2014 who had ferritinemia documented at AML diagnosis. Ferritin level was measured by spectrophotometry. Primary outcome was disease-free survival (DFS). To avoid the loss of information and the reduction in power introduced by the categorization of ferritinemia and to deal with the non-linearity in the relationship between outcomes and ferritinemia, we explored the relationship between ferritinemia and outcomes using restricted cubic spline.

Results: Median age at diagnosis was 59.4 years (interquartile range [IQR], 47.8-66.4); 303 of them (57.7%) were men. Disease status was *de novo* in 83.2% (N=437). Median white blood cell count (WBC) was 10.0x10^9/L (IQR, 2.5-41.5). Cytogenetic risk was favorable, intermediate and adverse in 9.2 (N=48), 71.8 (N=374) and 19% (N=99) respectively; ELN classification was favorable, intermediate-I, intermediate-II, adverse and unknown in 21.0 (N=110), 25.5 (N=134), 22.3 (N=117), 18.9 (N=99) and 12.4% (N=65) respectively. Median ferritinemia at AML diagnosis was 715 µg/L (IQR, 372-1304), ranging from 34µg/L to 70759 µg/L (upper normal limit [UNL]: 300µg/l). 421 patients achieved complete remission (CR; 80.2%). Early death and treatment failure rates were 7.8% (N=41) and 12% (N=63) respectively. 169 patients underwent allogeneic HSCT in first CR (32.2%). Median DFS was 19.8 months (IQR, 8.4-Not Reached). Ferritinemia had a significant impact on DFS: median DFS was 21.2 months in patients with ferritinemia ≤2100 µg/L (7-fold UNL), and 12.7 months with ferritinemia >2100 µg/L (HR, 1.6 [95%CI, 1.1-2.3], p=0.0253). After adjustment for age, AML status and cytogenetics or ELN classification, relapse or death rate significantly (p=0.0122) increased from ferritinemia superior or equal to 2141 µg/L (Figure 1). Ferritinemia had also a significant impact on early deaths, CR rate, EFS and OS after adjustment (≥4-fold UNL, p<0.0001; ≥7-fold UNL, p=0.004; ≥3-fold UNL, p<0.0001 and ≥3-fold UNL, p<0.0001 respectively).

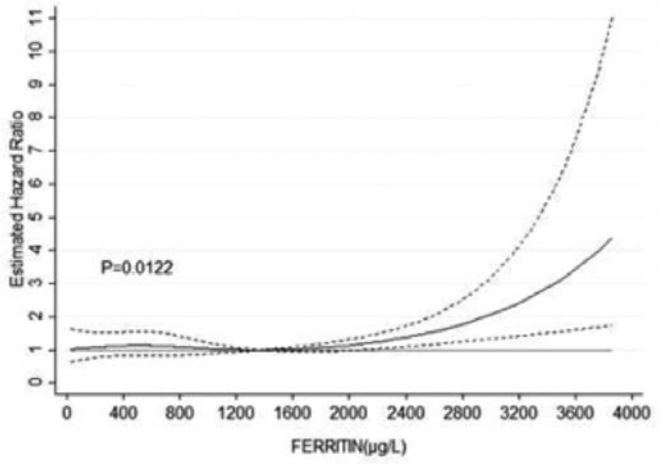

Figure 1.

Summary/Conclusions: In conclusion, hyperferritinemia is a prognostic marker independent from well-acknowledged factors, such as cytogenetics and molecular abnormalities. Ferritinemia should be included at AML diagnosis workup as it provides reproducible information on short and long-term outcome for AML patients of any subgroup. The putative link between hyperferritinemia, inflammation and chemoresistance should be investigated.

P558

NGS ANALYSIS OF 474 BONE MARROW SAMPLES FROM 157 AML PATIENTS TREATED WITH AZACITIDINE–IMPACT OF AGE ON MUTATIONAL LOAD

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Background: Recent publications have shown the prognostic value of performing molecular analyses in patients (pts) with acute myeloid leukaemia (AML) (Papaemmanuil et al, NEJM 2016). While recent data has been published on pts with myelodysplastic syndromes (MDS) and AML treated with decitabine, (Welch et al, NEJM 2016; Duncavage et al, Blood 2017) data on

AML pts treated with azacitidine (AZA) has only been presented in abstract form thus far (Tang et al, ASH 2016). Data on the impact of age on mutational load in AML are scarce.
Aims: To assess the mutational landscape in elderly AML pts treated with AZA; specifically, whether age has an impact on mutational load.
Methods: We analysed 474 bone marrow FFPE specimens from 157 AML pts in the Austrian Registry of Hypomethylating Agents from two centres (Salzburg, Wels-Grieskirchen) using a 53-gene panel (all exons). NGS was performed by Qiagen. Minimum coverage: 1.500x. All mutations were checked against COSMIC-v79, ClinVar, ICGC, DoCM, dbsnp and Varsome databases. For comparison of categorial variables Chi-squared test was used, for comparison of means Students T-test was used.
Results: The rate of secondary (s)AML was significantly lower in pts <75 (n=85), *vs* ≥75 years (n=54) (66.0 *vs* 77.8%, P<0.001). There was no significant difference in the rate of adverse cytogenetics or monosomal karyotype before AZA treatment between pts < *vs* ≥75 years, respectively (data not shown). Mutational load (average number of mutated genes and mutations per pt) assessed at/before initiation of AZA, was significantly higher in pts <75 *vs* pts ≥75 years (10.2 *vs* 8.6 mutated genes/pt; P=0.030 and 12.9 *vs* 10.5 mutations/pt; P=0.012; Figure 1A). This also held true when mutational load was assessed at any timepoint during the course of AML (including during/post-AZA treatment) (Figure 1B). In total, 139 pts had more than one marrow sample with NGS results. Analysis of paired samples revealed that mutational load was significantly higher during/post-AZA *vs* before AZA in both age groups (Figure 1C-D). In total, 60.4%, 15.8%, 8.6%, 3.6% and 11.5% of pts acquired 0, 1, 2, 3, 4-13 additional mutations, respectively. No relevant differences between pts < *vs* ≥75 years were found (data not shown). When comparing the delta of mutations before *vs* during/after AZA according to age group, no significant difference was found (Figure 1E).

Table 1.

Figure 1. Impact of age on mutational load in AML patients treated with azacitidine

1A (total cohorts)	Before AZA		
	<75 years	>=75 years	P-value
n pts. in cohort	85	54	n.a.
Mutated genes per pt., median (avg) [range]	9,0 (10,2) [3-28]	8,0 (8,6) [3-21]	0,030
Mutations per pt., median (avg) [range]	11,0 (12,9) [3-34]	10,0 (10,5) [4-25]	0,012

1B (total cohorts)	During the course of the disease		
	<75 years	>=75 years	P-value
n pts. in cohort	94	63	n.a.
Mutated genes per pt., median (avg) [range]	10,0 (11,0) [3-31]	10,0 (9,3) [3-21]	0,011
Mutations per pt., median (avg) [range]	12,0 (13,9) [3-40]	11,0 (11,4) [4-25]	0,008

1C (paired samples)	Before AZA vs during the course of the disease		
	<75 years		
	Before AZA	During disease	P-value
n pts. in cohort	85	85	n.a.
Mutated genes per pt., median (avg) [range]	9,0 (10,2) [3-28]	10,0 (11,2) [3-21]	<0,001
Mutations per pt., median (avg) [range]	11,0 (12,9) [3-34]	13,0 (14,2) [3-40]	<0,001

1D (paired samples)	Before AZA vs during the course of the disease		
	≥75 years		
	Before AZA	During disease	P-value
n pts. in cohort	54	54	n.a.
Mutated genes per pt., median (avg) [range]	8,0 (8,6) [3-21]	9,0 (9,3) [3-21]	0,004
Mutations per pt., median (avg) [range]	10,0 (10,5) [4-25]	11,0 (11,4) [4-25]	0,004

1E	Before AZA vs during the course of the disease		
	<75 years	≥75 years	P-value
n pts. with BM samples before & during/after AZA	85	54	n.a.
Delta mutated genes per pt., median (avg) [range]	0 (1,0) [0-8]	0 (0,6) [0-9]	0,157
Delta mutations per pt., median (avg) [range]	0 (1,3) [0-11]	0 (0,9) [0-13]	0,349

Summary/Conclusions: The observed mutational load per pt in our cohort is higher than that observed by others using targeted re-sequencing methods, which report an average of only 2-4 mutations per pt (Duncavage et al, Blood 2017; Conte et al, Leuk 2013; Au et al, Diagn Pathol 2016; Grove & Vassiliou, Dis Model Mech 2014). It seems however, that a higher mutational load (average: 13-14 [range 1-51]) per pt can be found using whole genome/whole exome sequencing (The Cancer Genome Atlas Research Network, NEJM 2013; Merlevede et al, Nat Commun 2016). We hypothesize that the higher observed number of mutations in our study may be due to the high coverage (minimum 1.500x) we used (most previous publications had a median/average coverage of ≤500x). While age <75 years seems to coincide with a higher mutational load both before AZA start and during/after AZA in our cohort, it does not seem to predispose to the acquisition of more mutations during/after AZA. Higher mutational load in AML pts <75 years did not go hand in hand with a higher rate of known/presumed adverse prognostic baseline factors such as adverse cytogenetics, monosomal karyotype, or sAML at AZA start. We thus hypothesize that the biology of the disease may generally be more aggressive in younger pts. Correlation analyses of age and mutational load with response and survival will be in our final presentation.

P559

PROGNOSTIC VALUE OF EARLY WT 1 RESPONSE IN AML PATIENTS UNDERGOING INTENSIVE CHEMOTHERAPY

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Background: Monitoring minimal residual disease based on quantitative PCR represents an important risk stratification tool in acute myeloid leukemia (AML) and enables the prediction of impending relapse. Besides common fusion genes and mutated genes, Wilms tumor 1 (WT1) gene is widely used to follow *de novo* AML.
Aims: The aim of our study was to evaluate the relevance of WT1 expression for the prognosis of patients with AML in a real life population.
Methods: Bone marrow samples from 174 consecutive adult AML patients (18-85 years) were used for WT1 mRNA quantification. APL patients were excluded. Of 143 patients with WT1 overexpression at diagnosis, those treated with intensive induction chemotherapy and achieving haematological remission after the first cycle of therapy were included in the retrospective follow-up analysis (n=129).
Results: The extent of WT1 expression at diagnosis had no prognostic relevance. In contrast, achievement of low WT1 levels after induction chemotherapy was associated with a significant better overall (OS) and disease free survival (DFS) as compared to persistent high WT1 expression at hCR1: 5 years OS 54% *vs* 0% (p<0.001); DFS 44% *vs* 0% (p<0.001). Additionally, compared with patients with a low WT-1 reduction (<5 log) at hCR1, the relative risk of death was 0.32 (95% CI 0.1-0.7) in patients with intermediate WT-1 reduction (5-8 log) and 0.15 (95% CI 0-0.05) in patients with high WT-1 reduction (>8 log), after adjustment for age, ELN-risk group, and stem cell transplantation in CR1. The corresponding 5-years OS for patients with low, intermediate and high WT-reduction were 10%, 42% and 71% (p<0.001), respectively. Even though numbers of patients were small (n=33), SCT at CR1 seems to overcome the adverse risk of persistent WT1 expression: DFS 5.3 years (0-12.9) for patients with SCT and 0.7 years (0.6-0.9) for patients without SCT (p=0.004).
Summary/Conclusions: Persisting WT1 expression in AML patients achieving a CR1 after induction chemotherapy is a strong, independent predictor for DFS and OS in patients with AML. Since 80–90% of AML patients exhibit WT1 overexpression at diagnosis, this marker is widely applicable for early risk re-evaluation and corresponding therapy adaption.

P560

EVALUATION OF THE IMPACT OF SIGNAL RATIO ON OVERALL SURVIVAL IN FLT3-MUTATION-POSITIVE RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA FOLLOWING ONCE-DAILY TREATMENT WITH GILTERITINIB

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Background: Fms-like tyrosine kinase 3 (FLT3) internal tandem duplications (ITD) in acute myeloid leukemia (AML) are associated with early relapse and short survival, particularly in the context of high allelic burden. Patients with high FLT3-ITD signal ratio are particularly sensitive to FLT3 inhibitors but the clinical effects of allelic burden on survival have not been validated in trials of these drugs. Gilteritinib is a highly specific, potent FLT3/AXL inhibitor with demonstrated activity against both FLT3-ITD and tyrosine kinase domain (TKD) mutations. A recent Phase 1/2 study (CHRYSALIS; NCT02014558) demonstrated that FLT3 mutation-positive ($FLT3^{mut+}$) patients with relapsed/refractory (R/R) AML treated with gilteritinib had high clinical response rates and prolonged overall survival (OS), especially at doses ≥80mg/d.
Aims: To evaluate the effect of FLT3-ITD and FLT3-TKD signal ratios on OS in $FLT3^{mut+}$R/R AML patients who had received gilteritinib doses ≥80mg/d.
Methods: Signal ratios were assessed in adult $FLT3^{mut+}$R/R AML patients who had received gilteritinib doses ≥80mg/d. Genomic DNA extraction and PCR with fluorescent primers were used to generate transcripts of *FLT3* alleles containing ITD and TKD mutations. *FLT3* alleles containing ITD mutations generated transcripts >330 bp in length. For TKD mutations, primers on either side of the TKD region were used to amplify the target region and an EcoRV endonuclease digest was performed. *FLT3* alleles containing TKD mutations generated shorter (130 bp) transcripts due to the lack of an EcoRV restriction site typically

present in the wild-type allele. Differential fluorescence detection enabled identification of the specific transcripts. Median ITD signal ratio (ie, FLT3-ITD:wild-type FLT3) was calculated from patients with ITD mutations (with/without concomitant TKD mutations); median TKD signal ratio (*i.e.*, FLT3-TKD:wild-type FLT3) was calculated from all patients with a TKD mutation. Median OS estimates were derived and stratified based on ITD and TKD signal ratios that fell above or below median signal ratio values reported for the trial.
Results: Signal ratio was assessed in 152 patients with FLT3-ITD and -TKD mutations who had received ≥80mg gilteritinib. Of these patients, 136 had FLT3-ITD mutations with or without concomitant TKD mutations, and 16 had FLT3-TKD mutations only. Median ITD and TKD signal ratios were 0.84 and 0.5, respectively. Patients with FLT3-ITD signal ratios that were above or below the median ITD signal ratio had OS durations of 216 and 213 days, respectively. No significant difference in median OS was observed between patients in the highest and lowest FLT3-ITD signal ratio quartiles (**Figure 1**). Patients with TKD signal ratios that were above the median value (0.5) had a median OS of 202 days; those with TKD signal ratios below the median value had significantly shorter median OS of 33.5 days (*P*=.0004; **Figure 1**).

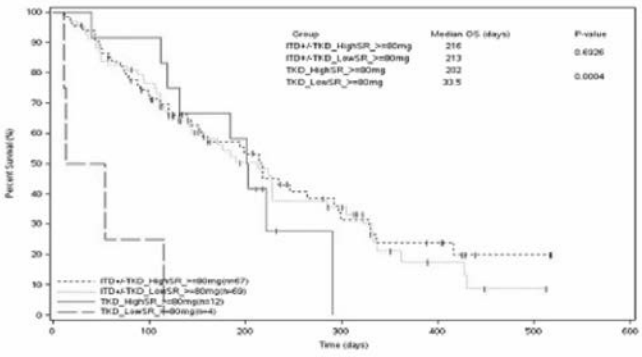

Figure 1.

Summary/Conclusions: These data show that FLT3-ITD signal ratio has little impact on survival in patients with FLT3-ITD mutations who received gilteritinib. In the small number of patients with FLT3-TKD mutations only, high TKD signal ratio was associated with a longer OS, similar to that observed in patients with FLT3-ITD mutations. These data suggest a possibility that oncogene addiction in FLT3-TKD$^+$ R/R AML requires a high allelic burden and clonal dominance. Also, it is possible that FLT3-TKD signal ratio in R/R AML may contribute to the response rate in patients with FLT3-TKD mutations only. Further investigation is warranted.

P561

CLINICAL OUTCOME OF HYPOCELLULAR AML AND AML WITH MYELODYSPLASIA-RELATED CHANGE (MRC) COMPARED TO DE NOVO ADULT AML WITH NORMAL CELLULARITY AFTER HEMATOPOIETIC CELL TRANSPLANTATION

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Background: Hypocellular acute myeloid leukemia (hypo-AML) and AML with myelodysplasia-related change (AML-MRC) accounts for small proportion of adult AML. As the characteristics and outcomes are not well recognized.
Aims: We tried to analyze these specific groups and compared to normocellular AML.
Methods: After exclusion of secondary AML, therapy-related AML, and AML M3, we retrospectively analyzed 1593 AML cases between 2002 and 2013. We found 101 (6.3%) patients with hypo-AML and 164 (10.3%) patients with *de novo* AML-MRC. Hypo-AML was diagnosed with blast counts ≥20% within hypocellular (<20%) bone marrow (BM) which was identified with at least two biopsy specimens and age-related correction was considered. *De novo* AML-MRC was defined with multilineage dysplasia ≥10% for each lineage with blast counts ≥20% without history of antecedent hematologic disease. Patients (n=20) with both AML-MRC and hypo-AML were distributed in AML-MRC group.
Results: Patients with hypo-AML were older (p<0.001) and significantly presented lower leukocyte and PB/BM blast counts (p<0.001). Patients with AML-MRC were older and lower hemoglobin level with lower PB/BM blast counts (p<0.001) compared to normocellular *de novo* AML. In both groups, the risk of karyotype was poorer. In untreated group (n=207), hypo-AML showed longer survival outcome compared to normocellular *de novo* AML and AML-MRC. In treated group (n=1386), hypo-AML and AML-MRC both showed higher relapse rate and inferior survival outcome compared to normocellular AML, and hypo-AML showed higher therapy-related mortality (TRM) rate. However, multivariate analysis showed that there were no significant differences between the three AML subgroups especially when the patients were treated with hematopoietic cell transplantation (HCT).

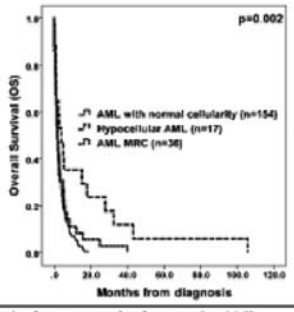

Figure 1. OS results in the untreated subgroup (n=207).

Figure 1.

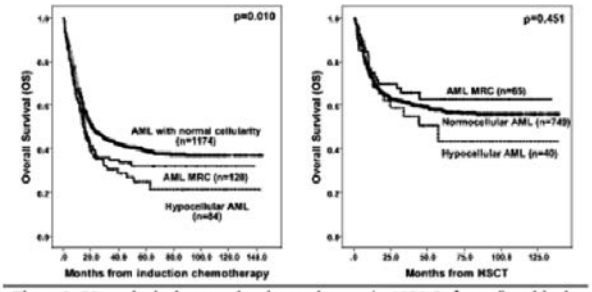

Figure 2. OS results in the treated patients subgroup (n=1386, Left panel) and in the HSCT treated subgroup (n=854, Right panel).

Figure 2.

Summary/Conclusions: The long-term outcome of hypo-AML and AML-MRC were poorer than normocellular *de novo* AML, mainly due to older age and large proportion of adverse-risk karyotype which caused unavailable condition for HCT.

P562

INITIAL RESULTS FROM A FIRST-IN-HUMAN STUDY OF IMGN779, A CD33-TARGETING ANTIBODY-DRUG CONJUGATE (ADC) WITH NOVEL DNA ALKYLATING ACTIVITY, IN PATIENTS WITH RELAPSED OR REFRACTORY AML

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Background: Acute myeloid leukemia (AML) accounts for the highest number of leukemia deaths in the United States annually. IMGN779 is an ADC that binds with high affinity and specificity to CD33, a validated therapeutic target in AML. IMGN779 comprises a humanized anti-CD33 antibody attached via a cleavable linker to the novel DNA-interacting payload DGN462. Once released within the target cell, DGN462 exerts potent antitumor activity via DNA alkylation, without cross-linking, resulting in cell cycle arrest and apoptosis.
Aims: This Phase I study is designed to establish the maximum tolerated dose (MTD) and determine the recommended phase 2 dose (RP2D) of IMGN779 when administered to patients with CD33$^+$ AML. Evaluation of the safety, tolerability, pharmacokinetics (PK), pharmacodynamics (PD), and preliminary antitumor activity of IMGN779 are secondary objectives.
Methods: Adult patients (≥ 18 years) with relapsed or refractory CD33$^+$ AML (defined by ≥20% of AML blasts expressing CD33 by flow cytometry) were eligible for enrollment. Informed consent was obtained from all patients. Dose-escalation, which follows a standard 3+3 design, began with a starting dose of 0.02mg/kg. IMGN779 was administered intravenously once every 2 weeks on days 1 and 15 as part of a 28-day cycle. Adverse events (AEs) were evaluated using NCI-CTC v4.03.
Results: As of February 2017, a total of 17 patients (9 female, 8 male) with a median age of 62 years have received IMGN779 treatment. Five dose levels have been completed, with escalation proceeding from 0.02–0.26mg/kg. AEs were as expected for this relapsed/refractory AML population including cytopenias and constitutional symptoms. No relationship between frequency or sever-

ity of AEs and IMGN779 dose level was observed. The most common AEs were nausea (41%), febrile neutropenia (29%), and rash (29%); pneumonia, respiratory failure, and constipation were additional AEs reported in 4 or more patients (≥ 24%). The most common serious adverse events (SAEs) were grade 3 febrile neutropenia (29%) and pneumonia (24%). No dose limiting toxicities (DLTs) have been reported. Importantly, no safety signals regarding liver or hematopoietic toxicity have been observed in laboratory assessments. In general, plasma concentrations (Cmax) of IMGN779 increased relative to dose. In the highest cohort examined thus far (Cohort 5, 0.26mg/kg), sustained exposure (AUC) was observed in all patients through 48 hours post-infusion. A pharmacodynamics (PD) assay demonstrated consistent saturation of residual free CD33 in all Cohort 5 patients past 48 hours, consistent with the PK results. Initial response data will be presented.

Summary/Conclusions: This is the first clinical experience of the next generation CD33-targeting ADC, IMGN779, in AML patients. No DLTs have been noted to date. AEs were generally consistent with the underlying disease. PK and PD are favorable and dose escalation is continuing.

Aggressive Non-Hodgkin lymphoma - Relapsed/refractory

P563

COMBINATION OF TGR-1202, UBLITUXIMAB, AND BENDAMUSTINE IS SAFE AND HIGHLY ACTIVE IN PATIENTS WITH ADVANCED DLBCL AND FOLLICULAR LYMPHOMA

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Background: TGR-1202 is a next generation, once daily, PI3Kδ inhibitor, active in patients (pts) with rel/ref hematologic malignancies that has demonstrated a notably differentiated safety profile, including in long-term follow up (Burris, 2016). Ublituximab (UTX) is a novel glycoengineered mAb targeting a unique epitope on the CD20 antigen. Bendamustine (Benda) is an active chemotherapy agent in pts with lymphoma. The combination of UTX + TGR-1202 is tolerable and active in pts with rel/ref hematologic malignancies and is under Phase 3 testing for patients with CLL and Phase 2b testing for patients with DLBCL.
Aims: This Phase 1 trial evaluates the safety and efficacy of UTX + TGR-1202 + Benda in pts with advanced Diffuse Large B-cell Lymphoma (DLBCL) and Follicular Lymphoma (FL).
Methods: Eligible pts had rel/ref DLBCL or FL with an ECOG PS ≤2 w/o limit to number of prior therapies. ANC of >750 and Platelets >50,000 was permitted. Pts refractory to prior PI3Kδ, Benda, or anti-CD20 therapy were eligible. UTX was dosed on Days 1, 8, 15 of Cycle 1, Day 1 of Cycle 2-6, followed by Cycle 9 & 12. TGR-1202 was started at 800mg QD with a -1 dose reduction cohort at 600mg if not tolerated in ≥2/6 pts. Benda was dosed at 90mg/m^2 on Days 1 & 2 of Cycles 1-6 only. Primary endpoints included safety and efficacy (Cheson 2007).
Results: Twenty-three pts were evaluable for safety: 15 diffuse large B-cell (DLBCL) and 8 follicular (FL). Med age 68 yo (range 31-81); 12 M/11 F; median prior treatment regimens=2 (range 1-6); 12 pts (52%) were refractory to their immediate prior treatment and to prior CD20 therapy, and 7 patients had progressed post-transplant. ECOG PS 0/1/2 (3/18/2). Initially 2/4 pts at 800mg TGR-1202 experienced AE's in Cycle 1 that led to treatment interruption (rash, neutropenia) thus the 600mg dose of TGR-1202 was explored. No additional Cycle 1 treatment delays were reported at the 600mg dose level, which was later expanded and the 800mg TGR-1202 dose is now being evaluated with stricter eligibility criteria to require an ANC of ≥ 1.0, and the use of growth factor support in cycle 1 is now encouraged. The most common AE's included diarrhea (39%; G3/4 4%), decreased appetite (35%; G3/4 4%), nausea (30%; G3/4 4%), asthenia (26%; G3/4 4%) and neutropenia (22%). The only Grade 3/4 AE reported in >10% of pts was neutropenia (22%). Two pts had a TGR-1202 dose reduction. Nineteen pts (11 DLBCL/8 FL) were evaluable for efficacy: ORR amongst all pts was 79% (15/19) with 42% (8/19) achieving a complete response (CR), of which 5 were DLBCL and 3 FL. ORR in the respective groups as follows:

Table 1.

Group	N	ORR (%)	CR N (%)	PR N (%)	SD N (%)
DLBCL	11	73%	5 (45%)	3 (27%)	1 (9%)
FL	8	88%	3 (38%)	4 (50%)	-

Median follow-up time on study is 6 mos for all pts (range 1–14+ mos).

Summary/Conclusions: The combination of UTX, TGR-1202, and bendamustine has exhibited manageable toxicity with significant activity in advanced DLBCL and FL pts including an encouraging 42% CR rate (45% in DLBCL and 38% in FL). Enrollment continues at the 800mg TGR-1202 dose level with the use of growth factor prophylaxis. Safety and efficacy data for all pts will be updated at the meeting. Based upon the early activity of the triplet, future registration directed studies are being planned.

P564

VENETOCLAX (VEN) IN PATIENTS WITH RELAPSED/REFRACTORY NON-HODGKIN LYMPHOMA (NHL)

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Background: VEN is a selective orally bioavailable BCL-2 inhibitor. The dose-escalation Phase 1 study of VEN in 106 patients (pts) with relapsed/refractory NHL reported an ORR of 44%. Most pts had diffuse large B-cell/follicular lymphoma.
Aims: We report on updated results in pts with less common NHL subtypes.
Methods: VEN was administered and continued until progressive disease (PD)/unacceptable toxicity, in dose cohorts ranging from 300-1200mg. Adverse events (AEs) were assessed by NCI-CTCAE v4.0 and response by 2007 Cheson IWG response criteria, utilizing CT scans beginning at wk 6.
Results: 35 of 106 pts had mantle cell lymphoma (MCL, n=28), marginal zone lymphoma (MZL, n=3) or Waldenström macroglobulinemia (WM, n=4). Most common all grade treatment emergent AEs were nausea (51%), diarrhea (49%) and fatigue (34%); grade 3/4 AEs in >10% of pts were neutropenia and anemia (17% each). Laboratory TLS was reported in a single pt (bulky MCL). MCL pts (median age: 72 years) had received a median of 3 (1–7) prior treatments (tx). Median time from start of prior tx to start of VEN was 13 mo (2–148) and time on VEN was 11 mo (0.2-42). ORR was 75%, 6 pts (21%) achieved CR and remain on study (DORs: 25-40 mo). One pt with a PR proceeded to elective allogeneic stem cell transplant and remained disease free at last protocol defined follow-up (24 mo after coming off study). Median PFS was 11 mo and DOR was 15 mo. MZL pts (median age: 63 years) had received a median of 4 (2-6) prior tx. Time from start of prior tx to start of VEN was 8, 14, 73 mo and time on VEN was 5, 1, 35 mo. One pt (6 prior tx) received VEN for <1 mo due to progressive cytopenias; 1 pt (4 prior tx) achieved a PR with VEN at wk 6 but had PD at wk 16; 1 pt (2 prior tx) achieved PR at wk 6 and is the only pt to remain on study (DOR:32 mo). WM pts (median age: 67 years) had a median of 4 (3-5) prior tx. Time from start of prior tx to start of VEN was 5, 18, 33, 67 mo and time on VEN was 42, 17, 54, 20 mo. All pts achieved PR (at wks 6 [n=2], 16 and 36), with DORs of 11, 12, 38 and 50+ mo (latter is ongoing and remains on study).
Summary/Conclusions: VEN monotherapy has a tolerable safety profile in MCL, MZL and WM pts. ORR were high and most responses durable; median PFS and DOR suggest significant activity in MCL pts. Further investigation of VEN in each disease is indicated.

P565

WHOLE BODY DIFFUSION-WEIGHTED MAGNETIC RESONANCE IMAGING IS A GOOD PREDICTOR OF TREATMENT OUTCOME AFTER ONE CYCLE OF IMMUNOCHEMOTHERAPY IN AGGRESSIVE LYMPHOMA

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Background: Early identification of non-Hodgkin lymphoma patients not responding to therapy may enable treatment adaptation which might impact on the prognosis and reduces exposure to ineffective drugs. Interim fluorodeoxyglucose-positron emission tomography/computed tomography (FDG-PET/CT) after 2-4 cycles of immunochemotherapy has prognostic value shown for some lymphomas, but its role in treatment adaption is still considered experimental. Disadvantages of this technique are the significant amount of false positive results due to a rituximab-induced inflammatory response as well as substantial patient radiation exposure.
Aims: To evaluate the use of whole body diffusion-weighted magnetic resonance imaging (WB-DWI/MRI) as an radiation-free imaging technique to predict treatment outcome in NHL after one cycle of ICT (2-3 weeks).
Methods: Forty-six patients with aggressive NHL (35 diffuse large B-cell lymphoma (DLCBL), 2 primary mediastinal B-cell lymphoma (BCL), 1 unclassifiable BCL, 1 Burkitt lymphoma, 4 Mantle cell lymphoma (MCL), 2 peripheral T-cell lymphoma (TCL) and 1 extranodal natural-killer TCL) were consecutively enrolled between 2011 and 2015. All patients had baseline and interim WB-DWI/MRI (after 1 cycle immunochemotherapy), and end-of-treatment FDG-PET/CT; 38/46 had an interim FDG-PET/CT. Additional International prognostic index (IPI), immunohistochemical markers Ki-67, Bcl-6 and Bcl-2 were evaluated for their predictive value. WB-DWI/MRI were assessed quantitatively with histogram analysis (both on high b-value signal intensity (b1000) and apparent diffusion coefficient (ADC)), and calculation of parameter changes between baseline and interim scan (Δpar). Statistical analysis consisted of Kaplan-Meier survival analysis univariate and multivariate Cox regression analysis with disease-free-survival (DFS) as outcome measure.
Results: Median follow-up time was 43 months (4-70 months). Thirty-three patients achieved complete remission (CR), 4 progressed and 9 had recurrent disease. Patients were non-responders according to WB-DWI/MRI in case of an ADC_{mean} decrease for lymphoid tissue or less than 10% $b1000_{mean}$ decrease in bone or a $b1000_{kurt}$ increase of less than 6% in extranodal lesions. WB-DWI/MRI predicted DFS correctly in 45/46 (96%) [p<0.001; hazard ratio (HR) 66.6, (CI 95% 8.5-523.2)]; end-of-treatment FDG-PET/CT was predictive in 37/46 (80%) [p=0.004; HR 5.1, (CI 95% 1.7-15.4)], and interim FDG-PET/CT in 27/38 (71%) [p=0.042; HR 3.5, (CI 95% 1.0-11.5)]. Nor IPI score neither histological or immunohistochemical parameters demonstrated a significant predictive value. Multivariate analysis revealed WB-DWI/MRI as the only independent prognostic factor (p<0.001).
Summary/Conclusions: WB-DWI/MRI can accurately predict treatment outcome in aggressive NHL after only one cycle of immunochemotherapy (2-3 weeks) without the burden of radiation exposure.

P566

CLINICAL OUTCOMES OF DIFFUSE LARGE B CELL LYMPHOMA, FOLLICULAR LYMPHOMA AND RICHTER'S TRANSFORMATION PATIENTS TREATED WITH IBRUTINIB: A REAL-WORLD EXPERIENCE OF OFF LABEL, IBRUTINIB USE.

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Background: Ibrutinib (IBR), a Bruton's Tyrosine Kinase (BTK) inhibitor, is FDA approved for chronic lymphocytic leukemia, Waldenstrom macroglobulinemia, marginal zone lymphoma and mantle cell lymphoma. Despite its limited data, IBR is increasingly being utilized as a treatment option for patients with relapsed/refractory (RR) diffuse large B-Cell lymphoma (DLBCL) and follicular lymphoma (FL).
Aims: To further characterize the efficacy of IBR in patients with RR DLBCL, Richter's transformation (RT) or FL.
Methods: We conducted a retrospective cohort study of DLBCL, RT and FL patients consecutively treated with IBR. Data collected included patient demographics, stage, IPI, genetic characteristics, prior treatments, IBR dose and duration, reasons for discontinuation, and response. PFS and OS were estimated using the Kaplan Meier method and survival analysis by the log rank (LR) test.
Results: 44 patients were identified (DLBCL: n=24, 54.5%; FL: n=12, 27%, RT: n=8, 18%) who received IBR monotherapy in the RR setting. Baseline characteristics included age (range 19–80), 61% male, 95% ECOG 0 - 1, 71% stage IV, 62% elevated LDH, and 48% R-IPI ≥ 4. DLBCL sub-types (Hans criteria) were 25% non-GC (n=11), 16% GC (n=7), and 14% unclassifiable (n=6). In the FL subgroup, 8% were grade 1, 58% were grade 2, 33% were grade 3a. Median number of prior therapies was 5 (range 1-11). All RT patients were not treated with IBR previously for CLL. The three most common reasons for IBR discontinuation were progression (35%), toxicity (20%), and bridge to CAR-T (10%). PFS and OS data are shown in Table 1. In DLBCL, cell of origin (IHC) did not impact outcomes (p=0.97, LR test). Patients with RT had better PFS as compared to *de novo* DLBCL (p=0.03, LR test).

Table 1.

Table 1: Survival data stratified by NHL Type

Subtype	Median PFS (months)	Median OS (months)
Entire cohort	3 (n = 41)	19 (n=41)
DLBCL (entire cohort)	1.1 (n = 21)	11.5 (n = 23)
DLBCL GC (CR + PR)	0.9	12
DLBCL Non-GC	1.1	7.6
RT	3 (n = 8)	15.6 (n = 8)
FL	10.5 (n = 12)	30.8 (n = 12)

Summary/Conclusions: In the largest single-center, real-world experience of IBR use in DLBCL, RT and FL, we validate findings reported in clinical trials. In FL, responses appear to be durable (median PFS of >10 months). Outcomes are extremely poor in DLBCL and use of IBR as monotherapy is not recommended. Perhaps IBR is best used as a short-term bridge to more definitive therapies. Cell of origin by immunohistochemistry does not predict PFS and should not be used to preferentially select non-GC DLBCL patients for IBR. Patients with RT appear to have more durable responses (*vs* DLBCL) suggesting differing dependence on BTK signaling for tumor survival.

P567

PREVALENCE AND PROGNOSTIC VALUE OF MYD88 AND CD79B MUTATIONS IN IMMUNE-PRIVILEGED SITE AND (EXTRA)NODAL DLBCLS

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Background: Activating mutations in CD79B and MYD88 are important molecular drivers of a subset of diffuse large B-cell lymphomas (DLBCLs), activating the B-cell receptor and toll-like receptor pathways, respectively. Interestingly, the frequency of these mutations differs greatly among DLBCLs at different anatomical sites, with a remarkably high prevalence at immune-privileged (IP) sites (central nervous system and testis). Recent studies suggest that these mutations are associated with an unfavorable prognosis. However, the prognostic value in relation to the site of presentation has not yet been explored.
Aims: To investigate if mutations in MYD88 and CD79B are independent prognosticators for overall survival (OS) in DLBCL, particularly in patients with lymphomas at IP sites, for which a high prevalence of these mutations was reported.
Methods: In this retrospective study, we investigated a large clinically annotated cohort of 189 consecutive primary DLBCLs, including primarily nodal (N=64), primarily extranodal (N=74) and IP localizations (N=51). Patients were diagnosed between 1990-2015 at the Academic Medical Center, (University of Amsterdam) or other Dutch hospitals. The vast majority was treated with (R-) CHOP (N=143) or other immune-chemotherapies (N=16). Detailed clinical characteristics of all patients were collected. For all patients BCL2, BCL6, and MYC translocations, Epstein Bar Virus (EBV) status and the mutational status of MYD88 and CD79 were assessed, employing methods described previously (Kraan *et al.*, BCJ 2013).

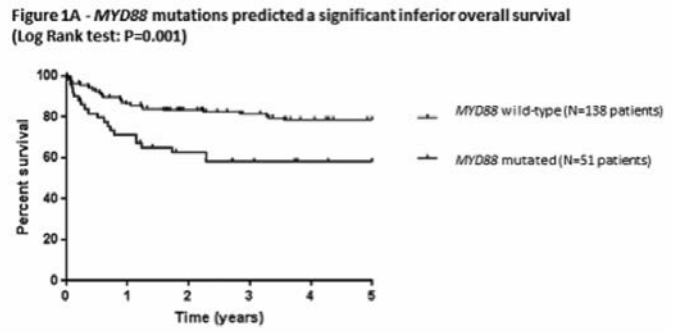

Figure 1.

Results: Translocations in BCL2, BCL6 and MYC were identified in 14, 32 and 13 patients, respectively and 23 EBV-positive cases were found. MYD88 and CD79B mutations were identified in 51 patients and 19 cases, respectively. Interestingly, there was hardly any overlap between the presence of translocations (BCL2, BCL6 and MYC) or EBV and that of MYD88 and/or CD79B mutations, indicating that these tumors represent distinct DLBLC subgroups. In accordance with previous studies, the incidence of MYD88 mutations was increased at IP sites (67%, Chi-square P=0.001), compared to nodal (13%) and other extranodal localizations (12%). In patients harboring a MYD88 mutation, we frequently found a coexisting CD79B mutation (N=14). Patients with a MYD88 mutation demonstrated a significantly inferior 5-years OS compared to DLBCL with wild-type MYD88 (Log Rank test (LR) P=0.001, Figure-1A). This prognostic significance was also found for DLBCLs with IP sites (Figure-1B, LR P=0.029). Coexistence of a CD79B mutation did not impact the prognostic significance of MYD88. Multivariable Cox regression analysis, including clinical and molecular characteristics (i.e. age, translocations, EBV, CD79B, etc.) identified MYD88, Ann Arbor Stage and localization (IP, nodal and extranodal) as independent prognostic parameters with Hazard ratios 1.8, 1.5 and 2.6, respectively (95% Confidence intervals: 1.0-3.6, 1.0-2.8 and 1.3-4.9, respectively).
Summary/Conclusions: Our study demonstrates that mutated MYD88 is an independent unfavorable prognostic factor for OS, in particular in DLBCL patients presenting at IP sites. These patients with MYD88 mutations display a relatively high prevalence of coexisting CD79B mutations. Interestingly, a recent study by Wilson *et al.* (Nat. Med. 2015), indicates that these patients are more sensitive to treatment with Bruton's Kinase inhibitors. Our study highlights the importance of investigating the mutational status of MYD88 and CD79B in larger prospective clinical trials with molecularly targeted agents, particularly in DLBCL patients with IP localizations.

P568

HIV-INFECTED PATIENTS WITH RELAPSED NON-HODGKIN LYMPHOMA (NHL) OR HODGKIN LYMPHOMA (HL): RESULTS FROM THE GERMAN HIV-RELATED LYMPHOMA COHORT STUDY

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Background: The outcome of HIV-associated lymphoma has undergone significant improvement in recent years beginning with the widespread use of combination antiretroviral therapy (ART). However, among AIDS-related deaths, non-Hodgkin lymphoma (NHL) is the most frequent event. HIV-positive patients (pts) with relapsed NHL or Hodgkin lymphoma (HL)) should be treated in a manner similar to immunocompetent pts.
Aims: To analyze the outcome of pts with HIV-related lymphoma who experienced a relapse after having achieved a complete response to first line therapy.
Methods: This prospective multicenter cohort study includes adult HIV- 1 infected pts with biopsy or cytologically proven HIV-related lymphoma diagnosed at 32 participating centers in Germany and Austria since January 2005. Data on HIV-infection and lymphoma characteristics, treatments and outcomes were recorded. Pts with indolent lymphomas and primary central nervous system lymphomas were excluded from the present analysis.
Results: Of 499 pts (463 males, 36 females) 394 had aggressive NHL and 105 HL. The median age at lymphoma diagnosis was 45.6 yrs (range, 22–74.7). 344 pts (69%) were diagnosed with advanced stage (III/IV) lymphoma and the median CD4-cell count was 271/µl (266/µl in NHL and 287/µl in HL). As of June 2015, 311 of 499 pts (62%) achieved a documented CR, 235 (60%) with NHL and 76 (72%) with HL. After a median follow-up of 17 months for NHL and 30 months for HL pts, 31 of 235 NHL (13%) and 8 of 76 HL (11%) experienced a relapse. Incidence of relapse was 6.9/100 patient years (PY) within the 1st year after primary diagnosis and 1.3/100 PY thereafter (*P*=0.0062). Median time to relapse was 7.3 months in NHL and 18.0 months in HL. Relapses beyond 12 months occurred in 6 of 31 NHL cases (19%) and in all 8 HL cases (100%) (*P*=0.045). Median overall survival (OS) of all relapsed pts was 29.0 months (95% CI 14.1-44.2 months) after primary lymphoma diagnosis. In pts with HL, OS was not reached, whereas it was 15 months in pts with NHL (*P*=0,024). Regarding the entire cohort of 311 pts with a documented CR, the 2-year OS rate was 57% in pts with relapse as compared to 97% in those without (*P*<0.001). The majority of relapsed pts died of lymphoma (68%).
Summary/Conclusions: Relapses from CR are relatively rare in pts with HIV-associated NHL and HL. In pts with NHL the majority of relapses occur within the first year after primary diagnosis, whereas in HL most relapses occur beyond 12 months. Overall, pts with relapsed HIV-related NHL have a worse outcome than pts with relapsed HL.

P569

RISK STRATIFICATION BASED ON NCCN-IPI AT THE TIME OF DIAGNOSIS IN COMBINATION WITH POST-TREATMENT PET-CT SCAN FOR THE TREATMENT OF NODAL PERIPHERAL T-CELL LYMPHOMA

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Background: Nodal peripheral T-cell lymphomas (PTCLs) are a heterogeneous group of neoplasms, which include PTCL not otherwise specified (PTCL-NOS), angioimmunoblastic T-cell lymphoma (AITL), anaplastic large-cell lymphoma-anaplastic lymphoma kinase positive (ALCL-ALK+), and ALCL-ALK-. Clinical assessments before and after treatment are essential to predict survival in nodal PTCL. However, limited data is available regarding the prognostic relevance of National Comprehensive Cancer Network International Prognostic Index (NCCN-IPI) and post-treatment PET-CT scan.
Aims: The study investigated the prognostic significance of baseline NCCN-IPI and post-treatment PET-CT scan, assessed by Deauville score, in patients with nodal PTCL. The primary aim was to establish a risk model for nodal PTCL patients based on NCCN-IPI, a clinical tool, and post-treatment PET-CT scan indicating tumor viability.
Methods: In this retrospective cohort study, patients with newly diagnosed nodal PTCL were consecutively enrolled from 11 hospitals in South Korea. Patients were eligible if they were histologically diagnosed with nodal PTCL from Jan 2005 to June 2016, received systemic chemotherapy, and had the results of PET-CT scan at the time of diagnosis and at the end of treatment. Post-treatment PET-CT was assessed using 5-point Deauville score. The study excluded ALCL-ALK+ due to well-known better survival.
Results: A total of 396 patients were screened for eligibility. Seventy patients were excluded from the analysis due to following reasons: unavailable pre- or post-treatment PET scans, no systemic treatment, uncertain histology, and ALCL-ALK+. Thus, 326 patients were analyzed. The median age was 61 years (range, 18-86) and 209 (64%) were male. PTCL-NOS (N=172, 53%) was the most common subtype included, and AITL (N=111, 34%) and ALCL-ALK- (N=43, 13%) followed. Three-fourths of patients (N=242) had stage III/IV. Majority of patients received anthracycline-based therapy. Patients were categorized into low (N=42, 13%), low-intermediate (LI, N=108, 33%), high-intermediate (HI, N=136, 42%), and high (N=40, 12%) risk groups according to NCCN-IPI. Based on the Deauville criteria, post-treatment PET scan was scored as 1 (N=130, 40%), 2 (N=47, 14%), 3 (N=60, 18%), 4 (N=27, 8%), and 5 (N=62, 19%). Because the number of progression in Deauville score 3 (40/60, 67%) was significantly different from score 2 (21/47, 45%; *P*=0.023) and 4 (24/27, 89%; *P*=0.030), we categorized patients into 3 groups: Deauville score 1-2, 3, and 4-5. With a median follow-up of 54.7 months (IQR, 30.2-84.5), 5-year PFS rate was 35.7% (95% CI, 30.0-41.4) and OS rate was 47.1% (95% CI, 40.8-53.4). NCCN-IPI risk and post-treatment PET-CT scan were independently associated with PFS in the multivariate analysis (for LI NCCN-IPI, hazard ratio [HR] 1.615, 95% CI 0.838-3.113; HI NCCN-IPI, HR 3.063, 95% CI 1.626-5.769; high NCCN-IPI 4.475, 95% CI 2.231-8.977; *P*<0.001: for post-treatment Deauville score 3, HR 1.895, 95% CI 1.281-2.801; score 4-5, HR 6.916, 95% CI 4.948-9.667; *P*<0.001). We stratified patients into 5 groups based on risk of progression: a low (low NCCN-IPI and Deauville score 1-2), INT-1 (low NCCN-IPI and Deauville score 3, or LI NCCN-IPI and Deauville score 1-2), INT-2 (HI NCCN-IPI and Deauville score 1-2), high (high NCCN-IPI and Deauville score 1-2, or LI to high NCCN-IPI and Deauville score 3), and very high (Deauville score 4-5). The risk model showed a strong association with PFS and OS (Figure 1).

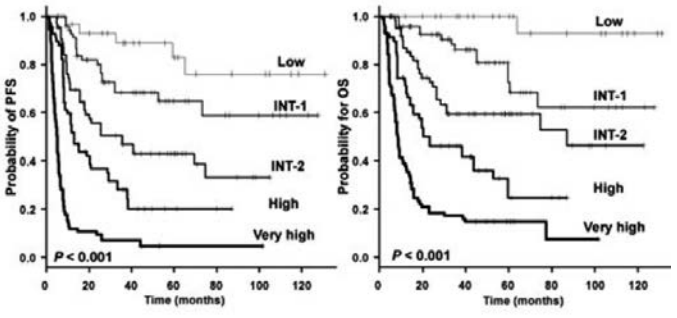

Figure 1.

Summary/Conclusions: This study proposes a new risk stratification model incorporating baseline NCCN-IPI in combination with post-treatment Deauville score on PET-CT scan in patients with newly diagnosed nodal PTCL.

P570

LONG-TERM EFFICACY AND SAFETY OF CRIZOTINIB IN RELAPSED ALK POSITIVE LYMPHOMA PATIENTS: CLINICAL AND BIOLOGICAL CORRELATES

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Background: Anaplastic large cell lymphoma (ALCL) is an aggressive disease with over 60% ALK-tyrosine kinase (TK) receptor positivity due to oncogenic fusions proteins containing ALK, mainly NPM-ALK. Crizotinib, an ALK inhibitor, proved to be effective in ALK positive tumors and in particular in ALK+ ALCL, with Objective Response Rates (ORR) in the 80-90% range. Long-term data on the safety and efficacy of crizotinib are lacking.
Aims: In this work, we analysed long-term clinical outcomes in 16 patients treated with crizotinib for ALK positive ALCL and we evaluated the presence of TK domain-point mutations in patients who relapsed during crizotinib treatment.
Methods: 16 patients were treated at the Clinical Research Unit, S. Gerardo Hospital, Monza (Italy) from June 2010 to December 2015 with a median follow up of 7.3 months (range 2.4-72 months). Survival curves were obtained using GraphPad Prism 5; differences were evaluated with Mantel-Cox test and Gehan-Breslow-Wilcoxon test. In 4/7 relapsed patients the TK domain of ALK were amplified from peripheral blood samples obtained at the time of crizotinib-relapse and subjected to deep sequencing. Subsequently we profiled *in-vitro* the activity of 2nd/3rd generation ALK inhibitors (brigatinib, alectinib, ceritinib and lorlatinib) with a murine BaF3 cell model and obtained their level of sensitivity/resistance to the drugs.

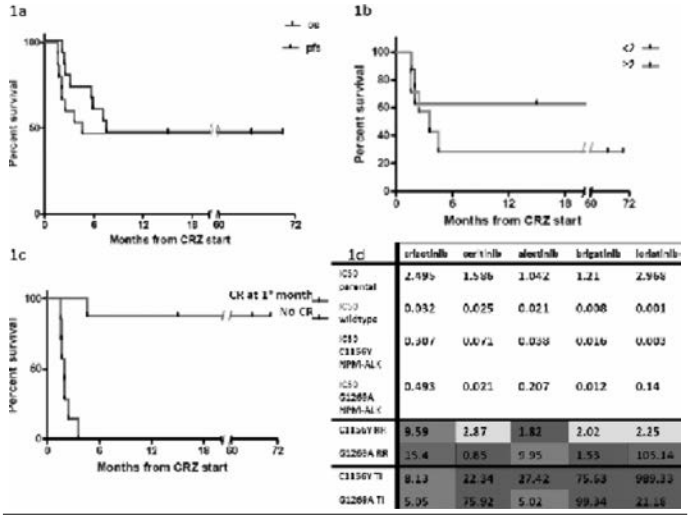

Figure 1.

Results: Median age at diagnosis was 25.5 years (range 16-38 years) and 8/16 patients were male. Median previous line-therapies were 2.5 (range 1-5). In the Intention-To-Treat population which includes 1 patients who died after the first crizotinib dose, 13/16 patients (81.25%, 95% CI 53-95%) and 8/16 patients (50%, 95% CI 25-75%,) achieved an OR and a complete response (CR) after 1 month of therapy, respectively. Median overall survival and progression free survival (PFS) were 7.53 months and 4.57 months respectively (fig 1a). Median time to progression was 50 days (range 47-137 days). OS and PFS at 1 and 3 years from treatment were 44%. At the last follow up 7 patients were still on treatment and in CR (median treatment duration 44 months [range 15-72 months]). There was a significant difference in 3 years PFS between patients in whom CR was obtained after 4 weeks of crizotinib and those who didn't (PFS at 3 years 87.5% *vs* 0%, p<0.001-fig 1c); patients with less than 2 previous lines of therapy showed a borderline better 3 years PFS (66% *vs* 33%, p=0.08-fig 1b). Crizotinib was well tolerated and there were no cumulative adverse events (AEs) over this long-term follow-up. The only G3 AEs reported were transient neutropenia and creatine-kinase elevation. The deep sequencing of 4 NPM-ALK in relapsed patients demonstrated the presence in 2/4 samples of ALK mutations G1269A and C1156Y, which were not present in samples before crizotinib treatment. The level of *in vitro* resistance of these mutations showed a high level of resistance to crizotinib (resistance index for C1156Y and G1269A: 9.59-15.4 respectively). The sensitivity *in vitro* of these mutations to ALK-inhibitors was also evaluated: all inhibitors, except alectinib for G1269A, were active with a therapeutic index (TI) >20 (fig 1d). TI values, as previously reported by Mologni L. et al (Oncotarget. 2015 Mar 20;6(8):5720-34), provide a view of the therapeutic impact of a mutation: the bigger the value, the more targetable is the mutation with the inhibitor.

Summary/Conclusions: Crizotinib confirmed to be an effective and safe therapy for advanced relapsed ALK+ ALCL, with durable responses up to 6 years after treatment initiation and no relapse later than 4 months. These results represent the longest available safety record for crizotinib. ALK point mutations can develop and 2nd/3rd generation inhibitors may be a therapeutic opportunity for patients who develop resistance to crizotinib.

P571

PRELIMINARY RESULTS FROM AN OPEN-LABEL, PHASE II STUDY OF TIPIFARNIB IN RELAPSED OR REFRACTORY PERIPHERAL T-CELL LYMPHOMA

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Background: Tipifarnib is a potent and selective inhibitor of farnesyltransferase (FT). FT catalyzes post-translational attachment of farnesyl groups required for localization of signaling molecules to the inner cell membrane. CXCL12 is a chemokine that is essential for hematopoietic stem cell (HSC) homing to the bone marrow and lymphoid organs and for maintenance of HSCs and immune cell progenitors. CXCL12 is known to signal in part through HRAS, a signaling protein that is uniquely farnesylated. Tipifarnib has previously been shown to be well tolerated and to have a 41% response rate (7 responses out of 17 patients) in patients (pts) with T-cell Non-Hodgkin Lymphoma, including 4 objective responses in 8 pts with peripheral T-cell lymphoma (PTCL) (Witzig et al, 2011). Building on this prior experience, we report herein the preliminary efficacy, safety and biomarker data from our ongoing Phase 2 study in PTCL.
Aims: This Phase 2 study is a multi-institutional, single-arm, open-label, two-stage (11+7) study designed to determine the efficacy and safety of tipifarnib in pts with relapsed/refractory (R/R) PTCL.
Methods: Pts with R/R PTCL after prior cytotoxic systemic therapy, aged ≥ 18 years old, and with a performance status of 0-2 were eligible. Informed consent was obtained. The following subtypes of PTCL were eligible for enrollment: PTCL, not otherwise specified (PTCL-NOS), angioimmunoblastic T-cell lymphoma (AITL), ALK-positive and -negative anaplastic large cell lymphoma (ALCL), hepatosplenic T-cell lymphoma, enteropathy-associated T-cell lymphoma (EATL), extranodal natural killer (NK) T-cell lymphoma, nasal type and subcutaneous panniculitis-like T-cell lymphoma. The primary endpoint of the study is overall response rate. Secondary endpoints include safety and tolerability, duration of response (DOR) and progression free survival (PFS). Based on activity observed in the first 18 pts in the study, the protocol has been amended and enrollment is ongoing to an expansion cohort in AITL (N=12). Enrolled pts are treated with tipifarnib 600mg administered orally twice daily on days 1-7 and 15-21 of 28-day treatment cycles until progression of disease or unacceptable toxicity. Biomarker studies included gene expression profiling of pretreatment tumor biopsies by RNASeq and DNA next-generation sequencing (NGS). Clinical trial information: NCT02464228.

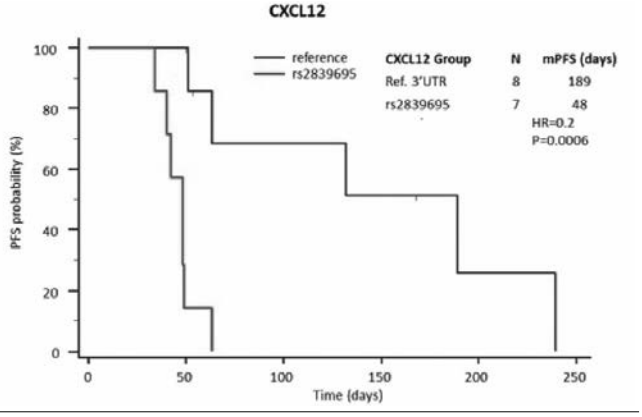

Figure 1.

Results: At data cut-off (2/15/2017), 18 pts (2 AITL, 1 ALK- ALCL, 15 PTCL-NOS) were treated with tipifarnib. Most common treatment-related AEs (grade ≥ 3) were myelosuppression, including neutropenia (61%), anemia (39%) and thrombocytopenia (39%). 3 pts achieved a partial response (2 AITL; 1 PTCL-NOS) and 3 additional pts experienced stable disease >6 months. Tumor DNA from 16 pts was sequenced using NGS. A high rate of CXCL12 3'UTR single nucleotide variation (SNV) was observed. Seven of 16 pts carried the rs2839695 variant while an additional patient carried a novel variant. The presence of 3'UTR SNVs was associated with low levels of CXCL12 gene expression and disease progression (Figure) while all pts deriving clinical benefit from tipifarnib carried reference (wild type) 3'UTR CXCL12 and had tumors that expressed high levels of mRNA for this chemokine. Testing of circulating CXCL12 levels is ongoing.
Summary/Conclusions: Although this study is ongoing, these preliminary data indicate that tipifarnib is generally well-tolerated and has antitumor activity, particularly in pts with AITL histology, absence of 3'UTR CXCL12 SNV and high levels of CXCL12 gene expression.

P572

BAM CONDITIONING BEFORE AUTOLOGOUS STEM CELL TRANSPLANTATION FOR LYMPHOMA: A RETROSPECTIVE STUDY ON BEHALF OF THE FRANCOPHONE SOCIETY OF BONE MARROW TRANSPLANTATION AND CELLULAR THERAPY (SFGM-TC)

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Background: High-dose chemotherapy before autologous stem cell transplantation (ASCT) is a therapeutic option as a consolidation in primary or relapsed lymphoma. BEAM conditioning is generally used. Alternative conditioning regimens have been published but few data are available.
Aims: To evaluate tolerance and efficacy of the BAM (Busulfan, Aracytin and Melphalan) conditioning before ASCT.
Methods: We conducted a retrospective study in 188 French patients treated between 2000 and 2015. Data were retrospectively collected from the Promise database. Informed consent was obtained from all patients.
Results: Indications for ASCT were diffuse large B-cell lymphoma (n=54, 29%), mantle-cell lymphoma (n=42, 22%), Hodgkin's disease (n=33, 18%), low-grade non-hodgkin lymphoma (n=26, 14%), T-cell lymphoma (n=17; 9%), Burkitt's lymphoma (n=8, 4%) and B-cell lymphoma (n=8, 4%). Median age at diagnosis was 50.9 years (35.7-59.9). Time between diagnosis and ASCT was 295 days (176-777). Patients received 1 (n=82, 44%), 2 (n=83, 44%), 3 or more (n=18, 10%), unknown (ND) (n=5, 2%) treatment lines before ASCT. Among the 138 B-cell lymphoma patients, 132 received rituximab before ASCT. Only 20 patients received prior radiotherapy. In all patients, ASCT was the first transplantation. In 11 patients, ASCT was planned as part of a multiple graft protocol. At the time of transplantation, 116 (62%) patients were in complete remission, 54 (29%) in partial remission, 13 (7%) in relapse or progression, and 5 (2%) ND. ASCT was documented in 186 (99%) patients. Median time to neutrophil and platelet (>50 Gigal/L without transfusion) recovery was respectively 11 days [10-12] and 19 days [14-32]. Infectious complications were found in 153 patients. One hundred (53%) patients had undocumented fever, 19 (10%) had sepsis, 150 (80%) had grade 1-4 mucositis during neutropenia with a WHO toxicity grading of 2 (42%), 3 (39%) and 4 (19%). Colitis with a median duration of 7 days [5-10], was reported in 73 patients, with a maximum toxicity grading of 1-2 (n=43, 59%), 3 (n=21, 29%) or 4 (n=4, 6%) and ND in 5 patients. Only 2 (1%) patients had non-fatal hepatic sinusoidal obstruction syndrome. Pulmonary toxicity was reported in 33 (17.6%) patients with 8 cases of respiratory distress syndrome. Respiratory distress was fatal in one patient but occurred more than 6 months after ASCT and salvage treatment. Seven (3.7%) patients reported secondary cancers (all were solid tumors except one acute leukemia). Median follow-up was 17.1 months [11.3-29.5]. At the time of the study, 47 (25%) patients had relapsed. Cumulative incidence of relapse was 6.24% at 3 months and 17.31% at 12 months. At the end of the follow-up, 149 (79%) patients were alive. The main causes of death were relapse (n=15, 41%) and toxicity (n=16, 43%). Median overall survival (OS) was not reached and progression-free survival was 71.5 months [47-NR]. Relapse-free mortality was 1.66% at 3 months and 4% at 12 months. In the univariate analysis, the number of treatment lines (1 or 2) before ASCT and previous use of monoclonal antibodies positively impacted the OS. Conversely, the multiple graft protocol had an unfavorable impact on OS.
Summary/Conclusions: BAM conditioning before ASCT for lymphoma helps to control disease activity without excessive toxicity. It may be a suitable alternative to BEAM in case of drug shortage. However, comparative studies are needed to confirm these findings.

Bone marrow failure syndromes incl. PNH - Clinical

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ANALYSIS OF MICRORNAOME, PROTEOME AND METABOLOME OF EXOSOMES FROM PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Background: Paroxysmal Nocturnal Hemoglobinuria (PNH) is a clonal disease caused by the lack of glycosyl inositol phosphatidyl anchored proteins at the cell membranethat leads to intravascular hemolysis upon complement activation. Patients have intravascular haemolysis with high risk of thrombosis, and a variable degree of bone marrow failure. Treatment with Eculizumab reduces intravascular hemolysis and also the thrombotic risk. The mechanism of thrombosis in PNH is still unknown. Exosomes are extracellular vesicles released by cells and whose secretion is closely related with the inflammatory status. Exosomes participate in cell communication by activating signaling pathways and transferring genetic material, *i.e.* miRNA, and proteins to host cells.
Aims: To describe the microRNAome, proteome and metabolome of exosomes from PNH patients to identify potential biomarkers of the disease and to investigate its relationship with the mechanism of thrombosis in these patients.
Methods: Plasma exosomes were isolated from 5 healthy controls and from 9 PNH patients(6 with Eculizumab, 3 with thrombosis –ET- and 3 without thrombosis –ENT-, and 3 without Eculizumab) using Total ExosomeIsolation kit (ThermoFisher).MiRNAsfrom exosomeswere purified using Nucleo Spin miRNA Plasma Kit (Macherey-Nagel).miRNA expression was evaluated by plasma/serum focus miRNAs PCR panel V4 (Exiqon). Proteomic analysis of exosomes was performed at the OMICs core facilities. Untargetted metabolomic analysis was performed by using combination of gas chromatography and liquid chromatography (LC) with mass spectrometry (MS). Additionally; latest advances were used combining LC-MS-solid phase extraction-nuclear magnetic resonance (UPLC-QTOF_SPE_NMR) 'on line' for unequivoque structural elucidation of unknown metabolites.
Results: Mir-16-5p and miR-451a had lower levels in patients *vs* controls. Eculizumab treatment increased their expression, particularly in the group with thrombosis. Eculizumab also decreased miR-223-3p (the most abundant miRNA in platelets and that has been associated with its reactivity)and increasedmiR-15a-5p levels (0.50- and 3.12-fold respectively). Those proteins differentially expressed in patients and controls were related with the complement system and the immune response. We identified an increase in the plasma hemoglobin levels in patients *vs* controls (4.9-fold), which is related with platelet activation. It is also noteworthy the decrease (1.5-fold) of the anticoagulant Protein S in patients *vs* controls. When the analysis was performed among the 3 groups of patients, only Ig heavy chain V-I region HG3 increased in 3.9-fold in the Eculizumab group *vs* without Eculizumab group, which could be related with the treatment. We identified quite few metabolites inside the exosomes, all of them associated with cell toxicity or immune response. The levels of Cholesterol, HydroxyTerbinafine-glucuronide and Diacyl-glycerol decreased in 17.3, 17.6 and 19.4-fold, respectively in patients treated with Eculizumab. Interestingly, the Aminoethylphosphonicacid, Cholesterol and PGF2 increased 16.7-; 21- and 19.4-fold in patients with thrombosis.
Summary/Conclusions: Our study supports that exosomes contain material that may influence the pathological status of the PNH patients. In concordance, most of the proteins, miRNAs and metabolites are related with the complement system or the inflammatory response. In future experiments, some of the proteins, miRNAs and metabolites should be validated to define whether they could be considered biomarkers.

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Abstract withdrawn.

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SEVERE CHRONIC NEUTROPENIA: THE ROLE OF PRIMARY IMMUNODEFICIENCY AS CAUSATIVE AGENTS. A SINGLE CENTER DATA

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Background: Severe Chronic Neutropenia may be a primary disease, usually defined as congenital (CN), or a condition mainly secondary to autoimmune disturbances (SN) (1,2). CN rises in early infancy, has a marrow block at pro/myelocyte, classically carries genes ELANE/HAX1 mutations in 70% of cases and is G-CSF dependent. SN is accompanied by extrahaematological signs and/or positivity of autoimmune markers; bone marrow has a normal morphology or is "left shifted". In spite of these categorization many cases do not fit either group and share features of both of them. These "Ovelap Neutropenia" (ON) patients are a diagnostic and management challenge.
Aims: Investigate the genetic backgroud of this ON from a cohort of chronic neutropenia subjects screened at Hematology Unit of Gaslini Hospital and characterize their clinical phenotype.
Methods: Patients with severe chronic neutropenia were seen prospectively in our center and diagnosed/followed-up according to published guidelines(3,4). Genetic diagnosis includes classical Sanger technique foe commonest severe chronic neutropenia genes and an enlarged NGS panel including also those gene responsible for PID.
Results: From 2008 to 2016, 24 patients (13 males) with median age at last follow of 18yrs (range 28 mo-51y) had a complete work up for severe chronic neutropenia (Table 1). Ten/24 subjects (43%) were diagnosed as classical CN with ELANE mutation found in the majority (80%) of cases. Seven/24 (29%) were diagnosed as SN and the remaining 7/24 (29%)as ON.A PID genes mutation was found in a total of 8/24 patients (30) with 5 patients belonging to 7 SN subjects (71%) and 3 to the 7 ON subjects (42%).Table 1 shows clinical hematological characteristic of the 3 categories of patients.
Summary/Conclusions: A considerable portion (30%) of subjects affected with severe chronic neutropenia have been identified as PID. In the group of ON subjects a mutated PID gene was found in 3/7patients and muations of ELANEin 2/7 patients. No mutation was found in the remaining 2.The phenotype of ON subjects is characterized by extra-hematological autoimmune symptoms, by maturation block and by the frequent involvement of more than one hematopoietic lineage. This phenotype my suggest to access to an enlarged genetic panel including PID genes for genetic diagnosis. An accurate immunological and genetic work may support diagnosis and management of these difficult patients.

Table 1.

Table 1. Clinical, biological and genetic characteristic of a group of Severe Chronic Neutropenia.

PT	Sex	Involvement of Hematopoietic Lineage	Extra-Haematological signs	Auto Imm Mrks	BM morphology Presence of block	GCSF/dose	response to G-CSF	Other drugs	GENE	status at last FUP
1	m	NP	no	no	Promyelocyte	low	yes	no	ELANE	alive
2	f	NP	no	no	Promyelocyte	high	yes	no	ELANE	alive
3	m	NP	no	no	Promyelocyte	high	yes	no	ELANE	alive
4	m	NP	no	no	Promyelocyte	standard	yes	no	ELANE	alive
5	f	NP	no	no	Promyelocyte	standard	yes	HSCT	ELANE	alive
6	f	NP	no	no	Promyelocyte	standard	yes	no	ELANE	alive
7	f	NP	no	no	Promyelocyte	low	yes	no	ELANE	alive
8	f	NP	no	no	Myelocyte	low	yes	no	ELANE	alive
9	f	NP	no	yes	Promyelocyte	standard	yes	no	UNKNOWN	alive
10	m	NP	no	nd	Promyelocyte	standard	yes	no	UNKNOWN	alive
11	m	NP, AN, THR	Thyroiditis	yes	No	low	yes	rapamycin/steroid	RTEL1	alive
12	m	NP, AN	Severe infections, Hypogb	yes	No	no	...	MMF/HSCT	RAG1	alive
13	m	NP, THR	no	yes	ND	no	...	no	UNKNOWN	alive
14	m	NP, AN, THR,	Lymphoproliphyferation	yes	ND	no	...	MMF	UNKNOWN	alive
15	f	NP,THR	Autoimmune hepatitis	yes	no	on demand	yes	MMF/rapamycin	LRBA	alive
16	f	NP,THR	Arthritis	yes	ND	on demand	yes	no	CECR1	alive
17	m	NP	Chronic diarrehs	yes	Metamyelocyte	on demand	yes	no	TACI	alive
18	f	NP,AN, THR	Autoimmune hepatitis	yes	Myelocyte	standard	yes	rapamycin	CARD 11	alive
19	m	NP	Chronic diarrea	no	Promyelocyte	standard	yes	HSCT	CD40	alive
20	f	NP	Eczema	yes	Myeloblast	high	no	Rapamycin+HSCT	PI3K	alive
21	m	NP, THR	no	no	Promyelocyte	standard	yes	no	UNKNOWN	alive
22	m	NP, THR	Vasculitis , thyroiditis	yes	Promyelocyte	low	yes	no	UNKNOWN	alive
23	f	NP , THR	Arthritis	yes	No	low	yes	NSAIDs	ELANE	alive
24	m	NP	no	yes	Metamyelocyte	on demand	yes	no	ELANE	alive

NP= neutropenia, AN=anemia, THR=thrombocytopenia, G-CSF doses: Standard 5-7,5 mcg/kg/die, Low dose ≤4mcg/kg/die, High≥8 mcg/kg/die, MMF =michophenolate mophetyl, HSCT=Haematopoietic Stem Cell Transplantation, NSAIDs=non steroid antiinflammatory drug. White background=Congenital Neutropenia , yellow background =Secondary Neutropenia, Graybackground=Overlap Neutropenias

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TREATMENT WITH HORSE-DERIVED ANTI-THYMOCYTE GLOBULIN LEADS TO ENDURING HEMATOLOGICAL RESPONSES AND A 1.5-YEAR SURVIVAL PROBABILITY OF 87% IN ADULT ACQUIRED APLASTIC ANEMIA PATIENTS IN THE NETHERLANDS

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Background: Acquired aplastic anemia (AA) is a rare disease characterized by bone marrow failure. First-line treatment is either an allogeneic stem cell transplantation (alloSCT) or intensive immunosuppressive therapy (IST) consisting of Anti-Thymocyte globulin (ATG) and ciclosporin. Based on studies from the National Institute of Health, the Dutch guidelines for diagnosis and treatment of aplastic anemia recommend horse-derived ATG (ATGAM) as the preferred type of ATG. Patients who are refractory after first-line treatment with IST can be treated with second-line alloSCT or rabbit-derived ATG (Thymoglobulin). Eltrombopag, a Thrombopoietin-mimetic, is registered for second-line treatment of AA since May 2016. In order to evaluate the guidelines, a national registry was started in 2014 in which seven university hospitals and two large non-academic hospitals collect data on all consecutive adult aplastic anemia patients, including those that received ATGAM and ciclosporin as first-line treatment.
Aims: To evaluate the safety and efficacy of first line treatment using ATGAM and ciclosporin in adult patients with acquired aplastic anemia in the Netherlands.
Methods: Data on adults with acquired aplastic anemia who received ATGAM as first-line treatment was collected in the LUMC, AMC, UMCG, UMCU, VUMC, UMCN, Erasmus MC, Medisch spectrum Twente and Antonius Ziekenhuis Nieuwegein. The data included baseline-characteristics and follow-up data at 3, 6, 9 and 12 months. After 12 months, follow-up data was collected at least yearly. All patients received first-line treatment with ATGAM (40mg/kg for 4 days intravenously) and ciclosporin. Response was defined as complete in case of normalization of blood values and as partial in case of transfusion independency and neutrophil count >0.5 x 10^9/L. Overall survival was evaluated with the Kaplan-Meier method.
Results: In October 2016, 70 patients were registered in the NVvH registry. Median age at start of treatment was 53 years (18-79) and median follow-up time was 18 months. Overall survival probability after 18 months was 87%. Fifty-nine patients were evaluable for a response at 6 months after treatment. Response was seen in 36 patients (61% (CI 49-73%)). Patients with a response at 6 months, had an overall survival probability of 94% at 12 months thereafter. After initial response at 6 months, aplastic anemia relapsed in 4 patients and 1 patient developed AML. From the 23 non-responding patients, 7 patients continued ciclosporin after 6 months without additional treatment. Five of these 7 patients became transfusion independent up to 16 months after treatment with ATGAM. Sixteen of the patients without a hematological response 6 months after ATGAM received second-line treatment consisting of alloSCT (n=8), Thymoglobulin (n=4), Eltrombopag (n=3) or Danazol (n=1). From the 16 patients that received second-line treatment, 10 patients eventually had a hematological response. Patients without a response at 6 months, had an overall survival probability 12 months thereafter of 84%. One patient developed secondary AML after second-line treatment with Thymoglobulin.
Summary/Conclusions: Six months after treatment with first-line ATGAM, 61% of the adult patients with acquired aplastic anemia is transfusion independent. Half of the remaining patients becomes transfusion independent after rescue treatment or after continuation of ciclosporin beyond 6 months.

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IMMUNE RECONSTITUTION IN PATIENTS WITH ACQUIRED SEVERE APLASTIC ANEMIA AFTER HAPLOIDENTICAL STEM CELL TRANSPLANTATION

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Background: Acquired severe aplastic anemia (SAA) is a rare disease that is characterized by bone marrow failure that results in pancytopenia and hypoplastic bone marrow. Hematopoietic stem cell transplantation (SCT) is one of the main treatment strategies for SAA. In recent years, for patients who require transplantation, but have no human leukocyte antigen (HLA) matched donors, haploidentical SCT (haplo-SCT) is an important alternative option. Delayed immune reconstitution (IR) after haplo-SCT played a crucial role in infections and graft-versus-host disease (GVHD) and was considered a barrier to the wider application of haplo-SCT in SAA. The assessment of immune reconstitution may provide tools to better predict and modulate adverse outcomes and subsequently improve survival after transplantation. However, the kinetics of immune recovery in patients with SAA after haplo-SCT have not been studied systematically.
Aims: We aim to provide the kinetics for immune reconstitution in SAA patients who receive haplo-SCT, investigate the factors that may affect immune recovery and assess the impact of immune cell subset recovery on transplant outcomes.
Methods: In this study, we examined immune cell subset counts and immunoglobulins in 81 SAA patients from day 30 to day 365 after haplo-SCT. The immune cells analyzed in this study including lymphocyte, monocyte, $CD3^+$ T cell, $CD8^+$ T cell, $CD4^+$ T cell, $CD4^-CD8^-$ T cell, $CD8^+CDC28^+$Tcell, $CD4^+CD28^+$Tcell, $CD4^+$ memory T cell and $CD4^+$ naïve T cells. Simultaneously, we determined which factors influence immune reconstitution and analyzed the effects of immune cell subsects on transplant outcomes.
Results: (i) The reconstitution of different immune cell subsets occurred at different rates after haplo-SCT. Monocytes were the first to recover, followed by $CD8^+$ T and $CD19^+$ B cells, and finally $CD4^+$ T cells. Early $CD4^+$ T cell recovery occurred at the expense of memory cells, whereas naïve $CD4^+$T cells rose only 9 months after SCT. (ii) In the multivariate analysis, lower recipient age, female gender, high mononuclear cell counts and $CD4^+$ T cell counts in the graft were associated with improved immune recovery after transplant. (iii) A CD4/CD8 ratio less than 0.567 on day 30 post-transplantation was associated with lower treatment related mortality and higher overall survival after haplo-SCT in SAA patients.
Summary/Conclusions: We provided the kinetics for immune recovery in SAA patients who received haplo-SCT. In general, our study demonstrated that the recovery of monocyte and CD8+ T cells was fast in SAA patients, whereas the recovery of the $CD4^+$ T cell subset was delayed. In addition, our data suggested that the CD4/CD8 ratio may be useful for predicting transplant outcomes in SAA patients after they complete haplo-SCT. Our results may be useful for making better predictions and modulating the IR of SAA patients, which would subsequently improve the outcomes after transplantation.

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DEVELOPMENT OF A SCREENING AND DIAGNOSTIC ALGORITHM FOR PAROXYSMAL NOCTURNAL HEMOGLOBINURIA USING A MODIFIED DELPHI PANEL METHODOLOGY

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare clonal hematopoietic stem cell disorder that manifests with hemolytic anemia due to uncontrolled complement activation, bone marrow failure, and thrombosis. Diagnosis is essential because PNH is a progressive disorder associated with substantial morbidity and mortality. The protean clinical manifestations of PNH complicate diagnosis, and subsequently the diagnosis is often delayed or missed. Although national diagnostic guidelines are available, international expert consensus on PNH screening and diagnosis is lacking.
Aims: An international panel of PNH experts was assembled to develop a clinically relevant, consensus-driven screening and diagnostic algorithm for PNH.

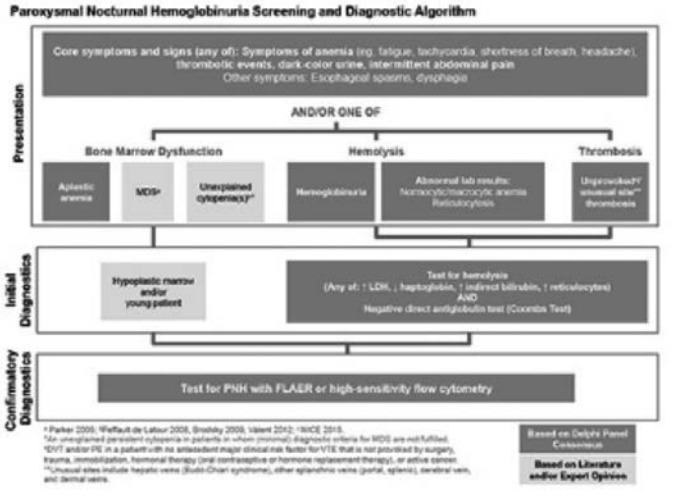

Figure 1.

Methods: An expert advisory committee of 4 PNH experts from North America, Europe, and Japan was assembled. Using a modified Delphi methodology, consensus was gained on the symptoms and signs of PNH and the laboratory tests required for screening and confirmation of diagnosis. Globally representative Delphi panelists were identified through a double-blinded screening process and asked to complete 2 rounds of web-based questionnaires. The questionnaires were developed by the expert advisory committee and presented to the Delphi panel in a case-based format. In the first round, Delphi panelists were given 5 blinded case studies—each including details on clinical presen-

tation and past medical history—and were asked to provide their differential diagnosis and the tests they would order to establish the diagnosis in free-text format. To reduce bias, Delphi panelists were blinded to the fact that the study was focused on PNH. Responses mentioned by ≥50% of Delphi panelists in the first round were included in the second-round questionnaire. For each case in the second-round questionnaire, Delphi panelists were presented with a series of consensus statements regarding potential diagnoses and the need for specific tests/data from a multiple-choice list and asked to respond with their level of agreement on a 4-point Likert scale. Consensus in the second round was attained if ≥80% of Delphi panelists agreed on a given screening or diagnostic approach.
Results: Twelve Delphi panelists from 6 countries, all of whom were clinicians with expertise in PNH, were recruited. Consensus was reached on 22 of 23 PNH screening and diagnostic decision points identified by the Delphi panelists. Specifically, consensus was gained on the core symptoms and signs of PNH at presentation, including hemolysis, bone marrow dysfunction, and thrombosis. Consensus was also reached for 36 of 38 specific screening and diagnostic tests required at each decision point to narrow the differential diagnosis and to confirm the diagnosis of PNH. The level of agreement on screening and diagnostic decision points and tests was sufficient to enable the development of a screening and diagnostic algorithm (Figure) that is consistent with the published literature and with the real-world experience of the international expert advisory committee.
Summary/Conclusions: The modified Delphi methodology facilitated development of a consensus-based, clinically relevant PNH screening and diagnostic algorithm. This algorithm provides clinicians with varying levels of expertise detailed guidance on how to screen for and diagnose PNH.

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DIAMOND-BLACKFAN ANEMIA IN THE NETHERLANDS: AN OVERVIEW OF CLINICAL CHARACTERISTICS AND UNDERLYING MOLECULAR DEFECTS

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Background: Diamond-Blackfan anemia (DBA) is a rare genetic disorder, characterized by bone marrow failure (anemia), congenital anomalies and a predisposition for malignancies. DBA is characterized by a highly heterogeneous nature, both clinically and genetically. Most of our understanding of this disorder stems from molecular studies combined with extensive data-input from international patient registries.
Aims: The aim of our retrospective study was to create an overview of the pediatric DBA population in the Netherlands.
Methods: Forty-four patients (age 0-18yr) diagnosed with DBA from all Dutch pediatric hospitals were included in this study and their clinical and genetic characteristics were collected from patient records.
Results: Congenital malformations were present in 19/41 patients (46,3%), varying from craniofacial and cardiac defects to urogenital and developmental disorders. An underlying genetic defect was identified in 23 patients (56,1%), the majority of which were found in the RPS19 gene (n=10; 45%). No significant diversities in malformations, course of disease or response to treatment were observed when comparing patients with or without identified genetic defects. In agreement with previous reports, two patients harboring defects in RPL11 displayed a more severe phenotype, including craniofacial malformations, thumb abnormalities, and cardiac defects. In contrast, our patient with a mutation in RPL5 has no associated congenital abnormalities, while previous studies reported a very high frequency (83%) of associated congenital defects. Furthermore we observed a relatively high number (12/23) of novel mutations in well-known 'DBA-genes', defined as novel variants. In addition, we have identified a novel 'DBA-candidate', describing a genetic defect in RPL9, in a patient with multiple congenital abnormalities (craniofacial defects, cardiac defects, colitis) in addition to severe anemia. Thirty-four (34/44) patients were treated with glucocorticoids, of which in thirty-one (31/34) patients a complete response was observed (91,2%). However, in 29% discontinuation was prompted by high-dosage-dependence, side effects, a weaning response, or a combination of these factors. Five patients (12,2%) were successfully transplanted with hematopoietic stem cells from either matched sibling donors (n=3) or matched unrelated donors (n=2), including two cases after the age of 10 years. Eleven patients (26,8%) were treatment-independent, defined as acceptable hemoglobin values without any therapy. No malignancies were thus far reported.
Summary/Conclusions: In line with previous reports, the Dutch pediatric DBA population is both clinically and genetically heterogeneous, with *RPS19* being the most frequently mutated gene. Interestingly, the majority of mutations in our cohort have not been described before, probably further underlining clinical heterogeneity. In addition, we have identified a novel DBA-candidate gene (*RPL9*), associated with a more severe phenotype, based on multiple associated congenital defects. While we created a comprehensive overview of the Dutch pediatric DBA population, limitations of our study include a relatively small number of patients, and the lack of complete genetic analysis (for all DBA candidate genes) in a relevant number of patients. Overall, to increase our understanding of genotype-phenotype correlation in DBA, and underlying pathophysiological mechanisms more generally, it crucial to further extend our genetic, and functional analysis of DBA-candidate genes, as well as compare, and share data from international registries.

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NEXT GENERATION SEQUENCING IN BONE MARROW FAILURE SYNDROMES

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Background: Inherited bone marrow failure syndromes (IBMFSs) are a heterogeneous group of genetic disorders, with similar clinical presentations, resulting in complex diagnosis. Molecular characterization is essential in order to establish diagnosis, treatment and prognosis. Next-generation sequencing (NGS) techniques seem to be a useful platform for genetically defining different IBMFSs.
Aims: To design a NGS panel with the objective of making a specific, fast and cost-effective diagnosis for these pathologies.
Methods: We developed a NGS panel of 164 genes involved in different IBMFSs. A total of 120 samples have been processed. Patients were classified into two groups based on the clinical presentation: classified IBMFS (CBMFS) for those with a clinical picture typical os some of these disorders, and unclassified IBMFS (UBMFS) for the others. For the NGS study the NextSeq platform of Illumina (Roche) has been used. Bioinformatic analysis has been oriented to the identification of point polymorphisms (SNPs) and insertions / deletions of small DNA fragments.
Results: Of the 120 samples processed, 10% (12/120) was not suitable for analysis. A total of 108 patients were studied. In 59,3% (64/108) causal mutations were detected. From the total samples analyzed (108), 75% (81/108) were included in the CBMFS patient group, obtaining a diagnostic yield of 64,2% (52/81). The remaining 27 patients (25%) were included in the UBMFS group and we found causal mutation in 37% (10/27). Therefore, it remains a percentage of patients without a genetic diagnosis, which seems more evident in the UBMFS group. This could be explained by the fact that the causal gene has not been described or due to the limitations of the technique.
Summary/Conclusions: NGS techniques are a fast and cost-effective option for the diagnosis of IBMFSs patients. In our series, we have reached a diagnosis rate of 59,3%, coinciding with that described in the literature. Undiagnosed patients should be included in new research projects.

P581

APLASTIC ANEMIA PATIENTS WITH MONOCYTE-DOMINANT PNH CLONES HAVE A UNIQUE PRESENTATION AND ARE LESS RESPONSIVE TO IMMUNOSUPPRESSIVE THERAPY

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Background: Aplastic anemia (AA) is a bone marrow failure syndrome that can be successfully treated with either immunosuppressive therapy (IST) or allogeneic bone marrow transplantation (BMT). In ~50% of patients (pts) with AA, a clone deficient in glycosylphophatidyl inositol (GPI)-linked antigens–a paroxysmal nocturnal hemoglobinuria (PNH) clone–can be detected (Young, *Blood*, 2006). In recent years, highly sensitive techniques have been developed to test for PNH clones that have primarily focused on evaluating peripheral blood white cells. Neutrophils are routinely tested for expression of GPI with fluorescent aerolysin (FLAER); monocytes may also be analyzed but are not always evaluated in PNH testing. Our centre has previously reported that 60% of PNH positive tests show a higher monocyte clone than granulocyte clone and that there was >10% difference in 20% of these discrepant results (Razavi, *ISLH Proceedings*, 2015). Whether pts with discordant monocyte and granulocyte PNH clones have different clinical characteristics and/or response to IST has not been reported to date.
Aims: To compare the granuloyte and monocyte PNH clones in pts with AA to determine whether there are differences in clinical presentation and/or response to IST for pts with discordant clone sizes.
Methods: A retrospective review was performed on all patients >age 16 treated for AA with IST at VGH, the tertiary referral centre for the Province of BC, between 11/09 and 10/15. All patients had central pathology review and metaphase cytogenetic analysis that confirmed a diagnosis of AA. High-sensitivity flow cytometry testing with a sensitivity of 0.1% was done on all patients

to detect the presence of a PNH clone. Granulocytes, monocytes and erythrocytes were interrogated with multi-colour flow panels including CD59 and FLAER. The criteria for determining discordant granulocyte and monocyte clone sizes was dependant upon the absolute size of the smaller clone. For clones 0.1-10%, discordance was defined as when the larger clone was either ≥2 x the smaller clone or at least 1% (absolute value) greater. For smaller clones >10%, the larger clone had to be ≥110% its size. IST was uniform - Cyclosporine (CSA, 2.5mg/kg p.o. b.i.d.), anti-thymocyte globulin (ATG; ATGAM 40mg/kg IV daily x 4 days) and (Methylprednisolone 1mg/kg/day x 10 days). CSA doses were adjusted to maintain whole blood trough CSA level of 200-300 ug/L for 12-months followed by slow taper based upon hematologic response. Non-responders at 6 months were eligible to proceed to either a second cycle of ATG or BMT, if a suitable donor was available. Severity of AA [very severe (VSAA), severe (SAA) or non-severe (NSAA)] and response to IST [(none, partial (PR) or complete (CR)] were determined according to published criteria (Marsh, *Br J Haematol*, 2009). Statistical comparisons were done using a standard Chi square analysis.

Results: 30 pts with AA and a PNH clone were identified, 18 females and 12 males with median age of 50.5 years (range 17-71). There were 14 pts with NSAA, 13 with SAA and 3 with VSAA. Responses were seen in 20/30 pts (66.7%) including 13 PR and 7 CR. Six pts relapsed with CSA tapering and 5 responded to intensified IST; 2 pts required Eculizumab after evolving to a classic PNH phenotype. Six pts underwent BMT for primary non-response and 4 pts have died (2 post-BMT, 1 from complications of AA and 1 from breast Ca); 26 pts remain alive and well with a median follow-up of 48 mos (15-86). There were 17 pts (56%) with concordant granulocyte and monocyte clone sizes (Group 1), 4 pts (13%) had granulocyte-dominant disease (Group 2) and 9 pts (30%) had monocyte-dominant disease (Group 3). Group 3 pts were significantly more likely to have NSAA and showed a trend toward an inferior response rate to IST (Table 1).

Table 1.

	Group 1	Group 2	Group 3	p value
Median age (yrs)	52	40.5	41	NS
Severity of AA				
VSAA/SAA	10/17	4/4	2/9	
NSAA	7/17	0/4	7/9	**0.028**
Response rate (%)	12/17 (70.6)	4/4 (100)	4/9 (44.4)	**0.096**

Summary/Conclusions: Flow cytometry for a PNH clone is routinely done in AA although it may be important to evaluate both granulocyte and monocyte clone sizes. Pts with a larger monocyte than granulocyte clone size more frequently have NSAA and appear to have a lower response rate to IST. This may have therapeutic implications and could identify a population of pts requiring a unique therapeutic approach.

P582

RESPONSE TO ANTI-THYMOCYTE GLOBULIN (ATG) IN PATIENTS WITH APLASTIC ANEMIA (AA): A SINGLE-CENTRE EXPERIENCE OVER THE LAST 28 YEARS

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Background: Aplastic anemia (AA) is a rare, usually acquired disorder characterized by bone marrow failure with bi- or pancytopenia and marrow hypoplasia. The classification into the three main subtypes is of prognostic and therapeutic relevance. Depending on disease severity, patient's age, and the availability of a potential HLA-identical donor, different therapeutic strategies are favored. Immunosuppressive therapy (IST) with anti-thymocyte globulin (ATG) and cyclosporin (CsA) is considered the initial standard treatment. A hematologic recovery is seen in up to 60-70% of the pts following horse-ATG (hATG) treatment, compared to 35-53% in rabbit-ATG (rATG) treated pts, considering hATG as first-line therapy in AA pts.

Aims: As response rates vary according to the different studies and the source of ATG being used, our aim was to retrospectively evaluate response rates in pts with AA receiving IST at the Department of Hematology at the University Hospital of Essen between 1988 until 2015.

Methods: In this single-center, retrospective analysis, approved by the institutional review board of the University Hospital of Essen, individual response rates to ATG, according to criteria reported by Camitta *et al.* 1975, were evaluated in 67 pts with AA (52% (35/67) females; median age 48 years (range 17-89 years)) being treated or monitored at the Department of Hematology between 1988 until 2015. 73% of the pts (49/67) were treated with hATG (ATGAM® (44/49) and Lymphoglobulin®). ATGAM® was administered at a dose of 40mg per kilogram (kg) body weight (BW) per day for 4 days and rATG (Thymoglobulin®) at a dose of 3.5mg/kg BW per day for 5 days, respectively. Pts in both arms simultaneously received CsA (5mg/kg BW) and prednisone (day 1-29).

Results: Following six months after primary ATG therapy, a hematologic recovery was seen in 66% of the pts (44/67). The hematologic response rate at 6 months was 75% (37/49) for hATG and 39% (7/18) for rATG (p=0.005). Irrespective the source of ATG we observed no significant difference in respect to gender (females: 71% (25/35) *vs* males: 59% (19/32)) or in the presence of a PNH clone (GPI-deficient granulocytes (FLAER) 67% (14/21) *vs* 79% (19/24) in pts with no detectable PNH clones), whereas in pts ≤50 years (yrs) a statistically higher rate in hematologic recovery was observed (≤50 yrs: 84% (31/37) *vs* >50 yrs: 43% (13/30); p<0.001). In primary refractory pts (34% (23/67)) (52% (12/23) in first-line treated hATG pts *vs* 48% (11/23) rATG treated pts) a second course with either hATG (3/9) or rATG (6/9) was initiated, achieving an overall hematologic recovery at 6 months in 3 pts (33% (1/3) hATG *vs* 33% (2/6) rATG treated pts). A disease relapse (median: 13 months after primary ATG therapy) was seen in 11 out of the 44 pts with primary hematologic recovery (25%) (82% (9/11) in first-line treated hATG pts *vs* two rATG treated pts). A salvage therapy with rATG was initiated in two pts, whereas in one other pt a second course with hATG was started. An overall response following relapse therapy was observed in 33% of the pts (1/3). Four refractory as well as relapsed pts were treated with eltrombopag respectively (final results are still awaited). A secondary HSCT (hematopoietic stem cell transplantation) was performed in 11 out of the 67 pts (16%), either being primary refractory or due to a disease relapse.

Summary/Conclusions: Our data are able to independently confirm the findings of previous studies concerning hematologic recovery rates in pts with acquired AA following IST with ATG by providing further evidence that rATG plus CsA is inferior to hATG plus CsA when administered as a first-line treatment. In addition, we were able to observe in pts ≤50 yrs, irrespective gender, an overall higher hematologic recovery. For this reason, it remains unclear why ATGAM® is still not approved in Germany as first-line therapy in pts with AA, as the only hATG product registered in Europe (Lymphoglobulin®) was withdrawn from the market in 2007.

Chronic lymphocytic leukemia and related disorders - Biology 2

P583

NOTCH1 MUTATED CHRONIC LYMPHOCYTIC LEUKEMIA CELLS ARE CHARACTERIZED BY A MYC-RELATED OVEREXPRESSION OF NUCLEOPHOSMIN-1 AND RIBOSOME ASSOCIATED COMPONENTS

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Background: Stabilizing mutations of *NOTCH1* have been identified in about 10% of chronic lymphocytic leukemia (CLL) cases at diagnosis, with a higher frequency in unmutated *IGHV* (*IGHV*-UM), immuno-chemorefractory or advanced disease phase CLL, and have been associated with particularly unfavourable prognosis (Rossi et al, Blood, 2012; Del Poeta et al, Br J Haematol, 2013; Stilgenbauer et al, Blood, 2014). In CLL, all *NOTCH1* mutations disrupt the C-terminal PEST domain and cause an accumulation of an active NOTCH1 isoform, resulting in a sustained pathway activation.
Aims: To identify molecular/biological features of *NOTCH1* mutated CLL
Methods: The presence of *NOTCH1* mutations was investigated by NGS. Gene expression profile (GEP) was performed by a one-color labeling strategy using the 4x44K platform. Specific gene/protein validations were performed by QRT-PCR, western blotting, flow cytometry and immunofluorescence. CLL-like MEC-1 cell line was transfected with a vector containing a *NOTCH1* intracellular domain (NICD) or with a control vector. Cell proliferation was evaluated by CellTrace assay. Cell size was estimated by flow cytometry from Forward Scatter (FSC) values.

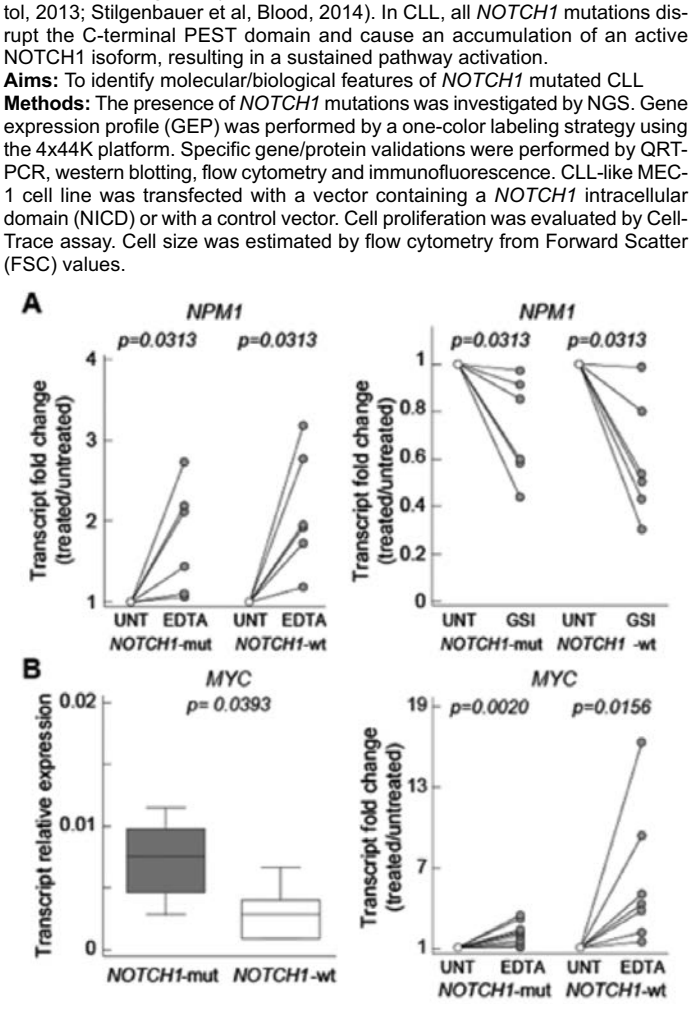

Figure 1.

Results: i) A GEP comparing purified cells of 10 *IGHV*-UM *CLL* cases (5 *NOTCH1*-mut; 15%>37% of *NOTCH1* mutated alleles) selected nucleophosmin-1 (*NPM1*) and genes codifying for several ribosomal proteins (*RNPs*) as significantly up regulated in *NOTCH1*-mut cases. A higher expression of *NPM1* and *RNPs* in *NOTCH1*-mut cases was validated in a wider independent series of 188 cases by QRT-PCR (76 *NOTCH1*-mut cases). In CLL, NPM1 expression was previously found higher in *IGHV*-UM cases (Rees-Unwin, Br J Haematol, 2009). In our series, no significant difference in *NPM1* transcript expression was found by comparing *IGHV*-UM and *IGHV*-M cases, but *NPM1* transcript expression was confirmed significantly higher in *NOTCH1*-mut than in *NOTCH1*-wt cases in the *IGHV* UM subgroup. **ii)** Western blotting in 11 CLL cases (5 *NOTCH1*-mut) confirmed a higher NPM1 protein expression in *NOTCH1*-mut cases, with a direct correlation with NOTCH1 expression (r=0.814). In *NOTCH1*-mut cases, the NPM1^{high}subpopulation, isolated by cell sorting, showed a higher *NOTCH1* mutational load than the NPM1^{low} subpopulation. **iii)** EDTA treatment of 12 CLL cases (6 *NOTCH1*-mut) activated NOTCH1 signaling (Rand et al, Mol Cell Biol, 2000), as from *HES1* and *DTX1* induction, and up-regulated *NPM1* and other *RNPs*. The same results were confirmed by co-culture of CLL cells with the JAGGED1-expressing M2-10B4 stromal cells. Inhibition of NOTCH1 signaling by gamma-secretase-inhibitor L-685,458 or by siRNA for *NOTCH1* reduced *NPM1* expression (Fig. A). **iv)** Previous studies identified *MYC* as a direct transcriptional target of NOTCH1 (Palomero et al, PNAS 2006) and, in turn, a transcriptional activator for both *NPM1* and *RNPs*. ChIP assays on MEC1-cells, transfected with exogenous NICD, revealed increased NICD binding to the *MYC* promoter, along with higher expression of *MYC*, *NPM1* and *RNPs*. Of note, after 48h culture, *NOTCH1*-mut CLL cases showed increased *MYC* transcript levels than *NOTCH1*-wt cases. *MYC* expression was further increased upon NOTCH1 activation by EDTA or by stromal cells co-cultures (Fig. B). *MYC* silencing by siRNA efficiently reduced *NPM1* transcript and protein expression. Moreover, CpG-ODN/IL-2 treatment, to induce MYC overexpression, also increased NPM1 transcript and protein levels in CLL cells. **iv)** *NPM1* silencing by siRNA was able to reduce proliferation rates and cell size of both NICD-transfected cells and control cells. In keeping with a *NOTCH1*-driven regulation of cell growth/ protein biosynthesis, activation of NOTCH1 signaling in 12 CLL cases (6 *NOTCH1*-mut) by EDTA or stromal cells co-culture, induced an increase in cell size.
Summary/Conclusions: *NOTCH1* mutations in CLL are associated with the overexpression of *MYC* and *MYC*-related genes involved in protein biosynthesis including *NPM1*, which are allegedly responsible for cell growth and/or proliferation advantages of *NOTCH1*-mut CLL.

P584

CLL-LIKE B-CELL CLONES FROM MBLLO INDIVIDUALS PERSIST AT INCREASED COUNTS AFTER SEVEN YEARS OF FOLLOW-UP

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Background: The presence of very low numbers of clonal B cells in peripheral blood (PB) of otherwise healthy individuals (low-count monoclonal B lymphocytosis -MBL^{lo}-) is a common finding in the general population. Since the vast majority of clonal B cells from MBL^{lo} subjects show a phenotype overlapping with CLL (chronic lymphocytic leukemia) cells, the former might represent either the normal counterpart of CLL or the earliest stages of the disease. Little information exists about both the clinical outcome of MBL^{lo} subjects and the biological features of their B-cell clones over time.
Aims: To gain insight into the biological and clinical significance of the presence of CLL-like MBL^{lo} clones, we re-evaluated the biological features of clonal B cells and the clinical outcome of MBL^{lo} individuals after 7 years of follow-up.
Methods: The baseline study was constructed in 2008, when 80 out of 639 (12.5%) healthy individuals (>40y) were found to carry at least one PB CLL-like clonal B-cell population, using high-sensitive flow cytometry. A subset of them (n=49) has been followed for a median period of 84 months (range: 67-95 months). Besides physical examination and flow cytometry analyses, the most frequent CLL-related cytogenetic alterations [del13q14.3(D13S25), trisomy 12, de11q(ATM) and del17q(TP53)] were studied at baseline and at follow-up.
Results: A total of 64 CLL-like MBL^{lo} clones (median size: 0.44 cells/ul, range: 0.027-66 cells/ul) were detected in PB of the 49 subjects at recruitment (in 15 cases ≥2 B-cell clones were detected in the same subject). In all subjects, B-cell clones persisted at reevaluation, phenotypically identical *vs* baseline. Interestingly, we found a near-3-fold overall increase in the size of CLL-like B-cell clones after a 7y follow-up *vs* baseline (median size: 1.22 cells/ul, range: 0.046-789 cells/ul; p:<0.001); in line with this, most clones (45/64; 70%) increased their size, while the remaining 30% maintained stable or slightly decreased numbers compared to time 0. From the genetic point of view, only 8/32 (25%) clones tested carried one cytogenetic alteration at baseline, del13q(D13S25) being present in 7/8 cases and trisomy 12 in the remaining one. Strikingly, re-evaluation after 7 years showed 36/56 clones (64%; p<0.01 *vs* baseline) with cytogenetic alterations; again, the most common abnormality was del13q(D13S25) (34/36) followed by trisomy 12 (1/36) and del 17p(TP53) (1/36). No statistical association (p>0.05) was found between the change over time in the size of these clones and the presence of cytogenetic lesions. Three subjects developed lymphocytosis (median: 5.3x10^{9}lymphocytes/l; range: 4.1x10^9-5.9x10^9/l) after 7 years; in these cases the clone size increased sub-

stantially over time and showed (2 of them) del13q14.3. Despite this, only 1/3 subjects evolved to more advanced stage of disease, who showed 5.9x10^{9}lymphocytes/l with >500 clonal B cells/ul (thus reaching MBL^{hi}) and del13q14.3.
Summary/Conclusions: Our results suggest that CLL-like MBL^{lo} is not a transient condition, as all clones detected at recruitment were systematically present also at follow-up with an increase frequency of cytogenetic lesions. Despite, B-cell clones slightly increased in size and even acquired cytogenetic abnormalities, the likely to progress into an over CLL seems to be extremely rare. Further research is needed to better understand the significance of CLL-like B-cell clones in healthy individuals and to clearly identify those MBL^{lo} subjects at risk to evolve to MBL^{hi}/CLL.

P585

NUCLEAR LAMINA REGULATES SOMATIC HYPERMUTATION AND PROGRESSION OF B CELL MALIGNANCIES

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Background: The nuclear periphery, containing the IgH and IgK gene clusters, is a unique compartment comprised of inner nuclear membrane proteins and nuclear lamina. Previous genome-wide and cytological studies revealed the regulatory role for some of these nuclear proteins in higher level genome organisation and gene regulation. In particular, Lamina Associated Domains (LADs) were identified at the nuclear periphery as transcriptionally silent, gene-poor domains associated with Lamin B1. More recent studies however revealed an important role of LADs in the regulation of gene expression and recombination.
Aims: Given the apparent topological coincidence between LADs and Ig variable clusters, we hypothesised that nuclear lamina might play a paramount role in the dynamics of Ig-encoding variable genome domains. In particular, here we tested whether Lamin B1, a principal LAD-associated component of the nuclear envelope, had any restrictive role on somatic hypermutation (SHM) and the expression of Ig genes. Due to the strong involvement of IgV mutations in the pathogenesis of B-cell malignancies, we also tested whether nuclear lamina is involved in the pathogenesis of germinal centre lymphomas and chronic lymphocytic leukaemia (CLL).
Methods: We used BL2 and naïve B cells as *in vitro* and *ex vivo* models for somatic hypermutation. ChIP-Seq, ChIP-PCR and ImageStream analyses were performed to establish Lamin B1 genome and nuclear binding dynamics in resting and activated BL2 and B cells. LMNB1 RNAi was used to obtain the functional evidence of the involvement of Lamin B1 in SHM in vitro. For *in vivo* studies, OVA immunised mice were used to study Lamin B1 dynamics in *de novo* formed spleen germinal centres. From a translational perspective, paired tissue microarray samples of diagnostic and transformed follicular lymphoma were analysed using immunohistochemistry and image analysis. Finally, comprehensive statistical analysis of CLL8 cohort patients was performed to test the impact of LMNB1 expression on various clinical parameters in CLL.
Results: We have found that genome binding of Lamin B1, a component of the nuclear envelope involved in epigenetic chromatin regulation, is reduced during B cell activation and formation of lymphoid germinal centres. ChIP-Seq analysis showed that kappa and heavy variable immunoglobulin domains were released from the Lamin B1 suppressive environment when SHM was induced in B cells. RNAi-mediated reduction of Lamin B1 resulted in spontaneous SHM as well as kappa-light chain aberrant surface expression. Finally, Lamin B1 expression level correlated with progression-free and overall survival in chronic lymphocytic leukaemia, and was strongly involved in transformation of follicular lymphoma.
Summary/Conclusions: In summary, here we report that Lamin B1 is a negative epigenetic regulator of SHM in normal B-cells and a "mutational gatekeeper", suppressing the aberrant mutations that drive lymphoid malignancy.

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MICROENVIRONMENT REGULATION OF PROGRAMMED DEATH-1 (PD1) RECEPTOR AND ITS LIGANDS PDL1 AND PDL2 IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background: The PD1 pathway is involved in the inactivation of immune effectors with potential reactivity against neoplastic cells in the tumor microenvironment (TME). PD1 binding to its ligand PDL1 inhibits proliferation, cytokine production, and cytotoxic activity of T-cell effectors. T-cells from CLL patients exhibit defective immunity, including impaired effector function leading to T-cell exhaustion. PDL1 checkpoint blockade restores immune dysfunction and impairs leukemia growth in the Eμ-TCL1 transgenic CLL mouse model. PD1/PDL1 ligation also affects BCR signaling, and because PD1 is also expressed on CLL cells, PD1 interference might directly influence tumor growth and proliferation directly. The PD1/PDL1 axis may be a therapeutic target, preferably in combination with BCR inhibitors (BCRi).
Aims: To investigate (1) expression of PD1 and PDL1/2 and their correlation with time to first treatment (TFTT); (2) the role of the TME in controlling the expression of the PD1 axis; and (3) the role of ibrutinib (IB) in the expression of PD1 and ligands.
Methods: CLL patients were prospectively enrolled at diagnosis (O-CLL1 protocol, clinicaltrial.gov identifier: NCT00917540). Gene expression (GE) analysis was performed using the GeneChipVR Gene 1.0 ST Array (Affymetrix) according to the manufacturer. Flow-cytometry (FC) was used to evaluate cellular phenotype (BD Biosciences) in an independent subset of patients. Lymph node samples from CLL patients were subjected to *in situ* immunolocalization analyses. Autologous T-cells (AAT) were obtained by *in vitro* exposure of patient T-cells with anti-CD3/CD28 and rIL2 in co-culture with CLL cells. Cultures were monitored daily until substantial clumping occurred and then tested for PD1 and ligand expression by FC. In selected experiments IB was added to cell culture.
Results: We evaluated GE of PD1 and PDL1/2 in 211 early-stage CLL patients. The impact of GE of PD1 and PDL1/2 on clinical outcomes in CLL cases [(n=211, median follow-up=39 months, range 6−82 months] indicated a significantly shorter TTFT in cases with higher levels of PDL2, while no significant impact was detected based on differences in PD1 or PDL1 gene levels. A Cox multivariate model showed that higher PDL2 gene expression retained an independent prognostic power (HR=1.9, 95%CI 1.1–3.4, P=0.022) in predicting TTFT together with IGHV-UM status, B-lymphocytosis≥5000/mm3 (P=0.020) and CD38 expression (P=0.021). *In situ* immunolocalization analysis of CLL tissue infiltrates indicated variable expression of PD1 mostly characterized small lymphoid elements,while PDL1 and PDL2 (with PDL2>PDL1) mostly characterized larger medium-sized elements within proliferation centers. Co-localization studies revealed PD1 co-expression on CD20+ CLL cells and in scattered CD3+ cells. Both ligands were variably expressed in CD20+CLL cells and few T-cells and macrophages showed expression of either ligand. AAT co-culture experiments showed a higher percentage of PD1+,PDL1+, and PDL2+ B-cells. Evaluation of T-cells detected an increase of CD3+, CD4+,and CD8+cells bearing both PD1 and PDL1,but not PDL2. IB (1uM) reduced the size of activated B- and T-cell clusters as well as PD1/PDL protein expression on CLL B-cells and only on the CD8+ T-cell subset.
Summary/Conclusions: Our findings indicate that expression of PDL2 characterizes a subset of high-risk early stage CLL patients. The expression of PD1/PDL1/PDL2 proteins is also characteristic of the CLL TME whereby, co-stimulatory signals derived from activated T-cells and/or the TME may modulate the PD1 axis, which is counteracted by IB.

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IL-4 INCREASES EXPRESSION OF POSITIVE REGULATORS OF BCR SIGNALLING IN CLL WHICH CAN BE OVERCOME BY CERDULATINIB

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Background: B cell receptor (BCR) mediated signalling is crucial for the pathogenesis of chronic lymphocytic leukemia (CLL). Drugs such as ibrutinib and idelalisib which inhibit BCR associated kinases have proved effective for the treatment of CLL but only suppress the disease without being curative. Some patients have developed resistance to these drugs following mutations, progress on therapy for unknown reasons, or cannot tolerate these drugs due to adverse events. We have shown that microenvironmental signals (*e.g.*, IL-4) can increase BCR expression and signalling, and can partially reverse the effects of BCR-kinase inhibition. GAB1, PTPN22 and FOXP1 can positively regulate BCR signalling in CLL; but the effect of IL-4 on these proteins has not previously been studied. Insight into how IL-4 promotes BCR signalling may allow the development of novel drugs that overcome resistance to kinase inhibitors. Cerdulatinib (cerd) is an inhibitor of both Syk (pivotal to BCR signalling) and JAK1/3 (integral for IL-4 signalling). Inhibition of Syk has been shown to induce apoptosis of CLL samples resistant to ibrutinib. Cerd is currently in phase II clinical trials in patients with relapsed/refractory B cell malignancies including CLL.
Aims: To investigate the effect of IL-4 on the regulation of BCR signalling in CLL and how this is modified by cerdulatinib
Methods: Eighteen primary CLL samples were treated with IL-4 +/-cerd (1μM) and expression of FOXP1, GAB1, PTPN22, SOCS1 and SOCS3 assessed by immunoblotting. The effect of cerd on apoptosis was assessed by flow cytometry and PI/Annexin V staining.
Results: Primary human CLL cells treated with IL-4 for 24hr significantly increased expression of positive regulators of BCR signalling FOXP1 and GAB1 in CLL samples with un-mutated IGHV (U-CLL); no change in expression in FOXP1 or GAB1 was seen in CLL samples with mutated IGHV (M-CLL). There was a 40% increase in PTPN22 expression in IL-4-treated U-CLL samples *vs* no change in M-CLL. Cerd, at therapeutic concentrations, blocked IL-4 mediated increases in FOXP1, GAB1 and PTPN22 and pSTAT6 (a positive control for IL-4 signalling). After 24hr IL-4 selectively increased expression of the negative regulators of IL-4 signalling, SOCS1 and SOCS3 in U-CLL, but not M-CLL cases, and this could be blocked by cerd. Cerd potently inhibited the signalling of other cytokines known to play a role in CLL biology (IL-6, IL-10, IL-15, IL-21 and IFNγ) which utilise either JAK1 or JAK3 for activation of STAT proteins. IL-4, CD40L and BCR ligation signals to CLL cells in lymph nodes can promote resistance to therapies such as the BCL2-inhibitor venetoclax. We have shown that cerd can overcome IL-4/CD40L induced expression of pro-survival proteins MCL1 and BCLxL and that cerd in combination with venetoclax induced apoptosis in a synergistic manner in the presence of IL-4/CD40L. We now extend these results to assess the important effect of this drug combination in the presence of BCR stimulation. The combination of cerd and venetoclax in the presence of either BCR signalling (bead immobilised anti-IgM) alone, or combined with IL-4 and CD40L, induced synergistic killing, with greater CLL cell death than with either drug alone.
Summary/Conclusions: These results provide evidence that IL-4 may increase BCR signalling by upregulating the expression of positive regulators of BCR signalling in U-CLL and that this can be overcome by cerd. These results support the continued use of cerd in clinical trials for the treatment of CLL, alone or in possible combination with venatoclax.

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INSIDE-OUT VLA-4 INTEGRIN ACTIVATION IS MAINTAINED IN IBRUTINIB-TREATED CHRONIC LYMPHOCYTIC LEUKEMIA EXPRESSING CD49D: CLINICAL RELEVANCE

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Background: VLA-4 (CD49d/CD29), a key molecule mediating cell microenvironmental interactions, can be activated via inside-out by BCR triggering in normal B cells. In chronic lymphocytic leukemia (CLL), nothing has been so far reported regarding the VLA-4 activation mechanisms and their modulation by ibrutinib, a drug that is known to determine an impairment of microenvironmental interactions with consequent shrinkage of tumor masses, and efflux of CLL cells into the blood stream.
Aims: To investigate in CLL the influence of VLA-4 expression/activation on ibrutinib response in-vivo.
Methods: VLA-4 activation was assessed by flow cytometry using conformation sensitive anti-CD29 mAbs (HUTS-21) and LDV-containing VLA-4 ligands, and measured as VLA-4 receptor occupancy (RO) (Chigaev *et al.* J Biol Chem, 2009). BCR engagement was performed using goat F(ab)2 anti–human IgM. In-vitro studies were carried out on purified VLA-4+ CLL cells exposed in-vivo to ibrutinib. The clinical impact of VLA-4/CD49d expression on ibrutinib treatment was evaluated by measuring the kinetics of absolute lymphocyte count (ALC), the reduction of lymphadenopathy measured as sum of products of the diameters (SPD) % reduction from baseline, and the clinical outcome, as defined by progression free survival (PFS) in CLL patients treated with ibrutinib single agent in the context of name patients program, clinical trials, and real world (n=97).
Results: BCR stimulation (n=27) induced VLA-4 activation (mean RO control *vs* stimulated: 0.40 *vs* 0.52, p=0.0006), and increased cell adhesion (stimulated/control: 4.7 *vs* 7.5; p=0.0002). By comparing day 30 (t30) in-vivo ibrutinib-treated CLL cells with pre-treatment (t0), we show that the ibrutinib-dependent BCR signaling impairment, although reducing the constitutive VLA-4 activation (mean RO t0 *vs* t30: 0.40 *vs* 0.30, p=0.02) and CLL cell adhesion (mean adhesion t0 *vs* t30: 4.7 *vs* 2.1, p=0.013), was overcome by exogenous BCR triggering, which re-activated VLA-4 at levels similar to those of ibrutinib naïve cells (mean RO: 0.49 at t30 *vs* 0.52 at t0). ALC data were available at pre-treatment and at days 30-60-90-120 on ibrutinib in 97 patients (52 CD49d+) (Fig.1A); CD49d+ CLL showed no ALC rise, whereas CD49d- CLL showed the typical ibrutinib-induced ALC peak. At day 30 median % ALC change from baseline for CD49d- and CD49d+ CLL were 126.8% and -4.4% (p=0.0002). When comparing the extent of nodal response, available for 57 cases (30 CD49d+), a significantly minor % SPD reduction from baseline was observed in CD49d+ *versus* CD49d- CLL: 70.5% *vs* 83% (p=0.033) at 6 months and 81.5% *vs* 92.0% (p=0.019) at 12 months (Fig.1B). The impact of CD49d expression on patient outcome was evaluated in the whole cohort (median follow-up, 24.5 months). PFS was inferior in CD49d+ compared to CD49d- CLL (median PFS 39.3 months, *vs* not reached; p=0.004), even when considering the concomitant presence of *TP53* disruption and CD49d+ expression (Fig.1CD). A multivariate Cox regression analysis confirmed the relevance of CD49d, along with *TP53* disruption and UM *IGHV* mutational status, as independent predictor of shorter PFS in ibrutinib-treated CLL.

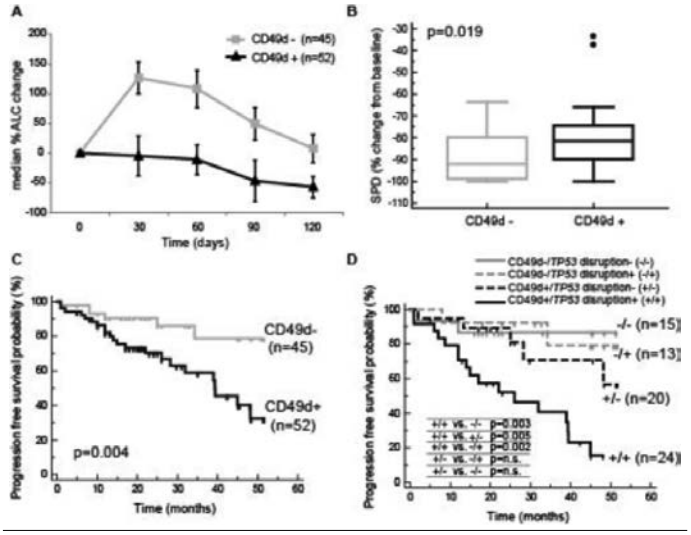

Figure 1.

Summary/Conclusions: Altogether, these data suggest that during ibrutinib treatment CD49d+ CLL cells residing in tissue sites keep receiving BCR-mediated BTK-independent stimuli that, by inducing inside-out VLA-4 activation, result in enhanced cell retention, with consequent reduced lymphocytosis, relatively lower and/or slower nodal response, eventually leading to inferior outcome for CD49d+ CLL patients.

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IBRUTINIB RESULTS IN REDUCTION OF PHOSPHORYLATION OF MULTIPLE KINASES IN THE B-CELL RECEPTOR PATHWAY IN CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL): RESULTS OF THE BLOODWISE TAP ICICLLE STUDY

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Background: Ibrutinib is an oral Bruton tyrosine kinase (BtK) inhibitor which has advanced the clinical management of CLL. Ibrutinib binds irreversibly to the cysteine 481 residue of the BtK protein, rendering it inactive. Btk inhibition affects the phosphorylation of other intracellular kinases resulting in an immediate redistribution of CLL cells and subsequent apoptosis. We investigated the impact of ibrutinib on the phosphorylation of upstream and downstream kinases in the B-cell receptor pathway in real time in the IcICLLe study (ISRCTN12695354).
Aims: The IcICLLe trial was a single arm, multi-centre feasibility study of ibrutinib in two cohorts of CLL patients: (A) 20 treatment-naïve (TN) requiring treatment (according to IWCLL criteria); and (B) 20 relapsed/refractory (RR). All patients received continuous oral therapy with ibrutinib (420mg once daily) from registration until disease progression. The primary endpoint of the trial was the proportion of patients achieving minimal residual disease (MRD) negative remission (depletion of CLL ≤0.01% in peripheral blood (PB) & bone marrow (BM)) within 6 months of trial treatment. Exploratory endpoints included the assessment of phosphorylation of intracellular kinases in the B-cell receptor pathway.
Methods: A panel of markers was assessed on PB & BM taken at screening, and 1 & 6 months. PB was also taken at baseline (0 hours), 4 & 24 hours, 7 & 14 days, and 2, 9 & 12 months. The phosphorylation of SyK pY348, BtK pY551, ERK1/2, AkT S473 was assessed in 4 conditions at each time point: unstimulated +/- ibrutinib, and stimulated with IgM/IgD +/- ibrutinib. $1x10^6$leukocytes were tagged to extracellular antibodies (CD3/CD19) conjugated to fluorochromes. Ibrutinib (10uM) was added to the cells for 30 minutes at 37ºC followed by anti-IgM/IgD stimulation (10ug/ml). The BD phosflow protocol was followed to lyse/fix/permeate the CLL cells. Antibodies to BtK pY551, Syk pY348, ERK1/2 pT204/pY204, Akt pS473 were used tagged to fluorochromes (from BD Biosciences). Cells were acquired on a BD Fortessa flow cytometer.
Results: The phosphorylation of BtK, Syk, Akt and ERK1/2 was analysed in cells at the specified time points and conditions for 20 TN and 20 RR CLL patients. Baseline phosphorylation of all kinases was similar in both PB & BM. Phospho-BtK showed no stimulation on addition of IgM/IgD 4h after initiating therapy. There was a strong (2-4 fold) increase in phosphorylation of Syk kinase with IgM/IgD even in the presence of ibrutinib *in vitro*. This effect was profound in the first 2 months of ibrutinib therapy with a general decrease in phosphorylation after 6 months. Baseline stimulation of ERK1/2 gave a 1.5-2 fold increase in phosphorylation but the effect was abrogated within 1 month of ibrutinib therapy. Akt S473 phosphorylation was maintained after 6-12 months of therapy although the degree of phosphorylation decreased at later time points. Syk, AkT and ERK1/2 phosphorylation was unaffected by the addition of ibrutinib *in vitro*. The pattern of phosphorylation was found to be relatively consistent in responding patients. One patient with progressive CLL had sustained phosphorylation in all markers despite ibrutinib therapy.
Summary/Conclusions: The effect of ibrutinib on the phosphorylation of various kinases in the B-cell receptor pathway was analysed in real time. Syk continued to be phosphorylated over the course of treatment, which is logical as this kinase is upstream of BtK. That the degree of phosphorylation declined over time (even with stimulation) suggests a general inhibitory effect of ibrutinib on CLL cells. ERK1/2 phosphorylation is effectively blocked and there is partial reduction of phosphorylation of AKT S473. Combinations of BtK inhibitor with a Syk or PI3 kinase inhibitor may result in complete BCR blockade. Phosphorylation patterns may also act as an adjunct to ascertain the response to therapy.

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EVALUATION OF COMBINATIONAL THERAPIES FOR RELAPSED/REFRACTORY CLL WITH MUTATED P53

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Background: Chronic lymphocytic leukemia (CLL) is a heterogeneous disease with survival ranging from months to decades. CLL patients harboring *TP53* alterations are well known to be refractory to standard therapies; however, recent studies indicate that ibrutinib, a Bruton's tyrosine kinase (BTK) inhibitor, suppresses the B-cell receptor (BCR) signaling pathway and is an effective treatment option for these patients. Unfortunately, many patients with *TP53* alterations will ultimately fail ibrutinib-based therapies. Similarly, we have used a mouse model of refractory p53 mutant CLL (*Eμ-TCL1;p53^{R172H}*), and reported that while ibrutinib is effective in reducing the CD5+CD19+ population and extending survival, these mice eventually succumb to the disease (Lee HJ, *BCJ* 2016). These incomplete therapeutic responses indicate that ibrutinib provides only a temporary respite for this refractory disease, and highlights our need to develop more potent and targeted combinations.
Aims: Ibrutinib is effective in delaying (but not eliminating) leukemic progression in p53 mutant CLL, suggesting that combinational therapies that inhibit BCR signaling and activate apoptotic programs may be effective therapeutic strategies. Thus, agents that do not require activation of p53 but are effective in blocking oncogenic pathways (BTK and BCL-2) are attractive options. Currently, ibrutinib and ABT-199 meet this criteria and thus, we *hypothesize that simultaneous inhibition of the BTK- and BCL-2-pathways will be an effective strategy in treating p53 mutated CLL.*
Methods: To test this, we used RNA-Seq to examine expression changes in B-cells from *Eμ-TCL1* mice carrying either wild type or a single *p53^{R172H}* hotspot mutation (corresponding to *p53^{R175H}* in humans) following ibrutinib treatment. qRT-PCR and IHC were used to validate expression of key targets within pathways amenable to combinational therapy. Hematopoietic tissues were subjected to combinational therapies to interrogate efficacy.
Results: We have shown that ibrutinib downregulates the BTK- and ERK-pathways regardless of p53 status. However, less is known in regards to global expression changes in p53 mutant CLL following BTK inhibition. To investigate this, we performed RNA-Seq analyses using malignant B-cells from untreated and ibrutinib treated *Eμ-TCL1;p53^{R172H}* and *Eμ-TCL1* mice. Pathway analyses revealed that CLL cells harboring a single *p53* mutant allele retained a partial ability to activate p53-dependent programs. qRT-PCR revealed robust activation of p53-dependent anti-proliferative targets like p21, but only modest activation of pro-apoptotic targets (*e.g.*; *PUMA*), suggesting these p53 mutant CLL cells have a diminish capacity to activate apoptosis or overcome apoptotic inhibitors. To explore this altered bi-modal p53 activation, we performed IHC and observed that apoptotic activation was hampered by increased BCL-2 expression. To examine whether this BCL-2-dependent inhibition could be overcome, malignant B-cells were treated with ibrutinib alone, ABT-199 (a BCL-2 inhibitor) alone, or in combination. Here, we observed that ABT-199 was sufficient to activate apoptosis, regardless of p53 status, and that its use in combination with ibrutinib drastically reduced cell viability.
Summary/Conclusions: Together, these data indicate that patients with a partially attenuated p53 pathway may retain the ability to activate apoptosis if molecular barriers are removed (*e.g.*; BCL-2 via ABT-199). Furthermore, these results suggest that combinations with BTK- and BCL-2 inhibitors may be therapeutically beneficial for patients with mutated TP53.

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THE DNA REPLICATION PATHWAY HAS POTENTIAL PREDICTIVE VALUE FOR TKI RESPONSE AND THERAPEUTIC INTERVENTION IN CHRONIC MYELOID LEUKAEMIA

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Background: Chronic myeloid leukaemia (CML) is a myeloproliferative disease which arises in a haemopoietic stem or multipotent progenitor cell with the t(9;22)(q34;q11) chromosomal translocation.Tyrosine kinase inhibitors (TKIs) were developed to target the constitutively active oncoprotein BCR-ABL, which is expressed as a result of this translocation. TKI therapy has significantly improved patient survival, however predicting response to therapy is one of the unmet clinical challenges in CML. Moreover, TKIs are unable to target the leukaemic stem cells (LSCs) which drive the disease; persistence of LSCs therefore remains a major obstacle to curing CML. Understanding the mechanisms that LSC employ to survive TKI treatment is necessary to design essential therapeutics to eliminate CML in the future.
Aims: To identify genes with predictive value for TKI response and to determine the efficacy of drug targeting one of the key pathways identified.
Methods: Microarray, Fluidigm, Real-time PCR, FACS based cell cycle and Annexin V apoptosis analysis, Trypan blue exclusion cell counts.
Results: Analysis of bulk CML patient microarray data (GSE 47927) identified 323 deregulated genes either in the stem cell population or during disease progression, important for self-renewal, DNA damage and repair, cell cycle and survival. These genes were validated in 60 samples from the SPIRIT 2 clinical trial [a multicentre phase III randomised trial comparing the TKI imatinib (400mg daily) *versus* Dasatinib (100mg Daily)] with 18 months follow-up data regarding molecular response to TKI treatment. Patients were stratified as good/intermediate/poor responders to TKI and the gene signature significantly differentially expressed was identified. These data highlighted the DNA repair genes as having potential predictive value, in particular, the minichromosome maintenance (MCM) protein and origin of replication (ORC) family of genes, involved in DNA replication and cell cycle regulation. Single cell analysis of CD34+ cells across the patient cohort identified considerable heterogeneity of expression of MCMs and ORCs, with ORC3, in particular, exhibiting a different expression profile in good/intermediate/poor responders (n=3 of each). In addition single cell analysis highlighted a significant difference in the expression of MCM2, -4, -7 & ORC2 in the most primitive LSC (CD34+38-90+93+) compared to CD34+38-90+93- cells. Next, we investigated the ability of heliquinomycin (HQ), a potent helicase inhibitor of MCM on its own and in combination with IM to target the CML cell line K562. Our extensive dose and time response studies followed by FACS-based apoptosis and cell cycle analysis prove the potency of HQ and its synergistic action in combination with imatinib. We also investigated the changes in a panel of cell cycle and DNA damage response genes at the transcript level in response to HQ and imatinib in the K562 cell line. Overall the data generated indicates that targeting the MCM pathway in combination with BCR-ABL inhibition is a rational approach for future therapeutic intervention in CML.
Summary/Conclusions: Global 'omics' experimental approaches are valuable for identifying novel pathways deregulated in CML. This combined with single cell 'omics' studies enables the heterogeneity of gene expression and the response of individual LSCs to TKI to be evaluated. Our data indicate that the DNA replication pathway plays an important role in CML, with levels of MCMs and ORCs having potential predictive value in TKI response and are a promising drug target in CML.

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SIGNAL TRANSDUCING ADAPTOR PROTEIN-1 (STAP-1) MAINTAINS CHRONIC MYELOID LEUKEMIC STEM CELLS

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Background: Signal transducing adaptor protein (STAP) -2 was cloned as a c-fms binding protein. Previously, we have demonstrated that STAP-2 binds to BCR-ABL, which is constitutively activated in chronic myeloid leukemia (CML), via its SH2-like domain and enhances BCR-ABL activity leading to activation of downstream molecules, including ERK, STAT5, BCL-xL and BCL2. The family of STAPs includes STAP-1, identified as a c-kit interacting protein, and STAP-2. While STAP-2 is expressed ubiquitously, STAP-1 has hematopoietic-specific expression in mice. It is still unknown whether STAP-1 plays a role in CML, although STAP-1 is expected to have similar functions based on the structural homology between STAP-1 and STAP-2.
Aims: To elucidate the role of STAP-1 in CML using mouse model and human samples.
Methods: We generated STAP-1 deficient mice of the C57BL/6J genetic background. For establishment of CML mouse model, we isolated Lineage (Lin)$^-$ Sca-1$^+$ c-kit^{high} (LSK) fraction of bone marrow (BM) cells from STAP-1$^{+/+}$ and STAP-1$^{-/-}$mice, infected them with retrovirus carrying MSCV-BCR-ABL-ires-GFP, and transplanted into congeneic recipients, that were named Wild type (WT) and STAP-1$^{-/-}$CML mice, respectively. Human BM samples were collected after informed consent, using protocols approved by the Investigational Review Board of Osaka University Hospital.
Results: Using Western blot and immunoprecipitation assay, we confirmed that STAP-1 binds to BCR-ABL. CML mice model was then employed to analyze the role of STAP-1. We found that STAP-1$^{-/-}$ CML mice showed significantly longer survival than WT CML mice (Fig. 1). STAP-1$^{-/-}$ CML mice displayed less severe splenomegaly and lung hemorrhages compared to WT, suggesting that loss of STAP-1 attenuates CML progression. To investigate how STAP-1 regulates CML progression, we evaluated leukemic stem cells (LSCs) in CML mice. The absolute numbers of STAP-1$^{-/-}$ LSCs (GFP+ LSK) in BM and spleen were significantly lower than those of control (WT *vs* STAP-1$^{-/-}$; 2090.3 ± 694.07 cells *vs* 412.57 ± 114.07 cells in BM, p=0.0291; 12.9 ± 1.75 ×10^4 cells *vs* 4.09 ± 0.72 ×10^4 cells in Spleen, p=0.0009). In colony-forming assay *in vitro*, STAP-1$^{-/-}$ LSCs generated less colonies in the first and second plating compared to WT LSCs. These data indicated that deletion of STAP-1 would impair self-renewal capacity of LSCs. When we transplanted 5,000 LSK cells from STAP-1$^{+/+}$ or STAP-1$^{-/-}$ mice without BCR-ABL transduction in the presence of competing BM cells, deletion of STAP-1 had no effects on engraftment at 28 days after transplantation. Furthermore, we measured the expression of STAP-1 in BM cells derived from patients in the chronic phase of CML. As a result, STAP-1 mRNA was abundant in the LSC (CD34$^+$ CD38$^-$ Lin$^-$) compartment.

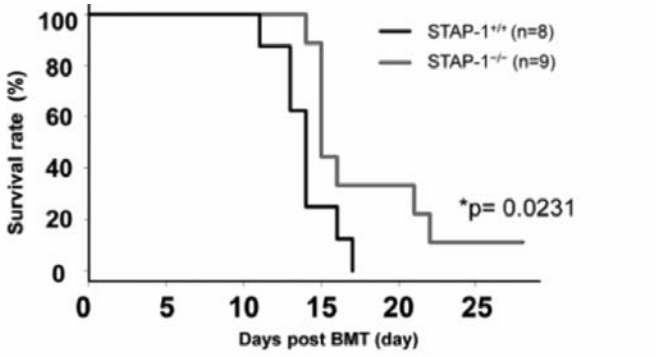

Figure 1.

Summary/Conclusions: In this study, we utilized CML mouse model and showed that STAP-1 is required for progression of CML. Our findings indicate that STAP-1 has an indispensable role in LSC maintenance, while normal hematopoietic stem/progenitors were not affected by STAP-1 deficiency. Although a majority of patients have a durable response to BCR-ABL tyrosine kinase inhibitors, the outcome of patients who fail the treatment due to primary or acquired resistance is still miserable. Our findings in mice and human suggests that STAP-1 could be a therapeutic target for CML. Further analysis will be needed to clarify the molecular mechanisms by which STAP-1 regulates the progression of CML and maintains survival of LSCs.

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TELOMERE SHORTENING IN CD34+38- BCR-ABL POSITIVE BONE MARROW CELLS FROM NEWLY DIAGNOSED PATIENTS WITH CML CORRELATES WITH THE CLONE SIZE OF THE LEUKEMIC STEM CELL COMPARTMENT

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Background: Chronic myeloid leukemia (CML) is a clonal stem cell disorder characterized by the BCR-ABL translocation. Previous work provides evidence that based on the size of the leukemic stem cell (LSC) clone within the CD34+38- population at diagnosis, chronic phase (CP) of CML can be stratified into early and late CP. Patients in late CP have a higher LSC burden going along with an inferior response to TKI therapy. Telomeres shorten with each

cell division and telomere length (TL) in peripheral blood cells has been shown to correlate with disease stage, response to treatment and duration of CP in CML patients. However, the use of TL as a routine clinical biomarker in CML has been complicated by considerable inter-individual, mostly genetic variability in TL ideally requiring non-clonal control cells.
Aims: Based on these considerations, we used a modified Q-FISH technique in order to be able to separately study TL directly in BCR-ABL+ LSC *vs* BCR-ABL- control cells within the CD34+38- hematopoietic stem cell compartment of diagnostic patients with CML in CP.
Methods: 15 patients (median age: 59 years, range: 41- 72 years) diagnosed with CML in CP of the NCT00852566 study (Nordic CML Study Group) were retrospectively analyzed. Of the patients studied, clinical data were available for 14 patients. Of those, 2 (14%) belonged to the Sokal high risk group, 5 (36%) to intermediate and 7 (50%) to the low risk group. CD34+38- cells sorted from bone marrow samples were tested with the standard FISH method using dual fusion dual color BCR-ABL1 probe following standard procedures. After capturing the BCR-ABL staining using confocal microscopy, samples were re-processed for TL analysis by Q-FISH using established protocols. TL staining was analyzed in all previously captured cells allowing the identification of BCR-ABL+/- cells within the same sample. Analysis and quantification of BCR-ABL FISH staining and TL measurement by Q-FISH were performed in blinded fashion.
Results: Overall, we observed significantly shortened TL in the BCR-ABL+ compared to BCR-ABL- cells (-4.9 arbitrary units (a.u.) range: -53.7 to 16.9 a.u., p=0.04). Next, we correlated the clone size (i.e. the proportion of BCR-ABL+ positive cells within the CD34+38- compartment) with the degree of telomere shortening in LSC. Mean clone size of the patients was 59.9 ± 32.0 % S.D. Of note, we found a significant negative correlation (R^2=0.36, p=0.02) between TL and clone size strongly supporting the notion that increased expansion of the BCR-ABL+ LSC pool leads to accelerated telomere shortening. Correlation with clinical prognostic data such as Hasford (R^2=0.07, p=0.41) or Sokal (R^2=0.04, p=0.38) score did not reveal any statistically significant correlation with the degree of telomere shortening probably due to the small sample size analyzed in this pilot study.
Summary/Conclusions: In this study, we provide further evidence for accelerated telomere shortening in BCR-ABL+ LSC as compared to their normal CD34+CD38- counterpart in CP CML samples at diagnosis. Interestingly, the degree of TL shortening linearly correlates with the clone size of the BCR-ABL+ LSC compartment. Thus, this retrospective study (now on the LSC level) further supports a role of TL as a prognostic and predictive biomarker in newly diagnosed patients with CML pending confirmation in prospective trials.

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GENOMIC CHARACTERIZATION OF CML AT DIAGNOSIS REVEALS PREEXISTING SOMATIC MUTATIONS THAT MAY PREDICT PROGRESSION TO BLASTIC PHASE INDEPENDENTLY OF BCR-ABL1 MUTATIONS

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Background: Blastic phase of chronic myeloid leukemia (BP-CML) remains mostly incurable even with newer generation tyrosine kinase inhibitors (TKI) and represents an unmet clinical need. Although in recent years a dramatic reduction in the transformation of chronic phase (CP-CML) to BP-CML has been observed, still up to 5% of patients will progress to BP-CML despite treatment with TKI. Prospective identification of such patients may have a significant clinical impact. There are only few reports to date which use next-generation sequencing (NGS) to look for somatic mutations - other than those affecting kinase domain of *BCR/ABL1* - at the time of diagnosis (Dx) which could have a prognostic/predictive value.
Aims: We analyzed the spectrum of somatic mutations in two groups of CML patients with clinically different disease course: first group (BP) comprised of 11 patients who progressed to BP-CML despite treatment with TKI and/or allo-HSCT (one patient) and died (paired samples from Dx and BP were analyzed); second group (MMR) included Dx samples from 36 patients who achieved major molecular response (MMR) on TKI within 6 months and remained in MMR for at least 48 months from Dx.
Methods: Targeted enrichment strategy using custom designed capture probes (SeqCap EZ, Roche NimbleGen) followed by NGS on Illumina platform was employed. More than 1200 genes implicated in human cancer were included. Common variants (>1%) gathered in large genomic databases and our internal database were filtered out and the subsequent analysis was focused on putative protein damaging variants, supported by variant effect prediction tools such as PolyPhen-2, SIFT or CHASM. All reported variants were reconfirmed by Sanger sequencing.
Results: BP group comprised of paired samples from 11 CML patients who progressed to BP and died despite treatment with TKI. Median age at diagnosis was 53y (range 26 -77), median time to progression for 9 patients (2 were diagnosed in accelerated phase or BP) was 17,5 months (mo) (range 4-108) and median survival was 22 mo (range 10 -116). None of those patients harbored *BCR/ABL1* mutation at the time of Dx and progression to BP-CML, 4 patients had additional chromosomal alterations at progression to BP including two frequent (trisomy 8 and monosomy 7). Targeted enrichment followed by NGS allowed us to achieve deep coverage (>80% ge50). Median number of rare variants was 26 (range 18-38) and 29 (range 23-32) for Dx and progression samples respectively. In majority of patients upon progression (72%, 8/11) we detected new and previously described mutations in selected genes, which are frequently mutated in myeloid malignancies, namely in *RUNX1* (36%, 4/11), *DNMT3A* (27% 3/11) *IDH1/IDH2 (18%, 2/11)* and *ASXL1* (18%, 2/11). In six patients (54%, 6/11) mutations in these genes (excluding *IDH2,* detected only in BP sample) were preexisting at the time of Dx. These results were compared to second, control group that comprised of diagnostic samples from 36 patients (median age at diagnosis 53y, range 23 -75) who were optimal responders to TKI and remained in MMR for at least 48mo (median time in MMR: 73mo; range 48-128). In MMR group, the median number of rare variants was lower than in BP group in Dx samples (21, range 14-32). However, in 2 patients (2/36, 5%) frameshift mutation in *ASXL1* (p. Gly643_Gly644fs) was detected, identical as in one of BP patients. Additionally, one patient harbored *RUNX1* mutation (p. Arg201Gln) which was not detected in the BP group.
Summary/Conclusions: Our results provide new insights into the already complex genomic landscape of blastic phase of CML. We suggest that a significant number of patients with poor disease outcome may harbor preexisting mutations in *DNMT3A, RUNX1* and *IDH1.* In contrast, mutations in *ASXL1* may be present at Dx in patients who will remain in long-term remission.

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INCREASED INDOLEAMINE 2,3-DIOXYGENASE (IDO1) ACTIVITY IN EARLY CHRONIC PHASE CHRONIC MYELOGENOUS LEUKEMIA (CML-CP) IS REDUCED BY NILOTINIB THERAPY AND PREDICTS MOLECULAR RESPONSE

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Background: Indoleamine 2,3 dioxygenase (IDO1) is the rate-limiting enzyme in the metabolism of the essential amino acid tryptophan (TRP). IDO1 is induced mainly by interferons during infection and inflammation. Strong IDO1 activity depletes tryptophan, which results in reduced T cell activation and proliferation as well as expansion of immunosuppressive regulatory T cells. Deregulation of IDO1 activity has been linked to cancer immune evasion, but its role in chronic phase (CP) CML has not been investigated in detail.
Aims: Determination of IDO1 levels and activity in plasma CML-CP patients in the course of tyrosine kinase inhibitor therapy and their correlation with clinical and immunological parameters as well as molecular response.
Methods: A large panel of circulating cytokines and components of the IDO-pathway (soluble IDO1=sIDO1 and kynurenine/tryptophan ratio=KYN/TRP as a product of IDO1 activity) as well as various leukocyte populations such as plasmacytoid dendritic cells (pDC) were analyzed alongside the prospective pan-european ENEST1st clinical study (NCT01061177). This substudy included 52 nilotinib-naïve chronic phase (CP)-CML patients that were subsequently treated with 300mg BID nilotinib and longitudinally analyzed at months 6 and 12 of therapy. Molecular responses were quantified in central EUTOS reference laboratories.
Results: Soluble IDO (sIDO1) levels and KYN/TRP ratio are significantly upregulated in newly diagnosed CP-CML and drop during nilotinib therapy. sIDO1 levels significantly correlate with increased KYN/TRP, suggesting increased IDO1 activity at diagnosis. Increased sIDO is linked to a pro-inflammatory status in CML patients, as it positively correlates with increased serum neopterin levels as well as to various other pro-inflammatory markers, such as IFN-g, IL-8, IL-10, IL-17A, sVEGF-A, sVCAM-1 and sTNFR-1. Albeit being an IFN-regulated gene, IDO1 activity (KYN/TRP) negatively correlates with the proportion of pDC, the main producers of IFN-α. Interestingly, a higher KYN/TRP is linked to superior molecular response, as demonstrated by a significant correlation

of the KYN/TRP ratio to BCR-ABL transcript levels. Patients having a high KYN/TRP ratio (> mean +2SD of post therapy levels) reach deep molecular response rates (i.e. $MR^{4.5}$) significantly earlier and at higher rates. Moreover, combining KYN/TRP with sCD62L levels, a recently identified predictive biomarker, resulted in a score robustly predicting the odds of achieving deep molecular response.

Summary/Conclusions: CML diagnosis in CP is linked to an increased inflammatory status, as shown by increased levels of sIDO and its metabolites kynurenine leading to an increased KYN/TRP ratio. In solid cancer increased IDO expression/activity is linked to inferior outcome by favoring immune evasion. In contrast, in CML an increased KYN/TRP ratio is associated with improved molecular outcome during nilotinib 1st-line therapy. One reason could be that IDO activity may reflect endogenous IFN-α production, a known factor favoring immune-mediated CML-control. The predictive potential of KYN/TRP is currently verified in an independent cohort.

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BCR-ABL1 COMPOUND MUTANTS DISPLAY DIFFERENTIAL AND DOSE-DEPENDENT RESPONSES TO PONATINIB

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Background: Despite the dramatic improvement of prognosis in CML patients due to the introduction of tyrosine kinase inhibitors (TKIs), resistance to therapy occurs in a considerable proportion of patients. The best-characterized mechanism of resistance is the acquisition of mutations in the BCR-ABL1 tyrosine kinase domain (TKD) affecting TKI binding. The third-generation TKI ponatinib exerts strong anti-neoplastic effects even in advanced CML stages and is capable of suppressing the kinase activity of BCR-ABL1 carrying any single mutation including T315I. Nevertheless, resistance to ponatinib can evolve in sub-clones carrying BCR-ABL1 variants with two or more mutations on the same allele, if the IC_{50} values for this TKI exceed the maximum achievable effective plasma levels (efC_{ave}). These co-called compound mutations (CMs) are associated with increased oncogenic potential in comparison to individual mutations, and represent a powerful mechanism of potential resistance to all currently available TKIs. The occurrence of compound mutations has been linked particularly to sequential treatment with different TKIs, and the identification of their responsiveness to ponatinib is of paramount importance for the subsequent clinical management.

Aims: 1. To determine the spectrum of highly TKI-resistant CMs. 2. Measure the responses of BCR-ABL1 CMs to ponatinib

Methods: We have established a BCR-ABL1 protein model facilitating assessment of the presumptive impact of 27 different CMs involving important functional sites of the BCR-ABL1 TKD, and including constellations expected to display high resistance to ponatinib. To assess the anticipated responses to ponatinib in vitro, we have introduced all BCR-ABL1 CMs into Ba/F3 cells using a recently published transposon-mediated approach (Byrgazov *et al.*, Oncotarget 2016, 7(47):78083-78094), and IC_{50} values of ponatinib were determined.

Results: Most CMs involving sites with no previous evidence for implication in resistance to ponatinib displayed IC50 values below 10 nM. This efC_{ave} is readily achievable even with the 15mg daily dose of ponatinib. CMs revealing elevated resistance to ponatinib *in vitro* almost invariably included T315I or F317L mutations. While most CMs involving T315I revealed very high IC50 values, some of the predicted compound mutations containing F317L displayed an IC50 for ponatinib in the range of the efC_{ave} achievable only with a daily dose of 45mg. These observations are supported by clinical findings in the PACE trial which revealed impaired responses of patients with CMs involving F317L who had received average daily doses of ponatinib below 45mg (Deininger *et al.*, Blood 2016, 127(6):703-12).

Summary/Conclusions: Current strategies that aim at decreasing the dose of ponatinib to prevent severe side effects should carefully consider the presence and type of mutations in the BCR-ABL1 TKD to enable effective treatment. It would be highly desirable, therefore, to implement testing of the effective plasma drug concentrations and monitoring the kinetics of mutant subclones covering also compound mutations in the routine diagnostic surveillance to provide a basis for optimized clinical management of patients treated with ponatinib.

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IS THERE EFFECTIVE IMMUNE SURVEILLANCE AGAINST CHRONIC MYELOID LEUKAEMIA? NO

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Background: *Immune surveillance* refers to a process whereby the innate and adoptive immune systems recognize and eliminate cancer cells on the basis of cancer-specific or related antigens or other molecules. Substantial data support the concept of immune surveillance in rodents but data in humans are less convincing and apply mostly to virus-induced cancers. However, considerable data indicate a strong anti-leukaemia effect in persons with chronic myeloid leukaemia (CML) receiving an allogeneic haematopoietic cell transplant. Most, if not all, of this anti-leukaemia effect is confounded with graft-versus-host disease (G*v*HD) and whether there is a allogeneic or host specific anti-leukaemia effect distinct from G*v*HD is controversial. Another interesting observation is about 40 percent of persons with CML and a deep molecular response to tyrosine kinase-inhibitors (TKIs) remain in molecular remission when TKI-therapy is stopped. Because TKI-therapy is very unlikely to eradicate every CML cell, some ascribe this therapy-free remission (TFR) a result of immune surveillance.

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Aims: Another interesting observation is about 40 percent of persons with CML and a deep molecular response to tyrosine kinase-inhibitors (TKIs) remain in molecular remission when TKI-therapy is stopped. Because TKI-therapy is very unlikely to eradicate every CML cell, some ascribe this therapy-free remission (TFR) a result of immune surveillance.

Methods: To test these hypotheses, we studied whether there was an increased incidence in CML in persons receiving immune suppression after solid organ transplants. IF immune surveillance is important in CML we would expect an increased incidence in this setting. We used a dataset from the Collaborative Transplant Study (CTS) which collects information on recipients of solid organ transplants beginning in 1985 from >300 transplant centers worldwide. Cancer incidence data were checked annually by questionnaire. Data for expected CML incidence were obtained from a cohort of identical size matched for age and sex from *Cancer Incidence in Five Continents* monitored for the same duration as the transplant cohort. Data collection and processing were approved by the Data Protection Agency in Germany and all participating centers complied with local ethical and privacy regulations.The CTS dataset consisted of 441,332 recipients of kidney (N=355,606), liver (N=47,846) and heart (N=37,880) transplants. Amongst kidney transplant recipients the standardized incidence ratio (SIR) for developing CML was 1.54 (95% confidence interval, 1.1, 2.1; p=0.01) representing 39 cases in 1,682,491 person-years at-risk *vs.* 25 expected (14 excess cases). Amongst liver transplant recipients the SIR was 1.72 (0.6, 4.0; P=0.34) representing 5 cases in 182,833 person-years at-risk *vs.* 3 expected (2 excess cases). Amongst heart transplant recipients the SIR was 3.47 (1.8, 6.1; p=0.0005) representing 12 cases in 173,015 person-years at-risk *vs*. 3 expected (9 excess cases). Data from recipients of kidney and liver transplants suggest immune-suppression does not increase risk of developing CML or does so very slightly. The increase in SIR in kidney graft recipients is generally-attributed to increased cancer surveillance including blood testing. Although the SIR of CML was substantially-increased after heart transplants, these persons receive high doses of ionizing radiations for diagnostic radiological procedures such as computer tomography (CT)-angiography. Ionizing radiations are a proved cause of CML which might explain the increased SIR.
Results: Our data, 25 excess cases of CML in 2,038,339 person-years at-risk observation suggest the magnitude of immune-surveillance do not support the hypothesis immune surveillance operates to an important extent to prevent CML in humans.
Summary/Conclusions: Consequently, the anti-leukaemia effect associated with allotransplants and the TFR observed after stopping TKI-therapy is unlikely to result from effective immune surveillance against CML.

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MUTATIONAL ANALYSIS IN BCR-ABL1 POSITIVE LEUKEMIA BY DEEP SEQUENCING BASED ON NANOPORE MINION TECHNOLOGY

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Background: In newly-diagnosed chronic phase (CP)-CML patients, 15–30% who start first-line tyrosine kinase inhibitors (TKIs) therapy will not reach an optimal response, and a BCR-ABL1 kinase domain (KD) mutation will be detectable in 25–50% of patients with treatment failure with an increased frequency of these mutations observed in accelerated phase and blast crisis patients. Currently, Sanger sequencing (SS) technique analyzing BCR-ABL1 is considered the gold standard for mutation detection knowing that this assay has a sensitivity of around 20 %, and therefore is unsuitable for identifying low-level variants (<20 % variant frequency). Recently next generation sequencing (NGS)-based assays have been reported for detecting BCR-ABL1 KD mutations; although these NGS strategies are more accurate and precise than SS, they are burdened by costs related to the initial investment, that is the sequencer purchase, the preparation of specific targets libraries, and the required reagents. MinION is a single molecule sequencer connected to a laptop through a USB3.0 interface, based on nanopore technology; it works by connecting two strands of DNA molecules by a hairpin, and sequencing them consecutively.
Aims: We describe a third- generation sequencing assay on MinON for detecting BCR-ABL1 KD mutations and compare the results to a SS-based test in 24 Ph+ leukemia cases.
Methods: Overall, 24 patients were included; among them, 12 (11 CML and 1 ALL cases) developed treatment resistance during the TKI's treatment course (Group 1) and 12 were at diagnosis (7 CML and 5 ALL cases) (Group 2). For ABL1 amplification and barcoding the total cellular RNA was extracted from peripheral blood cells. MinION library preparation, sequencing and data analysis were performed. All cases included in the study were analyzed by SS and MinION sequencing in blinded manner.
Results: Two sequencing runs were performed with the two different pools of patients: the first lasted eight hours and was carried out on the Group 1, whereas the second run included the Group 2 and lasted 24 hours to achieve a deeper sequencing. Sequencing results showed that 100% of ABL1 from exon 2 to 10 was covered and that the mean of the sequencing depth was around 150x and 1000x for Group 1 and 2, respectively. In any case, the depth of sequencing was never found below 50X. We found 10 BCR-ABL1 KD mutations in 9 patients belonging to the Group 1 (one case showed compound mutations). Notably, almost all mutations had a high allelic ratio. Despite a high depth of sequencing, MinION data analysis on the Group 2 was able to detect mutation only in a ALL case. Results from MinION and SS showed 92% concordance in all cases included in this study. Notably, mutations that were initially undetectable by SS became evident thanks to the indications coming from MinION analysis.
Summary/Conclusions:. Our findings demonstrate multiple advantages by using MinION approach, first of all the sensitivity: our comparison of MinION to SS identified mutations below the detection limit of SS (generally stimated around <20%) in 2 (22%) among the mutated cases, including mutations known to be clinically important. Another point on the side of the nanopore technology is the costs profile. Therefore, the main advantage of this technology is to allow a more efficient and sensitive analysis than SS at very competitive costs. In conclusion, we demonstrated that MinION is suitable for employment in hematology laboratory for detecting BCR-ABL1 KD mutation in Ph+ leukemias.

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THE AUTOMATED MOLECULAR TECHNIQUE "ULTRA" ALLOWS A SENSITIVE AND ACCURATE BCR-ABL1 QUANTIFICATION IN PATIENTS AFFECTED BY CHRONIC MYELOID LEUKEMIA

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Background: The chronic myeloid leukemia (CML) is characterized by the presence of the Philadelphia chromosome and the BCR-ABL1 fusion gene. The introduction of tyrosine kinase inhibitors (TKIs) significantly improved the survival, but 15% of patients don't reach the optimal responses at the defined end-points or develop secondary resistance. The 2013 ELN guidelines identified as fundamental the early molecular response (BCR-ABL1/ABL1 % ≤10% IS), the MR3 (<0.1%) and the deep molecular response (MR4<0.01%, MR4.5<0.032%, and MR5 <0.001%). Consequently, the molecular monitoring plays a crucial role in the clinical management of CML patients, with a consequent research of sensitive and standardized molecular techniques. The automated methods offer advantages in terms of reduced time for analysis, decreased manual steps, and reduction of possible errors and contamination.
Aims: We compared the automated technique GeneXpert "Ultra" (Cepheid, Maurens-Scopont, France) with the "Labnet" method that includes the "classical" manual real-time PCR techniques standardized in the Italian network (57 centers), according to the European guidelines [Cross N, 2015]. We compared the sensitivity of the two methods (based on the number of ABL1 detected copies), the classification of molecular responses, with particular attention to the deep molecular one.
Methods: We assessed the BCR-ABL1 transcript in 86 patients afferent to laboratories of Pisa, Napoli, Torino, and Bologna (Italy). For statistical analysis, the *t*-, the Pearson's and the Cohen's K test were adopted. Because our patients presented different transcript levels (from the >10% to the 0% (MR4, MR4.5, MR5) the two techniques have been compared in the different molecular subgroups.
Results: Firstly we compared the number of detected ABL1 copies, that are fundamental for definition of the molecular response categories, especially for defining the degree of deep response (32,000 for MR4.5, 100,000 for MR5). By the "LabNet" method, 51 (81%) samples exceeded the 100,000 copies of ABL1, while by the automated method 81 samples (94.2%) reached >100,000 ABL1 copies. Then, we compared the two methods in discriminating positive and negative samples (K Cohen=0.690; p <0.02): 77 samples were concordant (89.5%) and only 9 (10.4%) were discordant. Of the 18 negative samples with the "LabNet" method, 2 (11.1%) were in MR4.0, 10 (55.5%) in MR4.5 and 6 (33.4%) in MR5.0. On the other hand, of the 19 negative samples with the method "Ultra", 1 (5.3%) was in MR4.5 and 18 (94.7%) in MR5.0, confirming the higher sensitivity of the automated method. In the cohort of positive cases by the two methods, the median values of transcript expression were superimposable (p=0.55) and the linear regression coefficient was very satisfying (Pearson's r=0.9399; p-value <0.0001). Finally we compared the results produced by the two methods according to the "molecular classes" (MR1 *vs* MR2+MR3 *vs* MR4+MR4.5 *vs* MR5). This comparison showed a good concordance between the two methods (Cohen's k=0.78–good correlation). Variation analysis demonstrated high concordance between "Ultra" and "LabNet" methods using assay comparison criteria proposed by Müller *et al.* [Leukemia 2009] (Table 1).

Table 1.

Category	Sample	Percentage	EUTOS Criteria
Less than 2-Fold Difference	49	77.7%	>50%
Less than 3-Fold Difference	56	88.8%	>75%
Less than 5-Fold Difference	60	95.2%	>90%
Greater than 5-Fold Difference	3	4.8%	NA
Total Samples Analyzed	63	100%	NA

Summary/Conclusions: In a huge series of patients the automated and manual molecular methods, applied in 4 different laboratories, resulted comparable in terms of classification of patients in "molecular classes". The advantage of the "Ultra" technique is represented by the higher number of detected ABL1 copies and the easier standardization.

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ROLE OF THE AURORA KINASE A/PLK 1 AXIS INHIBITION IN RESTORATION OF CELL GROWTH CONTROL OF CHRONIC MYELOID LEUKEMIA PROGENITORS

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Background: Cell response to stress is a central component of genomic stability. The integrity of signaling pathways involved in cell cycle arrest, chromatin remodeling and DNA repair, are critical for the maintenance fidelity of replicated DNA. In this context, Gadd45 proteins function as stress sensors and gene transcription regulators. Gadd45a, in particular, intervenes in G2/M checkpoint induction and DNA repair, and it is required for efficient coordination of centrosome duplication hence preventing abnormal mitosis and aneuploidy. Such evidences let assume a putative role of Gadd45a in cancer development and progression. Interestingly, Gadd45a interacts with Aurora Kinase A (AK A), a key component of centrosome cycle and polar spindle assembly required for regulated progression from G2 tom and throughout M.
AK A is a member of a serine-threonine kinase family active during mitosis and it is frequently overexpressed in human cancers where correlates with a poor prognosis. Notably, AK A overexpression is always associated with defects in centrosome duplication, bipolar spindle and chromosomal segregation and aneuploidy, suggesting that it may enhance other oncogenic events by promoting genomic instability, one major trait of chronic myeloid leukemia (CML). Our results support the hypothesis that AK A cooperates with the constitutive TK activity of Bcr-Abl fusion protein by increasing DNA damage, promoting the occurrence of additional genomic alterations and driving TKIs resistance and disease progression to blast crisis.
Aims: Here we investigated AK A and Plk1 role in CML hematopoietic progenitor survival as potential targets to eradicate the transformed clone.
Methods: K562 cell line is a human cell line generated from a CML patient in blast crisis. Drug resistance was induced in K562 cell line by the exposure to progressively increasing doses of Imatinib (IM). It was validated by dose-response curves showing a significant difference in LD50 of IM-sensitive and IM–resistant cells. By mean of cytofluorimetric and immunofluorescence microscope analyses we investigated the events leading to AK/Plk1 deregulation. Protein expression and activation were detected by western blotting and immunoprecipitation. Apoptotic cell death was measured by using an Annexin V/PI staining; cell cycle distribution was observed by PI staining and subsequent cytofluorimetric analysis.
Results: Preliminary experiments were aimed to determine whether IM resistance in a BCR-ABL1 cell context is associated with the over-expression and hyper-activation of AKA/PLK1 axis. In our *in vitro* model drug resistance was associated with increased expression and phosphorylation of AK A (Y282) and Plk1 (T210). 24h exposure to IM significantly reduced expression and phosphorylation of both proteins in parental K562, but not in IM-resistant K562, indicating that AK A and Plk1 activation is only partly dependent on BCR-ABL1 TK activity. Subsequent experiments showed that the inhibition of AK A and PLK1 in response to specific inhibitors (Danusertib and Volasertib respectively) was associated with:
• significant increase of gadd45 a expression levels;
• reduction of cell survival;
• G2/M checkpoint arrest.
The findings support the role of AK A/PLK1 inhibition in restoration of signals involved cell growth control and apoptosis.
Summary/Conclusions: The advantage of using AK and Plk1 inhibitors in CML therapy mostly arises from effects independent from TK activity of Bcr-Abl protein. We proved that the AK and Plk1 inhibitors induce growth arrest and apoptosis in IM sensitive and resistant cell lines.

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DURABLE TREATMENT-FREE REMISSION (TFR) FOLLOWING FRONTLINE NILOTINIB (NIL) IN PATIENTS (PTS) WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP): ENESTFREEDOM 96-WK UPDATE

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Background: ENESTfreedom (NCT01784068) is evaluating the ability to stop NIL and remain in TFR in pts with a sustained deep molecular response (DMR) on frontline NIL. Previous results from ENESTfreedom showed that 51.6% of pts (98/190) who attempted TFR remained off treatment and in major MR (MMR; *BCR-ABL1* ≤ 0.1% on the International Scale [IS]) at 48 wk.
Aims: To analyze updated TFR data and predictive factors for remaining in TFR in ENESTfreedom.
Methods: Eligible pts had CML-CP with b2a2 and/or b3a2 *BCR-ABL1* transcripts, ≥2 y of frontline NIL, and $MR^{4.5}$ (*BCR-ABL1*^{IS} ≤0.0032%) prior to enrollment. All pts provided informed consent. After enrollment, pts continued NIL for 1 y (consolidation phase). MR was assessed every 12 wk during the 1-y consolidation phase; pts with no assessment worse than MR^4 (*BCR-ABL1*^{IS}≤0.01%), ≤2 assessments between MR^4 and $MR^{4.5}$, and $MR^{4.5}$ in the last assessment were eligible to enter TFR. Loss of MMR during TFR triggered reinitiation of NIL. To investigate potential predictors for remaining in TFR, pts were grouped according to Sokal risk score at diagnosis or depth of response prior to attempting TFR (based on response assessments in the consolidation phase), and 48-wk TFR rates in each subset were calculated. The current analysis was conducted when all pts who entered TFR had completed 96 wk of TFR, reinitiated NIL, or discontinued from the study (data cutoff, 31 Oct 2016).
Results: Of 190 pts who entered TFR, 93 (48.9% [95% CI, 41.6% - 56.3%]) remained in MMR and off treatment at wk 96, including 88 (46.3%) who were in $MR^{4.5}$. Three pts who were in TFR at 48 wk lost MMR by 96 wk, and 2 additional pts discontinued from the study between 48 and 96 wk without losing MMR. Among pts with low, intermediate, or high Sokal risk at diagnosis, 39/62 (62.9% [95% CI, 49.7% - 74.8%]), 25/50 (50.0% [95% CI, 35.5% - 64.5%]), and 9/28 (32.1% [95% CI, 15.9% - 52.4%]), respectively, remained in TFR at wk 48 (Sokal risk scores were missing for 50 pts). Among pts with $MR^{4.5}$ in all assessments during the consolidation phase, 90/170 (52.9% [95% CI, 45.2% - 60.6%]) remained in TFR at wk 48 *vs* 8/20 (40.0% [95% CI, 19.1% - 63.9%]) who had ≥1 assessment between MR^4 and $MR^{4.5}$ during the consolidation phase. Overall, of 88 pts who reinitiated NIL due to loss of MMR, 87 (98.9%) regained MMR and the remaining pt left the study 7.1 wk after NIL reinitiation without regaining MMR; 81 of 88 pts (92.0%) regained $MR^{4.5}$ by the data cutoff. Among pts remaining in TFR for >48 wk (n=100), adverse events (AEs) were less frequent during the second *vs* the first 48 wk of TFR; 2 (2.0%) and 1 (1.0%) of these pts had cardiovascular AEs during the first and second 48 wk of TFR, respectively; 34 (34.0%) and 9 (9.0%), respectively, had AEs in the predefined musculoskeletal pain grouping.
Summary/Conclusions: The majority of pts in TFR at 48 wk remained in TFR at 96 wk, and they reported fewer AEs during the second 48 wk of TFR than in the first 48 wk, affirming the durability and safety of TFR following NIL. No strong predictors for remaining in TFR were identified. Pts with low Sokal risk and pts with continuous $MR^{4.5}$ in the consolidation phase tended to have higher TFR rates than other pts, although these results must be interpreted with caution due to the small number of pts in some subsets and the wide 95% CIs. Additionally, the biological explanation for an association between Sokal risk score at diagnosis and a subsequent ability to remain in TFR is unknown. These results support TFR as a valuable option for pts in sustained DMR on frontline NIL.

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RESPONSE DIFFERENCES IN THE BCR-ABL1 E13A2 AND E14A2 VARIANTS MAY BE A TECHNICAL QPCR ARTIFACT

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Background: The t(9;22) translocation in chronic myeloid leukemia (CML) generally occurs in intron 12 or 13 of the BCR gene resulting in two different transcripts, the e13a2 or e14a2. It has been suggested that the two variants represent separate disease entities and that the transcript variants hold a prognostic value regarding treatment response, where e14a2 predicts a faster and deeper treatment response. However, no difference in overall survival has been observed and the issue remains controversial. Reverse transcription quantitative PCR (RT-qPCR) using the Europe Against Cancer (EAC) qPCR assay has been the gold standard for determining the levels of *BCR-ABL1* transcripts. The assay use common primers for amplification of the two variants resulting in a PCR product for the e14a2 variant that is 75 base pairs longer than the e13a2 variant. Under suboptimal PCR conditions, amplicons may be amplified with different efficiencies, which can result in an underestimation of especially the amount of longer qPCR products.
Aims: To study the accuracy of the EAC assay in quantifying the e13a2 and e14a2 transcripts.
Methods: Patient samples were screened for *BCR-ABL1* e13a2 and e14a2 transcript variants using either PCR with agarose gel separation or a droplet digital PCR (ddPCR) assay measuring the amount of e13a2 and e14a2 transcripts. The *BCR-ABL1* level was determined by qPCR using the QuantStudio instrument (Life Technologies) and expressed in the International Scale (%IS) using the EAC primers and assay conditions with *GUSB* and *BCR* as reference genes. Samples were re-measured by digital droplet PCR (ddPCR) on a QuantaLife instrument (Bio-Rad) using modified EAC primers multiplexed with *GUSB* and *BCR* as reference genes and expressed as %IS.
Results: Transcript levels from 124 *BCR-ABL1* positive patient samples were determined using the EAC qPCR assay (median: 0.08% IS, range: 0.001–159% IS) and ddPCR (median: 0.01% IS, range: 0.0002–124% IS). These included 59 samples with the e13a2 variant and 65 with the longer e14a2 variant. Comparing the expression levels obtained by the two techniques revealed ddPCR/qPCR ratio differences for e13a2 (median: 0.68, range: 0.35 - 3.2) and e14a2 (median: 3.43, range: 0–8.8), and a consistent 4.5 fold (>0.5 log) underestimation of the levels of the e14a2 compared to e13a2 when using qPCR (figure 1).

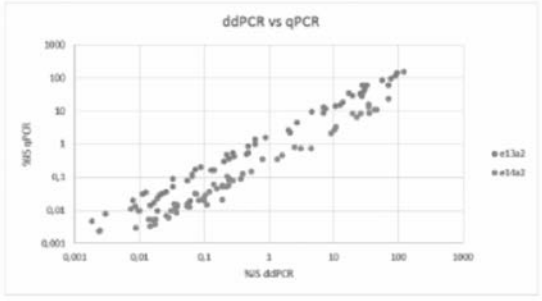

Figure 1. Scatter plot of %IS for 124 *BCR-ABL1* positive patient samples comparing values obtained by ddPCR (x-axis) and qPCR (y-axis). Samples with the e13a2 variant are shown in blue and samples with the e14a2 variant in orange. The plot clearly shows the underestimation of the longer transcript variant when using qPCR for quantification.

Figure 1.

Summary/Conclusions: When we compared the *BCR-ABL1* levels using qPCR and ddPCR, we observed a discrepancy between the e13a2 and e14a2 breakpoint variants. Since ddPCR is an endpoint measurement and not sensitive to variations in primer efficiencies, the most likely explanation for the discrepancy is a decreased qPCR efficiency of the longer e14a2 variant compared to e13a2 variant. Thus in qPCR analyses using the EAC protocol this may, at least on some analysis platforms, result in a consistently underestimation of the e14a2 level resulting in an appealingly better treatment response. A more than 0.5 log underestimation in a large subgroup of patients could have consequences in clinical decision-making *e.g.* by miss-grouping patients at different time points or when considering TKI discontinuation. Since many clinical laboratories uses the *BCR-ABL1* EAC protocol, the underestimation of the e14a2 variant could potentially be a widespread issue. We are presently working on an optimized *BCR-ABL1* qPCR protocol where the e14a2 underestimation is eliminated.

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5-YR RESULTS FROM THE PIVOTAL PHASE 2 PONATINIB PACE TRIAL: EFFICACY, SAFETY AND LANDMARK ANALYSIS IN HEAVILY PRETREATED PATIENTS (PTS) WITH CHRONIC-PHASE CHRONIC MYELOID LEUKEMIA (CP-CML)

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Background: Ponatinib is an oral tyrosine kinase inhibitor (TKI) approved for pts with CP-CML or Philadelphia chromosome–positive acute lymphoblastic leukemia (Ph+ ALL) for whom no other TKI therapy is indicated, or for pts with T315I. The ponatinib PACE trial (NCT01207440) enrolled pts with CML or Ph+ ALL resistant/intolerant to dasatinib or nilotinib, or with T315I. Long-term results in these heavily pretreated pts provide value in informing treatment decisions.
Aims: To report 5-yr efficacy and safety, and the association of early landmark responses with survival outcomes at 4 yrs past landmark, in heavily pretreated pts with CP-CML from PACE.
Methods: Ponatinib starting dose was 45mg/day. Dose reductions were instructed in Oct '13 to manage risk of arterial occlusive events (AOEs) observed with longer follow-up. Outcome measures were: 5-yr efficacy (n=267) and safety (n=270); post hoc landmark analysis (n=267) of the association of molecular responses (BCR-ABL^{IS} ≤0.1% [major molecular response (MMR)], >0.1–1%, >1%–10% and >10%) and cytogenetic responses (major [MCyR] and complete [CCyR]) at 3-, 6- and 12-mos with progression-free survival (PFS) and overall survival (OS) 4 yrs past landmark (log-rank *P* values). Data cutoff: 3 Oct '16.

Table 1.

Estimated PFS and OS at 4 yrs past landmark by BCR-ABL1 level

Landmark time	Response	n	PFS	*p–value	n	OS	*p–value
3 mo	BCR-ABL1						
	≤ 0.1%	32	97%	–	33	97%	–
	> 0.1–1%	47	57%	.32	48	85%	.54
	> 1%–10%	51	56%	.0050	55	80%	.10
	> 10%	82	51%	.0003	94	78%	.12
				overall: 0.0011			*overall: 0.27*
6 mo	BCR-ABL1						
	≤ 0.1%	57	83%	–	61	93%	–
	> 0.1–1%	42	53%	.011	44	83%	.021
	> 1%–10%	30	59%	.0004	32	90%	.18
	> 10%	57	50%	<.0001	74	78%	.0017
				overall: 0.0001			*overall: 0.0099*
12 mo	BCR-ABL1						
	≤ 0.1%	61	81%	–	63	97%	–
	> 0.1–1%	25	59%	.0086	27	85%	.014
	> 1%–10%	19	66%	.0092	22	95%	.058
	> 10%	41	52%	<.0001	50	80%	.0001
				overall: 0.0011			*overall: 0.0014*

*Calculated across the entire post-landmark timespan and unadjusted for multiple comparisons

Results: Baseline characteristics of the CP-CML pts included: median time from diagnosis, 7 yrs (range, 0.5–27 yrs); median age, 60 yrs (18–94 yrs); median %Ph+, 100% (2.5–100%); ≤10% Ph+, 20 pts (7%); 60% of CP-CML pts received ≥3 prior TKIs. At initiation of study closure, 99 pts (37%) were ongoing; among these pts, minimum follow-up was 52 mos, and most (78%) had 15mg/d as their last ponatinib dose. In efficacy-evaluable CP-CML pts, cumulative response rates as of the data cutoff were: MCyR, 60%; CCyR, 54%; MMR, 40%; and MR$^{4.5}$, 24%. Among pts who achieved MCyR (n=148) or MMR (n=108), the Kaplan-Meier (KM) estimated probability of remaining in response at 5 yrs was 74% (95% CI, 62–83) and 61% (95% CI, 51–70), respectively. Maintenance of response was high regardless of dose reductions in Oct '13. KM estimated 5-yr rates for PFS/OS were 49%/77%. Among pts with 3-, 6- and 12-mo landmark assessments, MCyR/CCyR was achieved in 48%/39%, 62%/52% and 71%/56%, respectively, and MMR achieved in 14%, 29% and 39%, respectively. Achievement of cytogenetic response and deep reductions in BCR-ABL1 levels (Table) at most landmark time points was associated with improved PFS and OS 4 yrs past landmark. Deeper responses at all landmarks were associated with achievement of MR$^{4.5}$ over time. Treatment-emergent adverse events (AEs) in ≥45% of CP-CML pts were rash 47%, abdominal pain 46%, and thrombocytopenia 46%. Most newly occurring AEs were observed within the first yr. The incidence of any AOE/serious AOE for CP-CML pts was

29%/23%. Among CP-CML pts with no prior AOEs who had a prospective dose reduction, 17% (11/63) had a first AOE occurring after Oct '13.
Summary/Conclusions: Long-term 5-yr results from PACE demonstrate that irrespective of dose reductions, ponatinib continues to show deep, lasting, clinically meaningful responses over time in heavily-pretreated pts with CP-CML. Achieving early cytogenetic response and deep reduction in BCR-ABL1 levels was associated with improved survival 4 yrs past landmark, demonstrating the prognostic value of early and deep response to ponatinib.

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LONG-TERM FOLLOW-UP IN VERY ELDERLY PATIENTS WITH CHRONIC MYELOID LEUKEMIA TREATED WITH IMATINIB FRONTLINE

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Background: Very elderly (>75 yrs) people are a substantial proportion of chronic myeloid leukemia (CML) patients that sometimes receive Imatinib (IM) at reduced doses based on physicians' judgment. However, data on long-term follow-up of these patients are still lacking.
Aims: To investigate the treatment response and outcome in a cohort of very elderly patients with newly diagnosed CML in chronic phase.
Methods: We revised in a retrospective database 263 CML patients aged ≥ 75 years and diagnosed from 2/2002 to 1/2016 and treated with IM frontline; among these, 121 patients (46%) were older than 80 yrs.
Results: Median age at diagnosis was 78.5 yrs [interquartile range (IQR) [76.3–81.3]. Sokal Risk at diagnosis was low in 1 patient (0.4%), intermediate in 171 (68.4%), high in 78 (31.2%) and not evaluable in 13 patients. As regards comorbidities, 63 patients had no or 1 concomitant disease, 147 patients 2 or 3 and 53 patients (20.1%) 4 or more. Median interval from diagnosis to IM start was 0.8 month (IQR 0.3–1.6): the initial IM dose was 400mg/day in 180 (68.4%), 300mg/day in 67 (25.5%) and <300mg/day in 16 (6.1%) patients. According to WHO, grade 3-4 haematological and extra-haematological toxicities were reported in 57 (21,7%) and 51 (19.4%) patients, respectively. As regards cumulative response, 13 patients (4.9%) discontinued IM due to early toxicity, 4 (1.5%) were resistant and 2 (0.8%) died from unrelated causes early after IM initiation; 250 patients (92.8%) achieved a complete haematological response (CHR). Among these, 208 (79% of all 263 patients) achieved a cytogenetic response (CyR), which was partial in 18 patients and complete (CCyR) in 190 (72.2%) after a median period of 6.1 months since IM initiation (IQR 3.4-9.1). Among the 190 patients in CCyR, 148 (56.2%) achieved a molecular response (MMolR) (ratio < 0.1) after a median period time of 13.7 months (IQR 9.0-22.3). Eleven patients (4.2%) developed a blastic phase (myeloid in 8 and lymphoid in 3 cases). After a median follow-up of 45.0 months from IM start (IQR 22.3–72.0), 93 patients have died (9 from disease progression and 84 from unrelated causes), 144 are alive and 104 of them are still in treatment with IM, while 8 discontinued for prolonged deep molecular response and 22 switched to 2nd generation TKI. Five-years event-free survival (EFS) and overall survival (OS) were 51.2% (CI95% 44.8-57.6) and 70.9% (CI95% 64.6-77.2), respectively. At univariate analysis Hb level at diagnosis (≥ 12 *vs* ≤ 12g/dl, p=0.021) and the initial dose of IM (400 *vs* ≤300, p=0.048) were significant predictive factors for CCyR achievement, while PLT count at diagnosis (>500 *vs* < 500 x 10^9/l, p=0.006) and female gender (p=0.046) were significant predictive factors for MMolR achievement. Multivariate analysis for EFS and OS are described in Table1.

Table 1.

Multivariate analysis for EFS	OR	95% CI	P
400 mg IM initial dose	0.656	0.459 - 0.938	0,021
PLTS < 500 x 10^9/l	1.517	1.064 - 2.161	0.021
Concomitant diseases ≤ 3	0.597	0.398 - 0.896	0.013
Multivariate analysis for OS	**OR**	**95% CI**	**P**
MMolR achievement	0.363	0.236 - 0.560	<0,001
Age < 80 years	0.622	0.397 - 0.975	0.038
Male gender	1.589	1.048 - 2.410	0.029

Summary/Conclusions: The long term follow-up of very elderly CML patients treated with IM suggests that any effort to treat these patients with standard doses should be made, in order to achieve cytogenetic and molecular responses as in younger subjects.

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IMPACT OF ARTERIAL THROMBOTIC EVENTS ON THE LONG-TERM OUTCOME OF CHRONIC MYELOID LEUKEMIA (CML) PATIENTS TREATED IN FIRST-LINE WITH NILOTINIB: AN ANALYSIS OF THE GIMEMA CML WORKING PARTY

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Background: Nilotinib has shown better efficacy compared to imatinib, but it has been associated to a higher incidence of arterial thrombotic events (ATEs). Little is known on the impact of ATEs on the outcome of nilotinib treated patients.
Aims: To investigate the characteristics of ATEs and their impact on the long-term outcome of CML patients treated with nilotinib first-line.
Methods: We analyzed 345 patients ≥ 18 years of age with CP CML enrolled in clinical trials of the GIMEMA CML WP investigating nilotinib as first-line treatment. Patients were treated with: nilotinib 400mg BID (n=73); rotation of nilotinib 400mg BID / imatinib 400mg OD (3-month periods for each drug)(n=123); nilotinib 300mg BID (n=149). The median follow-up was 58 (22-82) months. The median age at CML diagnosis was 53 (18–86) years. We analyzed the rate, type, management, and outcome of ATEs; moreover, we compared the molecular response rates and the long-term outcome of patients with or without ATEs. Definitions: ATEs: peripheral arterial obstructive disease (PAOD), coronary syndromes, significant carotid stenosis and ischemic stroke, or other significant ischemic events; major molecular response (MMR): BCR-ABL ≤0.1% (IS), with >10.000 ABL copies; MR4: BCR-ABL≤0.01% (IS), with >10.000 ABL copies.
Results: Overall, 30/345 (8.7%) patients had ATEs during treatment with nilotinib. The median age at CML diagnosis of these patients was 64 (43-85) years, and the median age at ATEs was 67 (47-89) years. The median duration of nilotinib treatment at ATE was 25 (1-78) months. ATEs were: 14 coronary dis-

ease (including 7 acute myocardial infarction), 8 PAOD, 4 carotid stenosis (asymptomatic), 2 avascular necrosis of femoral head, 1 optic artery ischemia, 1 atherosclerosis of aorta/right iliac artery. Overall, 21 patients were hospitalized for the management of ATEs; 15 patients received medical treatment only, while the remaining required invasive interventions: 9 coronary angioplasty with stent positioning, 3 lower limbs amputations, 2 peripheral vascular bypasses, and 1 prosthesis of femoral head. No patient died for ATEs. Overall, 24 patients (80% of patients with ATEs, and 7% of the whole cohort) permanently discontinued nilotinib because of ATEs. The median follow-up after ATE was 15 (1–58) months. Of the 30 patients with ATEs, 26 (87%) achieved a MMR and 18 (60%) obtained a MR4, during nilotinib treatment. These rates were comparable to those observed in patients without ATEs (MR3: 260/315, 83%; MR4: 113/315, 64%). The 5-year progression-free survival and overall survival were similar in patients with or without ATEs (PFS: 96% *vs* 92%, p=0.55; OS: 96% *vs* 93%, p=0.79).

Summary/Conclusions: After a median follow-up of 58 months, 8.7% of patients treated front-line with nilotinib had ATEs, being coronary disease and PAOD the most common. ATEs were more frequent in elderly patients (median age at ATEs: 67 years). Half of the patients required invasive procedures, including major surgeries in 6 patients. The other patients were successfully managed with medical treatment. Importantly, no patients died for ATEs, and ATEs did not affect the rates of MMR, MR4 and 5-year PFS and OS, which were all comparable to those observed in patients without ATEs. Taken together, these data suggests that ATEs, despite being sometimes associated with significant morbidity, did not significantly impact on response rates and on long-term outcome of CML patients treated with nilotinib front-line.

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ASSESSMENT OF CHRONIC RENAL INJURY IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN THE CHRONIC PHASE RECEIVING TYROSINE KINASE INHIBITORS

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Background: Long-term use of tyrosine kinase inhibitors (TKIs) may lead to chronic renal injury.

Aims: To evaluate the incidence of chronic kidney disease (CKD) in patients with chronic myeloid leukemia (CML) in the chronic phase (CP) receiving TKIs, and to identify the factors associated with the onset of CKD.

Methods: Data of CML-CP patients treated with TKIs as first-line or second- or third-line therapy for at least 3 months were analyzed. Glomerular filtration rate (GFR) was followed from the initiation of TKI-therapy. CKD was defined as persistent GFR less than 60 ml/min/1.73 m^2 or persistent more than 30% GFR reduction from baseline. CKD-free survival was used to evaluate the onset of CKD. Patients' characteristics and TKI used were analyzed to identify the factors associated with the onset of CKD by Cox regression model in those receiving first-line and second- or third-line TKI-therapy, respectively.

Results: 587 patients were included in this study. 383 (65%) were male. Median age was 40 (17-84) years.

464 patients received imatinib (n=363), nilotinib (n=88) or dasatinib (n=13) as first-line TKI-therapy. With a median follow-up of 35 months (range, 3-185 months), 136 of 416 (33%) patients with normal GFR at baseline developed CKD. Probabilities of CKD-free survival at 4 years were 62%, 78% and 100% in the patients receiving imatinib, nilotinib and dasatinib, respectively (p=0.004). Multivariate analysis showed that imatinib use (HR=2.4, 95% CI 1.4-4.3, p=0.002), male gender (HR=2.0, 95% CI 1.4-2.9, p=0.001), increasing age (HR=1.2, 95% CI 1.1-1.4, p=0.003) and prior administration of hydroxyurea, interferon or chemotherapy (HR=1.7, 95% CI 1.1-2.8, p=0.010) were factors associated with incident of CKD. In 48 patients with abnormal GFR or prior CKD before first-line TKI-therapy, 8 of 42 (19%) developed ≥30% GFR reduction from baseline during imatinib-therapy, while none of 6 during nilotinib- or dasatinib-therapy. In 123 patients receiving nilotinib (n=59) or dasatinib (n=64) as second- or third-line TKI-therapy after imatinib-failure, 13 of 110 (12%) with normal GFR at baseline developed CKD with a median follow-up of 19 months (range, 3-149 months). Probabilities of CKD-free survival at 3 years were 74% and 90% in those receiving nilotinib and dasatinib, respectively (p=0.059). Multivariate analysis showed that nilotinib use (HR=3.6, 95% CI 1.0-13, p=0.047) and a history of diabetes mellitus, hypertension or other renal diseases (HR=3.8, 95% CI 1.3~11.6, p=0.019) were factors associated with incident of CKD. 3 of 13 (23%) patients with abnormal GFR or prior CKD before second- or third-line TKI-therapy developed ≥30% GFR reduction from baseline during nilotinib (n=1) or dasatinib (n=2) therapy.

Summary/Conclusions: Our study showed that nilotinib and dasatinib were associated with less chronic renal injury compared with imatinib as first-line TKI-therapy, while dasatinib was related to less loss of renal function compared with nilotinib as second- or third-line TKI-therapy after imatinib-failure in CML-CP patients.

P607

COMPARATIVE MONITORING OF MINIMAL RESIDUAL DISEASE (MRD) BY QPCR AND DIGITAL-PCR (DPCR) IN CHRONIC MYELOID LEUKEMIA PATIENTS ACHIEVING MAJOR OR DEEP MOLECULAR RESPONSE WITH TIROSIN-KINASE INHIBITORS

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Background: Quantification of BCR-ABL1 transcript by qPCR is mandatory to monitor the response to TKIs therapy in CML patients. The achievement of Major or Deep Molecular Response (MMR or DMR) with TKIs is crucial for long-term survival and for treatment free remission (TFR). Currently, up to 30-40% of CML patients treated with TKIs can achieve DMR, but 50-60% of deep responders who discontinue the treatment loose their DMR and re-challenge continuous TKIs therapy. qPCR has some intrinsic limitations and it does not appear to be an optimal assay to select the best candidates to TKIs discontinuation. Digital PCR (dPCR) can give an absolute quantification of target nucleic acids by partitioning the PCR reaction mix over a large number of wells, each containing a single copy or no copies of the target region.

Aims: The aim was to comparatively monitor the BCR-ABL1 transcript levels by dPCR and qPCR in 57 CML patients treated with TKIs and achieving MMR or DMR in at least 3 time points.

Methods: Using qPCR and dPCR (QS3D Digital PCR System by Life Technologies), we comparatively analyzed 228 peripheral blood samples from 57 CML patients with MMR (n°14) or DMR (n°43). qPCR analysis were performed according to the last International Guidelines while absolute quantification of BCR-ABL1 transcript were obtained by dPCR and results were expressed as number of BCR-ABL1copies/ul of reaction. Patients were divided into 3 groups corresponding to the MR classes at the first time point: MR3.0, MR4.0 and MR4.5-5.0 groups. dPCR Positive Predictive Value (PPV) was also preliminary evaluated in 14 patients undergoing TKI discontinuation.

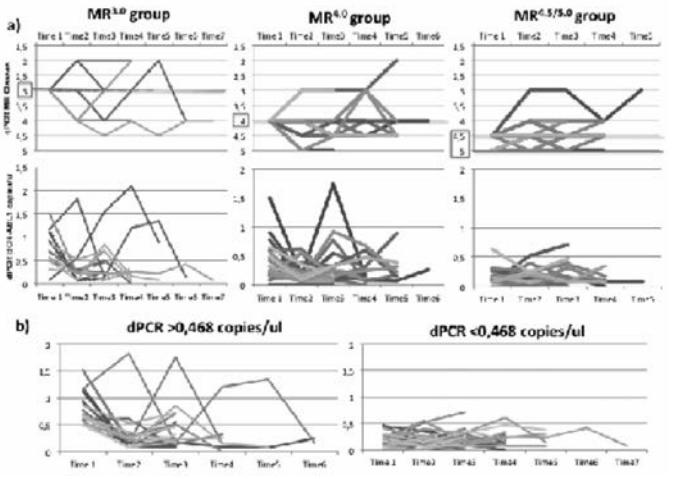

Figure 1.

Results: Analyzing comparatively the time course of MR in the patients of the three groups (MR3.0,MR4.0 and MR4.5-5.0) it was observed a similar trend, but the dPCR allowed to appreciate that, at the time of starting the monitoring the patients showed different levels of BCR-ABL1 copies/ml. Furthermore, those patients with MR4.5-5.0 undetectable by qPCR resulted with detectable BCR-ABL1 transcript levels when assessed by dPCR. Secondly, while MRD quantitations measured by qPCR appear to be more homogeneous, nearly due to a normalization effect of qPCR, the quantitations of MRD measured by dPCR appear to be more heterogeneous because of the high sensitivity and accuracy of dPCR. Therefore, dPCR values, reflecting the great heterogeneity of MRD level in patients belonging to the same MR group, suggest a higher accuracy in patients stratification (Figure 1a). dPCR value of 0,468 copies/ul, previously reported as value discriminating between major responders and deep responders, was used as threshold for dPCR data analysis. Patients with absolute value of BCR-ABL1 lower than 0,468copies/ul at the first time point presented more stable disease levels than the patients with absolute value of BCR-ABL1 higher than 0,468copies/ul (Figure 1b). In 14 CML patients who

discontinued TKIs, a preliminary analysis showed that 80% of patient with BCR-ABL1<0,468 copies/ul at discontinuation, maintained stable TFR (PPV of 80%).
Summary/Conclusions: This study suggests that dPCR is more precise and sensitive than qPCR when detecting levels of BCR-ABL1 transcript and that dPCR seems to be more robust and accurate for CML patients stratification. Larger and prospective studies are warranted to confirm the higher sensitivity and accuracy of dPCR and its usefulness to better select the candidates for TFR.

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OUTCOME OF BLAST PHASE CHRONIC MYELOID LEUKEMIA (CML-BP) IN THE TYROSINE KINASE INHIBITOR ERA

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Background: Primary goal of management in chronic myeloid leukemia (CML) is to prevent disease progression to blast phase CML (BP-CML). Current notion for management of BP-CML usually involves initiation of intensive chemotherapy regimen with addition of tyrosine kinase inhibitor (TKI). Despite treatment with intensive induction chemotherapy, outcome remains dismal.
Aims: We aimed to describe our experience with management of BP-CML and its outcome.
Methods: We included 58 patients from Moffitt Cancer Center from 2001 till 2016 with diagnosis of BP-CML and performed a retrospective chart review. Data elements including age, gender, peripheral blood and bone marrow parameters, phase of CML, treatment, cytogenetics and vital status were collected. Survival analysis using Kaplan-Meier method with log-rank test to determine significance by calculating two-sided p values was performed.

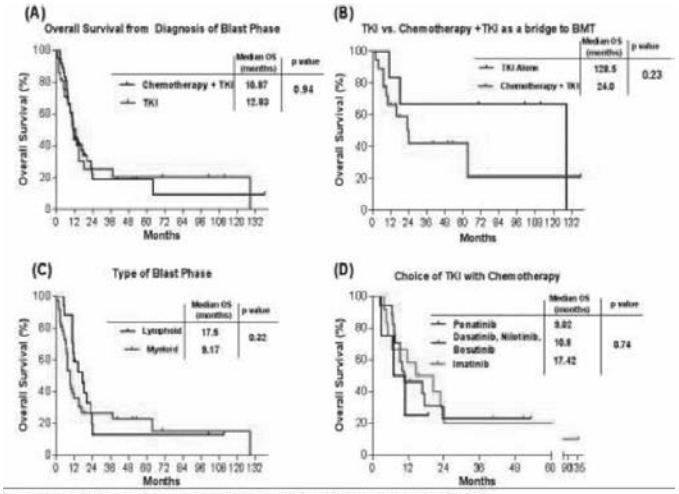

Figure 1: Overall survival in the era of TKI in management of Blast Phase CML.

Figure 1.

Results: The overall survival (OS) of our cohort was 31.87 months (mo). For patients with progression to BP-CML from previously known diagnosis of CML, median time to progression was 19.1 mo (range: 3.0-221.2 mo). The median OS from the diagnosis of BP-CML in this cohort was 10.8 mo, compared to *de novo* CML-BP cohort OS of 11.03 mo (p value=0.62). Myeloid blast phase CML had worse OS compared to lymphoid blast phase cohort but was not statistically significant (9.17 *vs* 17.5 mo, p=0.32). We further compared the treatment strategies of BP-CML including single agent TKI (n=21) and conventional chemotherapy regimens in combination with a TKI (n=36). The median OS of the cohort with single agent TKI was not statistically different from the combination with chemotherapy arm (12.83 mo *vs* 10.87 mo, p=0.73) as shown in Figure 1A. Additionally, combination of chemotherapy with TKI compared to single agent TKI did not have significant survival impact in either myeloid (9.17 *vs* 9.13 months, p=0.32) or lymphoid (14.47 *vs* 18.27 mo, p=0.24) BP-CML. Total of 26 patients (44.8%) proceeded to allogeneic bone marrow transplant, 26% (n=6) of which only received TKI prior to transplant compared to 76.9% (n=20) who received chemotherapy in combination with TKI. Use of single agent TKI rather than TKI in combination with chemotherapy prior to allogeneic transplant had a trend toward improved OS (128.5 *vs* 24 mo, p=0.23) (Fig 1B). Choice of TKI in combination with chemotherapy in treatment of BP-CML also did not identify any TKI combination resulting in superior survival (Figure 1D). Overall survival of the cohort stratified by presence of standard Philadelphia chromosome in comparison to additional cytogenetic aberrations did not detect difference in overall survival (10.87 *vs* 12.1 mo, p=0.51). Further evaluation of cytogenetic aberrations revealed monosomy 7 to be present in greater frequency in lymphoid blast phase compared to myeloid blast phase (35.71% *vs* 6.25%, p=0.02).
Summary/Conclusions: Our data suggest no survival difference when BP-CML is treated with a single agent TKI compared to a combination therapy, regardless of histology type. Therefore, single agent TKIs should be considered as an effective frontline therapy option for BP-CML, which may prevent the potential toxicity associated with chemotherapy. These findings need further validation in a larger prospective cohort.

P609

EFFICACY OF SWITCHING TO DASATINIB IN CHRONIC MYELOID PATIENTS WITH LATE WARNING RESPONSES TO IMATINIB. STUDY OF THE ASSOCIATION OF RESPONSE TO DASATINIB TO IMMUNOLOGIC STATUS

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Background: European LeukemiaNet (ELN) recommendations (2013) advised closely monitoring for patients with late warning response (patients with complete cytogenetic response without major molecular response after 12 months of treatment). Our trial, DASAPOST, has been the first one evaluating efficacy and safety of dasatinib in patients with late warning responses, and preliminary results have been reported (Garcia-Gutierrez et al, ASH 2016; P5450). Besides, many studies suggest that dasatinib may augment responses due to its immunomodulating effect. Although NK and CD8 cells seem to be involved, the specific mechanism remains to be clarified.
Aims: To evaluate the efficacy and safety of switching change to dasatinib in patients treated with imatinib first line during at least 18 months and having a late warning response, and to study the association between response to dasatinib and immune robustness, both baseline and during the therapy, and dasatinib-induced lymphocyte "mobilization".
Methods: Phase II, open, multicenter DASAPOST study (NCT01802450). Patients previously treated with imatinib after at least 18 months, with CCyR but without MMR, were included. All BCR-ABL/ABL (IS) measurements were centralized in a EUTOS laboratory. Patients not molecularly analyzed at a given time point were considered as non responders, Lymphocyte counts, subpopulations and migration studies were done at baseline (1st day of dasatinib), and every 3 months, and they were done both previous to the dose, and 2 hours after.

Table 1.

	Lymphocytes Baseline	CD8 Baseline	CD4 Baseline	NK Baseline
N (x 10^9/L)	1.78(0.83-3.24)	0.4(0.15-1.43)	0.68(0.43-1.59)	0,20(0.05-0.77)
Percentage	27.4(14.5-39.2)	32,2(15.4-64.8)	65.4(30.4-82.8)	14.1(2.9-40.3)

Results: From April 2013 to May 2015, 18 patients were enrolled in 12 centers. Median age was 59 years (39-77). The ratio of men to women was 13/5, and the Sokal risk groups were 48%, 30% and 22% for low, intermediate and high risk, respectively. Median time from diagnosis to switch to dasatinib was 2.6 years (1.6-23) and median time while on imatinib to achieve CCyR 1.4 years (0.2-12). Median exposure to imatinib was 2.4 years (1.6-14). Eight patients (44.4%) obtained MMR at 3 months, and 12 (66.7%) obtained MMR at 6 and 12 months. Of interest 9/18 patients (50%) achieved MR4 by 12 months. There were 3 study discontinuations because of toxicity (16%). Table 1 shows the median number of the most relevant lymphocyte populations in the pre-dose sample at baseline. Table 2 shows that the absolute number of CD8 cells was significantly superior at baseline in those patients having a MMR at 3 months, with a trend in the same direction of absolute lymphocyte count and percentage. There were no significant associations with response when considering CD4 T cells, NK cells, or the degree of mobilization after dasatinib dose either in total lymphocyte numbers or in subpopulations. Besides, lymphocyte number or proportions at 3 or 6 months were not associated with MMR at 6 or 12 months (data not shown).

Table 2.

	MMR 3m	No MMR 3m	p
Lymphocytes Baseline (x 10^9/L)	2.23	1.63	0.051
Lymphocytes Baseline (%)	30.4	23.3	0.053
CD8 Baseline (x 10^9/L)	0.62	0,29	0.037
CD8 3 monthd (x 10^9/L)	0,72	0,49	0.088

Summary/Conclusions: Our study shows that in patients treated with imatinib and with late warning responses, switching to Dasatinib induced MMR in 2 out every 3 patients, and MR4 in half of the patients, with a good safety profile. Contrarily to other group reports, we have not found any significant association between response and lymphocyte mobilization in any point studied. Interestingly, the absolute number of CD8 at baseline was significantly associated with the early obtention of MMR at 3 months, a finding which underscore the prognostic importance of baseline immune status, the relevance of CD8 cells in the antileukemic effect,and which suggest that this quite simple variable must be included in future studies with dasatinib in second line.

P610

GENETIC PREDICTION OF INSULIN RESISTANCE IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH NILOTINIB

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Background: Impaired fasting glucose (IFG) and type 2 diabetes (T2D) represents adverse events in Chronic Myeloid Leukemia (CML) patients treated with the second-generation tyrosine kinase inhibitor (TKI) nilotinib. A genetic risk score (uGRS) for the prediction of insulin resistance, consisting of 10 multiple single-nucleotide polymorphisms (SNPs), has been proposed.
Aims: We evaluated the uGRS predictivity in 45 CML patients treated with nilotinib.
Methods: Patients were genotyped for IRS1, GRB14, ARL15, PPARG, PEPD, ANKRD55/MAP3K1, PDGFC, LYPLAL1, RSPO3, and FAM13A1 genes. The uGRS was based on the sum of the risk alleles within the set of selected SNPs.
Results: $MR^{3.0}$ and CMR were achieved in 91% and 84% of the patients, respectively.Before treatment, none of the patients had abnormal blood glucose. During treatment and subsequently follow-up of 84.4 months (range 1-298), 5 patients (11%) developed diabetes requiring oral treatment, after a median of 11 months (range 3-95) since nilotinib. Nine patients (20%) developed prediabetes. Prediabetes/diabetes-free survival was significantly higher in patients with an uGRS below 10 compared to higher scores (100% *vs* 18%, p=0.004) (Figure). Each increment of 1 unit on the uGRS caused a 42% increase in the prediabetes/diabetes risk (HR=1.42; CI: 1.04-1.94; p=0.026).

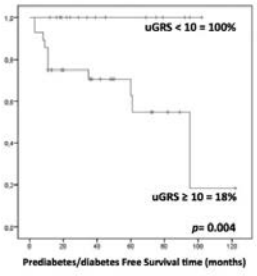

Figure 1.

Summary/Conclusions: Although nilotinib is not associated with a higher incidence of TD2 compared to a general population, it could be an early "highlighter" of genetic predisposition to the disorder. The presence of more than 10 allelic variants associated to insulin secretion, processing, sensitivity and clearance is predictive of prediabetes/diabetes developing. In clinical practice uGRS could help tailor the best TKI therapy.

P611

THE EUROPE AGAINST CANCER PROTOCOL FOR BCR-ABL P210 TRANSCRIPT MEASUREMENT MAY OVERESTIMATE RESULTS FOR E13A2 VARIANT

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Background: The quantitative PCR of *BCR-ABL* transcript has been the most useful technique for monitoring therapy in CML patients for over a decade. The numerous standardization projects have been undertaken in order to harmonize the molecular response results in laboratories all over the world. However, our data suggest that using the most common protocol may lead to overestimation of e13a2 transcript.
Aims: The goal of the study was to verify the observation that e14a2 transcript amplifies less efficiently than e13a2. The secondary goal was to validate the modification of Europe Against Cancer (EAC) protocol developed in 2011 which corrects observed artifacts.
Methods: The *BCR-ABL* measurements acquired using EAC and in-house modified EAC protocol have been compared with results from SA Pathology in Adelaide. The Adelaide protocol (Branford and Hughes 2006) consists of separate, optimized reactions for e13a2 and 14a2 transcripts, therefore it should be considered free of any PCR efficiency-related artifacts. The data originated from four independent sample batches exchanged between Poznan and Adelaide since 2009.
Results: The analysis of retrospective EAC protocol data showed that when e13a2 and e14a2 samples entered the exponential phase at the same time, the latter would cross the threshold approximately 2.2 cycles after the first one. Re-analysis of data from sample exchanges from 2009 revealed that after establishing a conversion factor (CF), all of the e14a2 measurements in Poznan were underestimated according to Adelaide. At the same time, almost all of e13a2 samples were overestimated (fig. 1). Still, the bias between methods was acceptable and a valid conversion factor (CF) was calculated. The method modification introduced 2011 eliminated this difference and increased concordance between laboratories. The last sample batch revealed significant difference between non-modified and modified EAC protocols in e13a2 measurements: 4,56 (+/- 0.96). Reanalysis of sample batch from 2009 (presented on fig.1) using 4.57 (2x2.28) factor (e13a2 results divided by 2.28, e14a2 results multiplied by 2.28) resulted in almost perfect data alignment. The results of modified EAC protocol, after CF recalculation, showed very good concordance with Adelaide (100% results of e14a2 and 88% of e13a2 within 2-fold of reference laboratory).

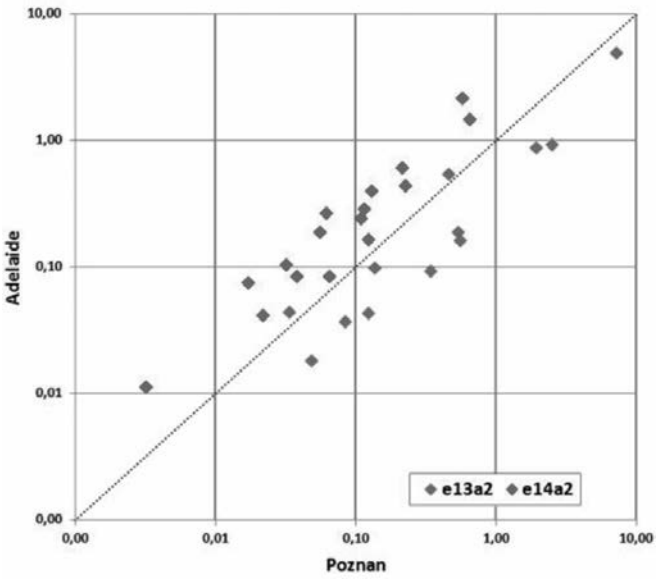

Figure 1.

Summary/Conclusions: In the EAC protocol, the e14a2 transcript amplifies less efficiently than e13a2. Since commonly used plasmids, including ERM-AD623, are based on e14a2, the standard curve is being shifted towards the latter cycles. It leads to overestimation of e13a2 by mean factor of 4.5 (over 0.5 log), which could be clinically significant. The reports of worse outcome of e13a2 patients are probably caused by this artifact, which can be easily eliminated by implementing an additional forward primer to EAC protocol. This overestimation cannot be detected in case of lab to lab validation when two centers are using EAC protocol. In case of method validation in Adelaide, those differences were not as obvious as well. The shift of 4.5 (fig. 1) means that results are 2.25 times different from the perfect concordance line and could easily fit into accepted 2-fold and 3-fold compartments. The CF calculated by Adelaide would depend on the percentage of each transcripts among the exchanged samples. The observed artifact should be also taken into consideration in clinical trials that rely on surrogate endpoints such as molecular response level at certain time points. Uneven transcript variant distribution between compared groups may lead to improper conclusions.

Enzymes and sickle cell disease

P612

ESTABLISHMENT OF IN VIVO AND IN VITRO MODEL OF X-LINKED SIDEROBLASTIC ANEMIA

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Background: Congenital sideroblastic anemia (CSA) is an inherited microcytic anemia characterized by the presence of bone marrow ring sideroblasts, reflecting excess mitochondrial iron deposition. The most common form of CSA is X-linked sideroblastic anemia (XLSA), which is attributed to mutations in the X-linked gene erythroid-specific 5-aminolevulinate synthase (*ALAS2)*. *ALAS2* resides on chromosome X and encodes the enzyme that catalyzes the first and rate-limiting steps in the heme biosynthesis pathway in erythroid cells. This pathway converts glycine and acetyl-coenzyme A to 5-aminolevulinic acid (ALA), which requires pyridoxal 5'-phosphate (PLP) as a cofactor. Although PLP has been used for treating XLSA, a marked proportion of patients with XLSA remain refractory to treatment (Ohba *et al.* Ann Hematol 2013). Thus, there is a need to establish a model of XLSA to reveal the detailed molecular mechanism contributing to RS formation as well as to explore novel therapeutic strategy for XLSA.
Aims: We explored to establish a novel model of XLSA by CRISPR/Cas9-based genome editing.
Methods: We targeted the GATA-1-binding region of intron 1 of the human *ALAS2* gene based on both *in vivo* mice and human induced pluripotent stem cell-derived erythroid progenitor (HiDEP) cells (Kurita *et al*. PLoS One 2013). The mutation diminished the binding of transcription factor GATA-1, which would lead to decreased transcription of the *ALAS2* gene, thereby causing XLSA (Kaneko *et al*. Haematologica 2014). Western blotting and quantitative chromatin immunoprecipitation (ChIP) analysis were performed using antibodies against GATA-1 (D52H6, Cell Signaling Technologies) and TAL1 (C-21, Santa Cruz). For transcription profiling, Human Oligo chip 25K (Toray) was used. Gene ontology (GO) analysis was performed with Genecoids (http://genecodis2.dacya.ucm.es/).
Results: We first generated a founder female mouse lacking the intron 1 enhancer region of *Alas2*, including the GATA binding domain (*Alas2*$^{\Delta int1/X}$). Whereas the heterozygous *Alas2*$^{\Delta int1/X}$ mice were viable and did not show anemic phenotype, hemizygous deletion (*Alas2*$^{\Delta int1/Y}$) in male mice led to an embryonic lethality, suggesting that this sequence is indispensable in the context of mice. As an alternate approach, we established a clonal line with HiDEP cells, which harbored 19-bp deletion within the intron 1 enhancer region of *ALAS2*, including GATA binding domain. Whereas wild-type HiDEP cells exhibited red color, the XLSA clone appeared pink/pale color, which were accompanied by the significantly decreased intracellular heme concentration. Despite no obvious change in the expression of GATA-1 protein in the XLSA clone, quantitative ChIP analysis demonstrated that the chromatin occupancies of GATA-1 and its cofactor TAL1 were significantly abrogated by the deletion of the GATA binding motif at intron 1 enhancer of the *ALAS2* gene. Quantitative real-time–polymerase chain reaction (RT–PCR) analysis demonstrated significant downregulation of *ALAS2* as well as globin genes (*HBA*, *HBG*, and *HBB*) in the XLSA clone. Microarray analysis revealed >2-fold up- and down-regulation of 619 and 274 genes caused by the 19-bp deletion, respectively. The downregulated gene ensemble included globins (*HBZ*, *HBG*, *HBE*, *HBD*, *HBM*, and *HBQ*) as well as genes involved in iron/heme metabolism (*ALAS2*, transferrin receptor: *TFRC*, coproporphyrinogen oxidase: *CPOX*, and mitoferrin 1: *MFRN1*). GO analysis revealed significant enrichment of cellular iron ion homeostasis (p=0.018), regulation of transcription (p=0.0021), and innate immune response (p=0.0018), implying that heme was involved in various biological processes in erythroid cells. Interestingly, ALA treatment significantly improved compromised heme production as well as downregulation of globin genes observed in the XLSA clone, suggesting that ALA may represent a novel therapeutic option for PLP-refractory XLSA.
Summary/Conclusions: The XLSA model established from HiDEP cells can be used as an important tool for clarifying the molecular etiology of XLSA and to explore novel therapeutic strategies.

P613

BENDAMUSTINE AND RITUXIMAB COMBINATION THERAPY FOR COLD AGGLUTININ DISEASE: RESULTS OF A PROSPECTIVE NORDIC TRIAL

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Background: Primary cold agglutinin disease (CAD) is an autoimmune hemolytic anemia in which a well-defined clonal lymphoproliferative bone marrow disorder (LPD) causes production of monoclonal cold agglutinins. Major clinical manifestations are anemia and, in most cases, cold-induced circulatory symptoms. Pharmacological therapy, although not indicated in patients with very mild disease, seems required in a majority of cases. Corticosteroids are ineffective. Rituximab monotherapy has resulted in approximately 50% response rate and 1-year median response duration. Fludarabine and rituximab combination therapy showed 70% response rate (20% complete responses) and very long response duration, but considerable toxicity.
Aims: We wanted to investigate whether bendamustine and rituximab combination therapy can result in favorable response rates and duration with an acceptable toxicity profile.
Methods: We conducted a prospective, uncontrolled multicenter trial with 16 participating hospitals from Norway, Finland and Denmark. Essential inclusion criteria were verified CAD with symptomatic anemia and/or severe cold-induced circulatory symptoms. Eligible patients received 4 cycles of rituximab 375mg/m^2 day 1 and bendamustine 90mg/m^2 day 1-2 with 28 days interval. Outcomes were classified into complete response (CR), partial responses (PR) and non-response (NR). The definition of CR included normalization of hemoglobin (Hb) levels with no hemolysis, complete histologic resolution of the bone marrow LPD and disappearance of monoclonal serum protein. The criteria for PR included increase in Hb levels by at least 2.0 g/dL or to the normal range, transfusion independence, at least 50% reduction of IgM and improvement of any circulatory symptoms.
Results: Forty-four patients (19 men and 25 women) were included, with a median age of 74 years (range, 48-86) and median disease duration 4 years (range, 0-18). Seventeen patients had received previous therapy. At baseline, median Hb level was 9.5g/dL (range, 4.5-14.8), bilirubin 45micromol/L, lactate dehydrogense (LDH) 468 U/L, haptoglobin undetectable, IgM 4.1g/L (1.0-27.2), CA titer 2048 (64-65536). Monoclonal IgM kappa was detected in 38 patients, IgG kappa in 1 and IgA kappa in 1. We observed CR in 16 patients (36%), PR in 15 (34%), while the remaining 13 (30%) were non-responders. Hb levels increased by a median of 4.0g/dL in the responders; 4.4g/dL in patients achieving CR and 3.9g/dL in those achieving PR. Median post-therapy Hb levels were 14.2g/dL (CR), 12.5g/dL (PR) and 10.5g/dL (NR). Acrocyanosis and Raynaud symptoms resolved completely in 16 patients and improved in 11 (47% and 32%, respectively, of those with such symptoms at baseline). Histologic regression of the LPD was complete in 17 patients (39%), partial in 5 (11%) and not evaluable in 18 (41%). Median time to response was 2 months (0.5-12). Only 3 responders experienced relapse; 2 after PR and 1 after CR. Median observed response duration was 32 months (range, 1-62) during median 32 months follow-up from response, consistent with a much longer expected response duration. Neutropenia grade >3 occurred in 14 patients (32%), of which 8 (18%) had grade 4. Three patients (7%) experienced 1-3 episodes of febrile neutropenia, which was readily manageable. Non-hematologic toxicity occurred in 17 patients (39%), mostly consisting of mild nausea or rash. Three non-neutropenic serious adverse events (SAE) were recorded; 1 was considered probably therapy related.
Summary/Conclusions: Bendamustine and rituximab combination therapy resulted in high response rates, a high rate of CR, long response duration and few relapses during the observation period, with a favorable safety profile. It might be considered in the first line for reasonably fit patients with CAD requiring therapy.

P614

EX VIVO TREATMENT OF RED BLOOD CELLS FROM 15 PYRUVATE KINASE (PK)-DEFICIENT PATIENTS WITH AG-348, AN ALLOSTERIC ACTIVATOR OF PK-R, INCREASES ENZYMATIC ACTIVITY, PROTEIN STABILITY AND ATP LEVELS.

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Background: Pyruvate kinase (PK) deficiency is a rare hereditary disorder affecting red blood cell (RBC) glycolysis. It is caused by mutations in the *PKLR* gene. PK-deficient RBCs are characterized by changes in metabolism associated with defective glycolysis, including a build-up of the upstream metabolite 2,3-disphosphoglycerate, and deficiency in the PK product ATP. It is hypothesized that insufficient energy production affects red cell homeostasis, promoting

premature removal of PK-deficient RBCs from the circulation. Affected patients display chronic hemolytic anemia of variable severity. Treatment of PK-deficient patients is generally supportive, focusing on the anemia and iron overload state, and there are no approved drugs that directly target mutated PK. AG-348 is an allosteric activator of the RBC isoform of PK (PK-R) and in clinical development for the treatment of PK deficiency

Aims: To evaluate the *ex vivo* effect of AG-348 treatment on PK-R enzymatic function, RBC metabolism and deformability

Methods: Observational case-control study, approved by the Institutional Review Board. All patients gave informed consent. Enrolled patients (N=15) were adults, transfusion-independent and compound heterozygous or homozygous for mutations in *PKLR*. Baseline metabolic profiling was performed by LC-MS/MS. Purified RBCs from patients and healthy control subjects were incubated with AG-348 (up to 10 µM) for 24 hours at 37°C. After 6 and 24 hours PK-R activity, ATP levels and RBC deformability (by Lorrca) were measured. For determination of PK-R thermal stability, RBC lysates were incubated for 2 hours with 2 µM AG-348 (37°C) prior to testing. Baseline protein levels of PK-R were assessed using antibodies against PK-R

Results: Baseline patient characteristics show strongly reduced PK-R activity in all patient cells, in particular taking into account the degree of reticulocytosis (Table 1). Distinct metabolic changes were consistent with a block of glycolysis at the PK-R step. Treatment of PK-deficient RBCs with AG-348 resulted in increased enzymatic activity in all patient cells after 24 hours (mean increase 1.8-fold, range, 1.2-3.4). Similar increases were observed in control cells (mean fold increase 2.3, range, 1.2-7.1). ATP levels in PK-deficient cells increased upon AG-348 treatment (mean increase 1.5-fold, range: 1.0-2.2) similar to control cells (mean increase 1.6 fold, range, 1.4-1.8). Generally, PK-R thermal stability was strongly reduced in PK-deficient patient cells, illustrated by a mean loss of activity of 72% (19% for control cells) after incubation at 53 °C for 60 minutes. *Ex vivo* treatment with AG-348 prior to incubation resulted in residual activity 1.4 to >10-fold higher than residual activity of vehicle-treated samples. Baseline protein level analyses suggests that a certain level of PK-R protein is required for cells to respond to AG-348 treatment *ex-vivo*, as treatment effects were minimal in patient cells with very low or undetectable levels of PK-R. In approximately half of the patients, *ex vivo* treatment with AG-348 was associated with an increase in RBC deformability, although there doesn't appear to be a clear correlation with enzymatic or metabolic response.

Table 1.

Table 1. Baseline characteristics and genotypes of PK-deficient patients

Patient/ Gender	*PKLR* mutation	Amino acid change	Hb (g/dL)	Retics (%)	PK (U/gHb)	HK (U/gHb)	PK/HK
AG090/F	c.1121T>C/c.1456C>T	L374P/R486W	9.7	10	9.0	5.0	1.8
AG018/M	c.331G>A/c.1456C>T	G111R/R486W	11.2	7	3.1	1.7	1.8
AG019/M	c.1178A>G/c.1456C>T	N393S/R486W	9.4	>35	16.9	6.2	2.7
AG044/F	c.1462C>T/c.1529G>A	R488*/R510Q	6.6	>34	15.9	7.4	2.1
AG032/F	c.1121T>C/c.1706G>A	L374P/R569N	10.9	7	2.1	1.5	1.4
AG008/F	c.507+1G>A/c.1436G>A	Splicing/R479H	7.7	75	1.9	6.0	0.3
AG057/M	c.376-2A>C/c.1529G>A	Splicing/R510Q	8.0	55	1.7	5.6	0.3
AG029/F	c.331G>A/c.1456C>T	G111R/R486W	11.9	5	2.2	1.4	1.6
AG020/M	c.331G>A/c.1492C>T	G111R/R498C	10.3	40	2.5	4.1	0.6
AG023/M	c.1154G>A/c.1529G>A	R385K/R510Q	13.4	15	2.7	2.0	1.3
AG006/M	c.721G>T/c.1529G>A	E241*/R510Q	8.2	85	2.2	6.1	0.4
AG046/M	c.142_159del/c.494G>T	T48_T53del/G165V	12.9	20	10.0	3.5	2.8
AG010/M	c.1269G>A/c.1654G>A	Splicing/V552M	9.7	55	1.8	5.8	0.3
AG089/F	c.401T>A/c.1529G>A	V134D/R510Q	9.6	60	1.2	5.2	0.2
AG060/M	c.1529G>A/c.1529G>A	R510Q/R510Q	12.8	40	1.6	2.8	0.6
		Reference	M: 13.8-17.2 F: 11.9-15.5	1-2	9.2-16.9	0.9-1.4	9.0-16.6

Summary/Conclusions: These data support the hypothesis that drug intervention with AG-348 effectively upregulates PK-R enzymatic activity and increases stability in PK-deficient RBCs over a broad range of *PKLR* genotypes. The concomitant increase in ATP levels suggests that glycolytic pathway activity may be restored. AG-348 treatment may represent an attractive way to correct the underlying pathologies of PK deficiency

P615

IDENTIFICATION OF NEW PATHOGENIC MUTATIONS IN PATIENTS WITH RED BLOOD CELL MEMBRANE DISORDERS USING NEXT-GENERATION SEQUENCING

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Background: Red blood cell (RBC) membrane proteins deficiency or structural alterations lead to RBC membrane disorders such as hereditary spherocytosis, hereditary elliptocytosis or hereditary xerocytosis among others. Genetic analysis of these patients was not usually performed before next-generation sequencing due to the laborious task it consisted to sequence by Sanger the several membrane related genes, considering that they all contain a high number of coding regions.

Aims: The aim of this study is to perform the molecular diagnosis of the patients included in the study as well as to identify new pathogenic mutations leading to RBC membrane disorders.

Methods: 116 patients from 74 unrelated families were studied with a next generation sequencing (NGS) based panel that contained genes already described as disease causing for RBC membrane disorders (ANK1, EPB41, EPB42, SLC4A1, SPTA1, SPTB, PIEZO1, KCNN4, RHAG) as well as for enzymopathies (ADA, AK1, ALDOA, BPGM, CYB5A, G6PD, GCLC, GPI, GSR, GSS, HK1, NT5C3A, PFKM, PGK1, PKLR, TPI1), hemoglobinopathies (HBA1, HBA2, HBB) and congenital diseritrhopoietic anaemias (CDAN1, C15orf41, SEC23B, KLF1, GATA1, KIF23). The patients analysed were oriented as hereditary spherocytosis (63 patients), hereditary elliptocytosis or piropoikilocytosis (8 patients) and hereditary xerocytosis (3 patients). There were also 42 patients were the combination of phenotypic laboratory results was suggestive of membranopathy but it didn't suggest any specific RBC membrane pathology.

Results: A total of 74 pathogenic variants leading to RBC membrane disorders were identified, of which 14 had already been reported as disease causing. Of the remaining 60 variants, 42 had never been identified neither by 1000G or ExAC projects and therefore are novel mutations. Beta-spectrin, ankyrin and alpha-spectrin were the proteins that gathered most part of the mutations, we identified 23 variants in SPTB, 20 variants in ANK1 and 16 variants in SPTA. 48% (36/74) of the identified variants were missense changes, mostly from SPTB gene (11 variants), while a 38% (28/74) of the variants were nonsense changes or frame shifting mutations, mostly from ANK1 (12 variants) and SPTB (9 variants). Of special interest, only 2 variants were identified in more than one unrelated family: 1) SPTB c.647G>A, leading to spherocytosis, was identified in 8 patients of 2 unrelated families, 2) SPTA1 c460_462dupTTG, leading to ellyptocytosis, was identified in 6 patients from 5 different unrelated families. Finally, with the NGS panel results, the genetic diagnosis of 89% (103/116) of the included patients could be determined and only in 11% (13/116) the mutation was not identified or the variant correlation with disease was not clear. It is worthy to highlight that 10 of the 13 undiagnosed patients had been oriented as unclear membranopathy.

Summary/Conclusions: According to the results, there is a high genetic heterogeneity in patients with RBC membrane disorders, as almost each family carries a unique mutation that is not observed in any other no related family. The present study reveals the usefulness of NGS panel, which allows the molecular diagnosis of almost the 90% of the patients and it would avoid misdiagnosis that could lead to splenectomy in contraindicated cases such as in hereditary xerocytosis. Moreover, the 11% of undiagnosed patients will be analyzed through a second NGS gene panel including potential new genes leading to chronic haemolysis and/or sequenced by whole exome sequencing with the aim to identify new disease causing genes.

P616

CLINICAL FOLLOW-UP OF 378 PATIENTS WITH AUTOIMMUNE HEMOLYTIC ANEMIA: PROGNOSTIC IMPACT OF HEMOGLOBIN LEVELS, AUTOANTIBODY CLASS, AND RETICULOCYTOPENIA AT ONSET ON THE RELAPSE RISK AND OUTCOME

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Background: Autoimmune hemolytic anemia (AIHA) is greatly heterogeneous, from mild/compensated to life-threatening, due to autoantibody class/thermal amplitude, efficiency in activating complement, activity of the reticuloendothelial system, and efficacy of bone marrow compensatory response.

Aims: Here we analysed predictors of first relapse, complications, and fatality in a large AIHA series.

Methods: We retrospectively studied 378 patients (135m and 243 F, median age 61 yrs, range 19-100) from 10 sites, followed-up for 4.3 yrs (range 0.5-27). Patients were classified in warm (w)AIHA (DAT positive for IgG and IgG+C), cold agglutinin disease, CAD (C), mixed (IgG+C with high titer cold agglutinins) and atypical (DAT-, IgA+, wIgM). Cases were also grouped in very severe (Hb<6 g/dl), severe (Hb 6-8 g/dl), moderate (Hb 8-10 g/dl) and mild (Hb>10 g/dl). LDH was expressed as fold increase upper the limit of normality (ULN), and reticulocytes as absolute count and reticulocyte index. The following therapy lines were considered a) steroids +/-IvIg, b) rituximab c) splenectomy, d) immunosuppressive drugs (azathioprine, cyclophosphamyde, cyclosporin), and e) transfusions, plasma exchange, erythropoietin.

Results: Table 1 shows clinical and laboratory characteristics of AIHA cases at onset and distribution of thermal types. Hb values were significantly lower in IgG+C wAIHA and atypical cases (p<0.001), LDH higher in IgG+C wAIHA, mixed and atypical forms (p=0.01), and Hb and LDH values were negatively correlated (r= -0.25, p<0.001). Absolute reticulocytes were reduced in CAD, mixed and IgG+C wAIHA (p<0.001) together with inadequate reticulocytosis (p=0.01). Moreover, the reticulocyte index was lower in cases with Hb<6 g/dL (65 *vs* 98, p<0.001), along with more frequent inadequate reticulocytosis (87 *vs* 70%, p=0.01). First line therapy was administered in almost all cases but 25 CAD. A second therapy line was mostly required in IgG+C wAIHA, mixed, and to a lesser extent in CAD (p=0.005). The ultra-refractory cases requiring 4 or more lines of therapy were mainly mixed, atypical and CAD. Considering anemia severity, patients with Hb<8 g/dL more frequently required treatment after first-line (51 *vs* 33%, p=0.004; p=0.03), or even 3 or more therapy lines (52/71, 73% *vs* 19/71, 26%, p<0.001). The following hazard ratios (HR) emerged from multivariate Cox regression analysis: HR 3.2 (95% CI 1.4-7), 2.9 (1.4-6.2), 3.4 (1.6-7.5), for Hb <6, 6-8, and 8-10 g/dL compared to patients with Hb >10, respectivelyAs regards complications, infections were observed in 14% of cases, mostly mixed AIHA (p=0.02); thrombosis occurred in 10% and acute renal failure in 3% of patients, with no relationship with AIHA type/Hb values. Evans' syndrome was more frequent in mixed or atypical cases (p=0.04) and in severe forms (74% with Hb<8 g/dL *vs* 26%, p=0.005), and was associated with higher relapse risk (HR 2.3, 95% CI 1.4-3.9). Seventy patients died during the follow-up, and 12 because of AIHA-related acute complications. Higher mortality was observed for infections (HR 5.8, 95% CI), acute renal failure (HR 7.6, 95% CI) and Evans' syndrome (HR 8.3, 95% CI).

Table 1.

	WAIHA (n=193)		CAD (n=109)	Mixed AIHA (n=56)	Atypical AIHA (n=20)
	IgG (n=159)	IgG+C (n=34)			
Hb (g/dL) median (range)	7.3 (2.1-14)	5.8 (2-10.7)	8.2 (4-13.5)	7 (2.9-11.5)	6.2 (3-9)
LDH ULN median (range)	1.7 (0-27)	1.8 (1-5)	1.4 (0-12)	1.9 (1-10)	2 (1-18)
Ret (x10^6/L) median (range)	192 (22-644)	156 (53-495)	122.5 (13-644)	150 (45-210)	202 (29-780)
inadequate reticulocytosis (n of pts, %)	85 (53%)	23 (37%)	71 (65%)	27 (48%)	11 (55%)
Anemia					
Very severe anemia (Hb<6 g/dL)	44 (28%)	20 (58%)	10 (9%)	18 (32%)	10 (50%)
Severe anemia (6<Hb<8 g/dL)	63 (40%)	10 (30%)	41 (38%)	21 (38%)	7 (35%)
Moderate anemia (8<Hb<10 g/dL)	37 (23%)	2 (6%)	34 (31%)	14 (25%)	3 (15%)
Mild anemia (Hb>10 g/dL)	15 (9%)	2 (6%)	24 (22%)	3 (5%)	0 (0%)
Therapy					
No therapy	8 (5%)	0 (0%)	25 (23%)	2 (3%)	1 (5%)
1 line of therpy (n of pts, %)	151 (95%)	34 (100%)	84 (77%)	55 (98%)	19 (95%)
2 lines of therpy (n of pts, %)	59 (37%)	19 (56%)	51 (47%)	36 (64%)	7 (35%)
3 lines of therpy (n of pts, %)	23 (15%)	6 (18%)	26 (24%)	15 (27%)	4 (20%)
4 or more lines of therpy (n of pts, %)	7 (4%)	0 (0%)	11 (10%)	5 (9%)	2 (10%)
Complications					
Infections (n of pts, %)	23 (14%)	5 (15%)	10 (9%)	14 (25%)	0 (0%)
Thrombosis (n of pts, %)	23 (14%)	7 (20%)	15 (14%)	11 (20%)	2 (10%)
Acute renal failure (n of pts, %)	5 (3%)	2 (6%)	2 (2%)	2 (4%)	0 (0%)
Evans' (n of pts, %)	11 (7%)	4 (12%)	1 (1%)	5 (9%)	2 (10%)
Death (n of pts, %)	31 (19%)	7 20%)	22 (20%)	6 (11%)	3 (15%)
Death for AIHA (n of pts, %)	5 (3%)	2 (6%)	2 (2%)	3 (5%)	0 (0%)

Summary/Conclusions: In conclusion, we found that severity of anemia at onset was the major determinant of relapse risk. The lowest Hb levels were observed in patients with IgG+C WAIHA and atypical cases along with higher LDH levels and inadequate reticulocytosis, advising strict clinical observation in these patients.

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HEME BINDS ANNEXIN-A5 DURING HEMOLYSIS AND PREVENTS ITS INTERACTION WITH CELL MEMBRANE PHOSPHATIDYLSERINE DURING SICKLE CELL DISEASE

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Background: Intravascular hemolysis, such as in sickle cell disease (SCD), is characterized by red blood cell damage, high levels of cell-free heme and extracellular vesicles in plasma, along with inflammation and tissue injury. Stressed leukocytes, platelets, endothelial and red blood cells shed microparticles (MP) that bear externalized phosphatidylserine (PS) at their surface and promote tissue injury. Conversely, intracellular annexin-A5 acts as an inhibitor of externalized PS at the surface of cells and MP. Annexin-A5 is thought to orchestrate vesicle trafficking, promote cell membrane repair, protect against PS-mediated effects and enforce anti-inflammatory and anti-thrombotic control.
Aims: We investigated a possible functional relationship between intravascular hemolysis and annexins. We hypothesized that annexins, and annexin-A5 activity in particular, is blocked by extracellular heme as it is released in plasma during intravascular hemolysis.
Methods: In order to test the heme-annexin-A5 relationship, we measured PS-, PS+, CD235a+ and annexin-A5+ circulating MP in adult SCD patient and matched control plasmas. We explored annexin-A5 expression in plasma and blood cells by Western blots and ELISA, and also quantified the PS-binding functionality of plasma annexin-A5 using a self-designed immunocapture assay and purified PS+ MP. Moreover, we investigated molecular interactions between purified heme and recombinant human annexin-A5 by surface plasmon resonance (BiaCore and Proteon), absorbance shift assay and protein autofluorescence quenching. Finally, we put forward a model of heme-annexin-A5 docking by 3D molecular rendering.
Results: Immunocapture of plasma annexin-A5 revealed an association with heme (Abs398 nm signature) during SCD, especially during acute hemolytic events. In SCD plasma, we found increased total annexin-A5, but virtually undetectable levels of functional annexin-A5, contrary to controls. This implied a greatly reduced ratio of functional annexin-A5/circulating PS+ MP. Moreover, purified heme bound readily to annexin-A5 with relatively high affinity in vitro, as demonstrated using absorbance shift, autofluorescence quenching and plasmon surface resonance assays, with human serum albumin and hemopexin in competition tests. Annexin-A5 autofluorescence was partly quenched by heme addition, which also produced a significant red-shift in heme absorbance wavelengths, implying that a tight and direct molecular interaction was possible. Hemoglobin and heme also triggered annexin-A5 aggregation in vitro, producing high molecular weight and heat-resistant multimers, observed by western blot. Surface plasmon resonance studies revealed that annexin-A5 contains several sites for heme binding, some with very low affinity, while others are estimated with a Kd in the 10-6m range, rather similar to that of albumin. Part of the heme bound to annexin-A5 remained in place, even in the subsequent addition of the high-affinity heme-scavenger hemopexin. 3D molecular docking rendering suggested that heme may bind to the PS-binding groove of annexin-A5, thereby preventing further interactions with PS. Finally, heme completely prevented the binding of exogenous annexin-A5 to purified PS+ MP and plasma MP, as well as their subsequent detection by flow cytometry.
Summary/Conclusions: Together, our data suggest that PS-neutralizing annexin-A5 is inhibited by cell-free heme. This heme-mediated inhibition of annexin-A5 may display physiopathological relevance, contribute to the accumulation of PS+ MP in plasma during intravascular hemolysis, and more specifically of RBC MP during SCD which can participate to the degradation of the vascular function.

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USE OF PEGYLATED-CARBOXYHEMOGLOBIN BOVINE FOR THE TREATMENT OF SICKLE CELL DISEASE ASSOCIATED LEG ULCERS: RESULTS FROM A PHASE 2 SAFETY STUDY

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Background: Leg ulcers are a common complication of sickle cell disease (SCD). The pathophysiology of SCD leg ulcer is complex and may include obstruction of blood vessels by sickled red cell, chronic anemia, depleted nitric oxide bioavailability (resulting in impaired endothelial function), infection, thrombosis and excessive vasoconstriction. These events lead to progressive peripheral de-vascularization and tissue necrosis, such that even minor lower-leg wounds can become persistent ulcers, with no tendency to heal after months of appropriate treatment. PEGylated-Carboxyhemoglobin bovine (PEG-COHb; SANGUINATE) is an oxygen carrying agent with anti-inflammatory activity. A study of safety and effectiveness was undertaken in SCD patients with chronic leg ulcers to determine the safety of this investigational drug administered in as a once weekly infusion for either 4 or 6 weeks.
Aims: To assess the safety and efficacy of repeated doses of PEG-COHb on SCD leg ulcers.
Methods: The study was an escalating, repeated-dose, open-label, Phase 2 study to test PEG-COHb at 320mg/kg (8 mL) in subjects suffering from leg ulceration associated with SCD. It was conducted in Panama and the Dominican Republic. All enrolled subjects underwent a 3-week Run-In Period, during which they received standard of care treatment for wound management. During the Treatment Period, subjects were assigned sequentially to Cohort 1 or Cohort 2. Cohort 1 received 4 once-weekly doses by 2-hour intravenous infusion of SANGUINATE. Following the completion of Cohort 1, the safety findings were reviewed prior to initiating Cohort 2. Cohort 2 received 6 once-weekly infusions. In addition to the study drug, subjects continued to receive standard of care during the Treatment Period. One week after the end of Treatment, subjects returned to the study center for a Final Visit. The following assessments were done: safety: Safety was assessed by recorded adverse events (AEs), laboratory assessments (hematology, chemistry, and urinalysis), vital signs, concomitant medications, and 12-lead electrocardiograms (ECGs), efficacy: Wound pain, wound appearance and condition, wound size, wound vascular status (Venous Clinical Severity Score; VSCC), quality of Life: Quality of life was assessed using the Short Form-12 v2 Health Survey (SF-12).
Results: The administration of once-weekly infusions of PEG-COHb was well tolerated. Treatment emergent adverse events (mild pyrexia, moderate wors-

ening anemia) considered related to study drug were report in 2/10 patients. Increases in mean arterial pressure were anticipated due to the oncotic effects of this colloidal drug, but with no consistent pattern to the changes. Changes in ECG intervals were seen in a few subjects, but those changes were not considered clinically meaningful. There were no clinically meaningful changes in laboratory values, physical examinations, or concomitant medications. There were no statistically significant changes from Baseline in leg ulcer pain and wound surface area for either Cohort. All of the wound assessments remained relatively consistent throughout the study. There were slight decreases in total VCSS at most time points, indicating slight improvement in vascular status. Results were similar for the individual scores.

Summary/Conclusions: The administration of 4 or 6 once-weekly infusions of PEG-COHb at a dose of 320mg/kg was generally well tolerated. Slight improvements in total and individual VCSS are promising and may warrant further study with prolonged repeated doses of PEG-COHb.

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NON-RENAL DETERMINANTS OF ENDOGENOUS ERYTHROPOIETIN LEVELS IN SICKLE CELL DISEASE

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Background: Sickle cell disease (SCD) is characterized by chronic hemolysis and inflammation. Elevated levels of erythropoietin (EPO) drive expansion of erythropoiesis to compensate for increased red cell destruction. EPO is produced in response to anemia and tissue hypoxia. Previous studies in SCD suggest that EPO is inappropriately low for the degree of anemia but the reasons are unclear.

Aims: To perform a retrospective analysis of data collected as part of routine clinical care to examine the relationship between serum EPO and degree of anemia, HbF levels, oxygenation status, hemolytic rate, alpha globin status, inflammation, serum ferritin and renal function.

Methods: King's College Hospital (London, UK) has a large SCD population. All patients with HbSS or HbSβ^0 thalassemia who had a serum EPO level measured between 2007 and 2013 were included. Sickle genotype, alpha genotype, "baseline" HbF (no hydroxycarbamide, transfusion or pregnancy) and demographic data were recorded. Other clinical variables were obtained from the same day as EPO levels (medications, laboratory values and oxygen saturation). Serum EPO was measured by chemiluminescence immunoassay (Siemens Immulite XPi). Exclusion criteria were: active vaso-occlusive crisis, transfusion within 8 weeks, chelation, erythropoiesis stimulating agent therapy, home oxygen, pregnancy and renal disease (eGFR<60mls/min or urine albumin creatinine ratio>30). Data analysis was performed in IBM SPSS Statistics 22. Skewed variables were log transformed and estimated GFR was calculated using the MDRD formula. Normalized variables were correlated with Ln EPO using Pearson's correlation, ordinal variables using one way ANOVA, and binary variables using independent samples 2-tailed t-test. Multivariate linear regression using Ln EPO as the outcome was performed.

Results: 245 adult (≥17 years) SCD patients (all of African or African-Caribbean origin) met the inclusion criteria. Of the 245, 241 had HbSS and 4 HbSβ^0, 100 were male and 145 female, and mean age was 31.2 +/- 11.6 years. 221 had known alpha globin status: 21 α-/α-, 81 αα/α-, 118 αα/αα, 1 αα/ααα. Univariate analysis revealed a weak/moderate negative association between Ln EPO and Hb (*r*=-0.383, p<0.001). Significant associations were also seen between Ln EPO and: negative correlation with PCV, oxygen saturations, Ln HbF; and positive correlation with Ln CRP, LDH, STFR, Ln uACR, cystatin C. One way ANOVA showed alpha globin status to be associated with EPO (higher EPO with more alpha chains). There was no significant association between EPO and: age, sex, eGFR, white cell count, and use of hydroxycarbamide. Multivariate linear regression (N=175) revealed alpha globin status, Hb, CRP and HbF remained independently associated with Ln EPO level, see table.

Table 1. Multivariate analysis of EPO.

	Beta (95% CI)	p-value
Alpha globin category	-.188 (-.311 to -.066)	.003
Hb	-.016 (-0.022 to -0.011)	.000
LnCRP	.115 (0.008 to 0.222)	.035
LnHbF	-.117 (-.209 to -.024)	.014

where alpha= 0 if aa/aa; 1 if aa/a-; 2 a-/a-

Summary/Conclusions: In our SCD cohort without renal dysfunction EPO was elevated. Unlike the non-sickle setting where Ln EPO is very strongly (negatively) correlated with Hb levels, in our SCD cohort we have found only a mild/moderate correlation. Instead, additional associations were seen between EPO and alpha globin status, CRP and HbF. Our findings suggest that in addition to Hb, other SCD severity markers influence EPO production. This may provide explanation for relative EPO deficiency, and have implications for considering therapeutic EPO in SCD.

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THE PHARMACOKINETICS (PK) OF GBT440 ARE SIMILAR IN ADOLESCENTS AND ADULTS WITH SICKLE CELL DISEASE (SCD)

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Background: Sickle cell disease (SCD) is caused by polymerization of Hemoglobin S (HbS), resulting in hemolysis and vaso-occlusion. Currently, no therapy achieving pancellular, direct inhibition of HbS polymerization is available for adults or children with SCD. GBT440 is a novel small molecular inhibitor which increases hemoglobin oxygen affinity, thereby preventing HbS polymerization and red blood cell sickling. This study represents the first evaluation of GBT440 in a pediatric population.

Aims: This study was designed to evaluate the safety and PK of GBT440 following a single and multiple doses in adolescents. In addition a population PK (PPK) model, based on data derived following single doses of GBT440, was developed to support the identification of future GBT440 dosing regimens for pediatric populations with SCD.

Methods: This is an ongoing, open-label, Phase 2a study in adolescents (12 to 17 years) with SCD (HbSS or HbS β^0thalassemia). Participants were enrolled after obtaining written informed consent/assent. This study is being conducted in 2 parts: Part A, single-dose, and Part B, multiple-dose for 24 weeks. The primary objective of Part A is PK and the primary objectives of Part B are safety and efficacy. PK samples to measure whole blood and plasma GBT440 concentrations were collected up to 15 days following single dose administration. Separate PPK models were developed to describe the concentration *versus* time profiles of GBT440 in whole blood and plasma using non-linear mixed effects modeling (NONMEM, version 7.3). The allometrically scaled adolescent PPK model was also used to estimate the appropriate single dose for subsequent evaluation in pediatric participants (6 to 12 years).

Results: Part A has been completed; 7 adolescents (3 males/4 females) received a single dose of GBT440 600mg. The median age of participants was 16 years (range 14 to 16 years) and the mean weight was 52.8 kg (range 44.6 to 65.8kg). GBT440 was well tolerated; there were no drug-related ≥Grade 3 adverse events (AE) or serious adverse events and the most common AE was Grade 1 nausea. A 2-compartment model with first order absorption best described the PK of GBT440 and is the same model structure as previously used for adults with SCD. GBT440 PK parameters (Table 1) are comparable to those derived in adults, suggesting that GBT440 PK in adolescents and adults are similar. Model validation confirmed this result with good agreement between the observed adolescent PK data and simulated profiles based on the adult GBT440 PK model.

Table 1.

Table 1 **Parameter Estimates from Whole Blood and Plasma GBT440 Population PK Models, Adults versus Adolescents Participants with SCD**

PK Parameters	GBT440 in Whole Blood Adults (RSE %)	GBT440 in Whole Blood Adolescents (RSE %)
CL (L/h)	0.43 (5)	0.37 (13)
V (L)	21.4 (6)	15 (17)
Ka (1/h)	0.34 (13)	0.19 (30)
	GBT440 in Plasma Adults (RSE %)	**GBT440 in Plasma Adolescents (RSE %)**
CL (L/h)	7.63 (5)	5.4 (6)
V (L)	373 (5)	329 (7)
Ka (1/h)	2.83 (14)	1.37

CL = apparent clearance, Ka = absorption rate constant, RSE = relative standard error, V = apparent volume of distribution; L= liters; h= hours

Summary/Conclusions: This is the first study used to develop a GBT440 PPK model in adolescent participants with SCD. Data suggests that similar GBT440 doses can be used in adolescents and adults. Part B has been initiated to evaluate multiple doses of GBT440 in adolescents. This PPK model can potentially be used to estimate individual PK parameters (*e.g.*, AUC) to support future GBT440 dose selection for evaluation in the pediatric population.

Gene therapy, cellular immunotherapy and vaccination

P621

DEVELOPMENT OF TAX-REDIRECTED T-CELL IMMUNOTHERAPY FOR ADULT T CELL LEUKEMIA

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Background: Adult T cell leukemia/lymphoma (ATL) is an aggressive peripheral T-cell neoplasm caused by HTLV-1 virus infection and its prognosis remains very poor. Tax, which is the most important regulatory protein of HTLV-1, is associated with aggressive proliferation of host cells and is also a major target antigen for $CD8^+$ cytotoxic T-cells (CTLs). We previously analyzed the Tax-specific T-cell receptor (TCR) repertoire, phenotypes and functions of Tax-specific CTLs at the single-cell levels in HLA-$A24^+$ ATL patients who underwent allogeneic stem cell transplantation (allo-SCT). We found that a particular amino acid sequence motif (PDR) in the CDR3 region of TCR-ß was conserved in different patients and also within the same patient before and after allo-SCT, and the PDR(+) Tax-specific CTL clone selectively expanded in ATL long-term survivors as less-differentiated effector memory CTLs. Actually, the PDR(+) CTL showed not only strong binding activity for the Tax-tetramer but also strong killing activity against patients' HTLV-1-infected T-cells without any reaction against normal cells.
Aims: Currently, we are planning a redirected T-cell immunotherapy using the PDR(+) TCR genes for ATL. Therefore, we prepared donor-derived PDR(+) TCR-transduced T-cells and evaluated their cytotoxic efficiency against HTLV-1-infected T-cells and ATL-cells both *in vitro* and *in vivo* mouse model.
Methods: HLA-A24:02 restricted and Tax301-309-specific TCR-α/β genes were cloned from an established PDR(+) CTL clone and integrated into a retroviral siTCR vector (Tax-siTCR vector) encoding small-interfering RNAs (siRNAs) to knockdown endogenous TCR genes for the efficient expression of therapeutic TCRs. Then, $CD8^+$ T-cells of healthy volunteers were transfected with Tax-siTCR vector (Tax-siCTLs). First, cytotoxicity and cytokine production capability of the Tax-siCTLs against HTLV-1 infected T-cells or ATL-cells were evaluated using calcein-AM-based assay and flow-cytometric analysis, respectively. Next, to evaluate the *in vivo* anti-ATL effects by the Tax-siCTLs, the bioluminescence assay (*in vivo* imaging system) was performed. We generated a luciferase-gene transduced HLA-$A24^+$HTLV-1 infected cell-line, MT-2 (Luc-MT-2), and injected 1×10^6Luc-MT-2 cells into six-week-old NOD/Shi-scid,IL-2RγKO Jic (NOG) mice intraperitoneally. After the 3 weeks, 2×10^6Tax-siCTLs were administered via the tail vain weekly, for a total of 3 times. For comparison, non-integrated T-cells (Mock) were administered in the same way. These mice were monitored for tumor growth using IVIS system weekly.
Results: Tax-siCTLs showed specific and strong killing activity against both HTLV-1 infected T-cells and patients' ATL-cells without any reaction against control normal-cells. In addition, Tax-siCTLs produced a sufficient amount of cytokines such as IFN-γ, TNF-α, and IL-2 against HTLV-1 infected T-cells. In mice experiments, the bioluminescence of Luc-MT-2 in the mice treated with Tax-siCTLs had started to reduce gradually after 7 weeks, and finally became undetectable after 9 weeks. In addition, macroscopic anatomical findings in the treated mice were normal after 12 weeks. In contrast, the amount of bioluminescence in the mice treated with Mock or in the control mice without treatment had rapidly increased and all mice died by 9 weeks.
Summary/Conclusions: We confirmed that Tax-siCTLs could exert a strong anti-ATL effect without significant reaction against normal cells both *in vitro* and *in vivo*. The therapy using this PDR+ Tax-siCTLs has a potential to be a novel immunotherapy for ATL patients.

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Abstract withdrawn.

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NHEJ-BASED GENE EDITING: A NOVEL GENE THERAPY APPROACH IN FANCONI ANEMIA HEMATOPOIETIC STEM AND PROGENITOR CELLS

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Background: Allogenic transplantation of hematopoietic stem and progenitor cells (HSPCs) is the only current curative treatment for the bone marrow failure of patients with Fanconi Anemia (FA). However, the risks of GVHD and increased incidence of subsequent cancer, and the limited availability of matched donors hamper the application of this therapy in FA patients. For this reason correction of patients' HSPCs by gene therapy is considered a promising therapeutic alternative for these patients. In this context, gene editing constitutes a new step in the development of safe gene therapy approaches. Since non-homologous end joining (NHEJ) is the preferred DNA repair mechanism in HSPCs, and given that this mechanism has been reported to be enhanced in FA cells, we have tested the efficiency of a NHEJ-mediated gene editing approach to generate compensatory mutations that can restore the FANCA protein function in HSPCs from FA patients, mimicking reversions observed in mosaic patients.
Aims: To demonstrate the feasibility of using a NHEJ-based gene editing strategy to correct FA-A HSPCs as a result of the insertions and deletions (INDELs) generated in edited *FANCA* sequences in these cells.
Methods: Two different *FANCA* mutations from FA-A patient-derived lymphoblastic cell lines (LCLs) and primary HSPCs were targeted by the CRISPR/Cas9 system. INDELs generated as a consequence of the NHEJ repair were analyzed at different time points.
Results: Initial studies conducted in a FA-A LCLs carrying the biallelic c.295C>T point mutation that generates a premature stop codon (p.Q99X) showed targeting efficiencies around 20%. Next Generation Sequencing (NGS) not only revealed the generation of frame-restoring repair events, but also that these corrective NHEJ events conferred a marked *in vitro* proliferative advantage to the edited cells. Moreover reversion of the characteristic MMC hypersensitivity and restoration of the FANCD2 foci formation were observed in these cells. In addition, western-blot analysis confirmed the stable expression of FANCA protein. To further demonstrate the feasibility of the approach, a second *FANCA* associated mutation was targeted (c.3558insG, producing a frameshift and a premature stop codon -p.R1187EfsX28-) with even higher gene targeting efficiencies. Finally similar studies were conducted in three HSPCs samples from FA-A patients harboring the c.295C>T mutation, that showed targeting efficacies up to 36%. Moreover, NGS detected the presence of corrective NHEJ-repair events immediately after editing and evidenced up to 50-fold expansion of corrected cells after nine days in culture, confirming the functionality and proliferative advantage conferred by the frame restored alleles.
Summary/Conclusions: Our results demonstrate for the first time that NHEJ gene correction is feasible in FA HSPCs. The high efficacy of the NHEJ repair pathway in HSPCs together with the simplicity of the strategy, make this approach clinically relevant for the future treatment of the hematopoietic defects in FA patients.

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NOVEL, ENHANCED AND DUAL TARGETING CAR INVARIANT NKT CELL-BASED IMMUNOTHERAPY FOR CD1D+ B CELL MALIGNANCIES

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Background: Anti-CD19 chimeric antigen receptor T cell (CART19) immunotherapy has shown promising clinical potential in relapsed/refractory mature B cell malignancies. However, only about half of patients benefit, highlighting the need for more effective CAR-based strategies. iNKT cells are rare but powerful immunoregulatory and cytotoxic T lymphocytes, playing a pivotal anti-tumor role. iNKT cells are restricted by CD1d, a non-polymorphic, phospho-/glycol-lipid-presenting HLA I-like molecule. We previously showed that CD1d, as well as on normal B cells, is also expressed on malignant CD19+ B cells in mantle cell lymphoma (MCL), marginal zone lymphoma (MZL) and chronic lymphocytic leukaemia (CLL).
Aims: We tested the hypothesis that bi-specific CAR*iNKT*19 cells, targeting simultaneously CD19 and CD1d via the CD19-specific CAR and their natural invariant TCR respectively, would be more effective than CART19 cells against CD19+CD1d+ B cell malignancies.
Methods: We optimized a novel protocol for manufacturing 2^{nd} (CAR2) and 3^{rd} (CAR3) iNKT cells expressing CAR19. Their *in vitro* reactivity was assessed in cytotoxicity (flow cytometry-based) and cytokine and cytotoxic granule release assays (intracellular staining and Luminex technology). *in vivo* reactivity was assessed in NSG xenograft assays, with monitoring of C1RCD1d tumour cell growth by bioluminescence imaging.
Results: Our optimised protocol for selection, lentiviral transduction and clinical scale expansion of CARiNKT cells within 3 weeks is suitable for frozen and fresh lymphocytes, derived from either healthy donors or cancer, including lym-

phoma patients. The manufacturing process consistently allows high CAR transduction efficiency of iNKT and T cells (75.31%±4.294 and 76.95%±14.76 respectively, n=8) and ensures the preservation of CD4− iNKT cells, which have been associated with a higher cytotoxic potential and anti-tumour activity. *In vitro* validation, using singly- or dual-positive CD1d and CD19 targets, demonstrated that CARiNKT19 cells are CD19-specific, retain their natural CD1d-restricted reactivity, and exert additive dual-specific cytotoxicity against CD1d+CD19+ targets. Additional functional dissection showed that activated CARiNKT19 cells, both fresh and cryopreserved, have the ability to produce cytotoxic granules and IFNγ faster and in larger amounts than same donor activated CART19 cells. Likewise, CAR2- and CAR3-iNKT cells are equally or more effective than their CART counterparts in killing CD19+CD1d+ lymphoid cell lines (B-lymphoblastoid C1RCD1d and lymphoma-derived Farage cells) and consistently more effective against primary MCL, MZL and CLL cells. Finally, in an *in vivo* NSG xenograft model of lymphoma, while survival of T- and NKT cell-treated animals was the same as that of untreated animals (P=0.23), both CART19 and CARiNKT19 cell-treated animals had significantly and comparably improved overall survival (P<0.001). However, compared to CART19, CARiNKT19 immunotherapy led to a better disease control, with earlier, more profound and sustained responses resulting in a significantly improved tumour free-survival (P=0.03).

Summary/Conclusions: In our pre-clinical *in vitro* and *in vivo* lymphoma models, CARiNKT19 are more effective than CART19 cells against CD19+CD1d+ B cell malignancies. Further, dual targeting by CARiNKT19 cells may mitigate against CD19-focused tumour escape after CAR immunotherapy, while the previously demonstrated role of donor iNKT cells in protection from aGVHD supports the development of CARNKT19 cells for 'off-the-shelf' use.

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A NOVEL CHIMERIC ANTIGEN RECEPTOR ENDOWS T CELLS WITH NK CELL-LIKE SPECIFICITY AND ATTACKS A WIDE RANGE OF HEMATOLOGICAL MALIGNANCIES AND CANCERS

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Background: Engineered T-cells expressing CD19-specific chimeric antigen receptors (CARs) have shown high response rates against relapsed and refractory B cell acute lymphoid leukemia (ALL). However, similar success has not yet been demonstrated in solid tumors, and the reasons for this are currently being investigated. One major obstacle is the difficulty in determining appropriate surface antigens that are effectively targeted by CAR-transduced immune cells. NKp44 is an activating receptor on human NK cells that is only expressed when the NK cells are activated, and which confers a marked increase in cytotoxicity against various tumors. Ligands for NKp44 have been reported to be expressed in various types of cancers, but not in healthy cells. Effective use of the ligand-binding domain of this receptor as an antigen recognition site of a CAR would thus allow a wide range of cancer cells to be attacked.

Aims: To determine the optimal CAR construct including the NKp44 immunoglobulin domain as a ligand-binding domain (NKp44-based CAR), with a view to developing effective CAR-T therapy against hematological malignancies and solid cancers.

Methods: We created several NKp44-based CAR constructs. Human T cells from healthy donors were stimulated with anti-CD3/CD28 beads and recombinant interleukin-2. Human NK cells were stimulated using K562-mb15-41BBL feeder cells, as previously reported (Imai C, 2005). Activated T cells or NK cells were then subjected to retroviral transduction with the CAR gene and the phenotypic and functional characteristics of CAR-T cells engrafted with the various NKp44-based CARs were compared. We determined if NKp44-ligands were present on the cell surface of various types of malignant cell lines using recombinant human NKp44 Fc chimeric protein.

Results: Surface expression of ligands for NKp44 was confirmed in a wide range of tumor cell lines including acute myeloid leukemia (AML; KG-1, THP-1, U937, K562, Kasumi-1, Kasumi-6), T-cell ALL (MOLT-4, HSB2, Peer, Jurkat), B-cell ALL (OP-1), Burkitt's lymphoma (Raji), osteosarcoma (NOS-10, NOS-1, NOS-2, SaOS-2, U2OS,mg-63), rhabdomyosarcoma (RMS-YM, Rh28), and neuroblastoma (NB1, NB16, IMR-32, SK-N-SH). Different expression levels of CAR were observed among the NKp44-based CARs created in this study, in which the major CAR domains, except for the ligand-binding domain, were derived from various components including NKp44, CD8α, CD28, or CD3ζ. A combination of the hinge domain from NKp44, transmembrane domain from CD28, and intracellular signaling domain from CD3ζ yielded the highest surface expression of CAR on both T cells and NK cells. T cells transduced with this CAR showed enhanced cytotoxicity against various target cells including AML, T-cell ALL, and B-cell ALL, but did not attack normal T cells. CAR-T cells also showed increased production of interferon-gamma and granzyme B. The hinge domain of NKp44 has been suggested to play a role in ligand binding (Koch J, 2013), but the details are poorly understood. Intriguingly, replacement of the hinge domain from NKp44 significantly reduced cytotoxic function, though CAR expression levels remained similar.

Summary/Conclusions: T cells transduced with NKp44-based CARs show enhanced activities against various tumor cells. The extracellular hinge region of NKp44 appears to play an important role in ligand binding and/or recognition. NKp44-based CARs may represent a promising candidate for novel immune therapies targeting a wide range of cancers.

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NKP30-CAR REDIRECTED HUMAN T LYMPHOCYTES INDUCE POTENT ANTITUMOR IMMUNITY TO LEUKEMIA CELL LINES AND PATIENT-DERIVED ACUTE MYELOID LEUKEMIA IN NSG XENOGRAFT MODELS

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Background: Adoptive cellular therapy (ACT) of chimeric antigen receptor (CAR)-redirected T cells has evolved as a highly effective individualized immunotherapy for leukemia and solid cancer. In particular, clinical trials using CD19 CAR expressing T lymphocytes to combat CD19$^+$ lymphomas have revealed compelling results. However, suitable antigens for an effective and specific CAR-mediated therapy to acute myeloid leukemia (AML) are still warranted as *e.g.* CD33 and CD123 CAR expressing T cells induce potent immune responses not only to AML blasts but also recognize normal hematopoietic stem cells (HSC). In contrast, B7H6, a member of the B7 family, is frequently expressed on various tumor cells including AML blasts while not detectable on normal tissues, and is recognized by the natural killer (NK) cell activating receptor NKp30. Moreover, NKp30 recognizes human leukocyte antigens (HLA)-B-associated transcript 3, a nuclear factor that is secreted and translocated to the cell surface in stressed and transformed cells.

Aims: In the current study, we thus explored the use of human T cells redirected to express a NKp30-CAR for inducing effective antileukemic immunity *in vitro* and in vivo to the erythrogenic leukemia line K562 and primary AML blasts in NSG xenograft mouse models following ACT.

Methods: PBMCs or MACS® purified human T cells were polyclonally stimulated and reprogrammed with a CAR composed of the extracellular region of the NKp30 receptor fused to the CD3ζ chain signaling domain (kindly provided by Dr. S. Klobuch, Dept. of Internal Medicine 3, Medical University Regensburg, Germany) by retroviral gene transfer. Transduced T cells were further selectively expanded utilizing puromycin resistance present on the retroviral backbone, and NKp30 expression was determined by flow cytometry. IFN-γ ELISPOT analyses and cytotoxicity assays were performed to assess antileukemic responses to leukemia lines and primary AML blasts *in vitro* and *in vivo* using NSG xenografts and adoptive transfer of redirected T cells. Expression of B7H6 in target cells was confirmed by RNA-based RT PCR.

Results: Following transduction and puromycin selection ≥90% of CD3$^+$ T cells expressed the NKp30 CAR. In addition, most T cells displayed an effector-memory phenotype. Upon coculture with the B7H6 expressing targets such as K562 and HL-60 (myelogenous leukemia cell lines), NALM 16 (pre-B-ALL) and patient-derived AML samples (*e.g.* MZ506 and MZ987) NKp30-redirected T cells elicited potent IFN –γ release and exhibited cytolytic activity to both leukemia lines and primary AML blasts in vitro. These responses were specific as *e.g.* no reactivity to the B7H6 negative myeloma line U266 was observed. We then evaluated antitumoral responses of NKp30-redirected T cells in vivo. Upon adoptive transfer of NKp30-CAR T cells into NSG mice engrafted with K562 significant reduction of tumor burden was observed. Moreover, injection of 1 - 5x10^6 HLA-matched, NKp30-CAR redirected CD3$^+$ T cells into NSG mice showing up to 5% engraftment of patient derived AML blasts and thus resembling a clinically relevant minimal residual disease status at time of ACT resulted in clear leukemia regression. Further experiments *e.g.* to elaborate to what extent CD4$^+$ and CD8$^+$ T cells contribute to this antileukemic immunity are in progress.

Summary/Conclusions: These studies demonstrate that human T lymphocytes can be successfully redirected to acute leukemia by NK cell activating receptor based CARs such as the NKp30-CAR. As its ligand B7H6 has not been reported to be expressed on CD34$^+$ HSC, this antigen might be an interesting target for adoptive immunotherapy to AML.

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PRECLINICAL TESTING OF ADOPTIVE T-CELL RECEPTOR GENE TRANSFER IN COMBINATION WITH CHECKPOINT INHIBITORS AS A NOVEL THERAPY FOR MULTIPLE MYELOMA

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Background: Adoptive cellular therapy (ACT) based on T-cell receptors (TCR) or chimeric antigen receptor (CAR)-engineered T cells has achieved tremendous success in the treatment of cancer, especially B-cell malignancies. The

impressive therapeutic results recently obtained with checkpoint inhibitors have opened a new era in the field of cancer immunotherapy. Yet, clinical responses are still often observed either transiently or in a minority of patients. This underscores the need for an improved understanding of underlying factors limiting the efficacy of T cell-based immunotherapy and its wide application.
Aims: We explored an immunotherapeutic combination strategy to unleash the full anti-tumor response of adoptively transferred antigen-specific T cells. We propose to target multiple myeloma (MM) tumor cells in our established xenograft *in vivo* adoptive cell therapy model by T cells equipped with two optimized TCRs specific for HLA-A2.1-restricted MDM2 and p53 epitopes in combination with checkpoint inhibitors.
Methods: Human T cells from healthy donors were retrovirally transduced with MDM2- and p53-specific TCRs and expression levels were analyzed by flow cytometry. MDM2 and p53 protein expression in MM cell lines was determined by Western blot. The therapeutic efficacy of adoptive TCR transfer was evaluated in NOD-scid IL2R gamma chain^{null}(NSG) mice engrafted (s.c) with HLA-A2.1-expressing NCI-H929 MM cell line. In the combination checkpoint inhibitor approach, mice were treated (i.p) with anti-PD-1 (Nivolumab). Tumor growth was monitored and intratumoral alterations (in particular expression of relevant tumor and T cell antigens) in ex-vivo tumors were analyzed by flow cytometry. Tumor-infiltrating lymphocytes (TILs) were also characterized by flow cytometry.
Results: Adoptive transfer of dual MDM2/p53-specific TCR equipped T cells showed a superior anti-tumor response *in vivo* compared to single TCR treatment, demonstrating the need to target multiple MM antigens to circumvent tumor escape mechanisms associated with down-regulation of antigen. Yet, we observed a strong up-regulation of PD-L1 expression in tumor cells *in vivo* and enhanced PD-1 expression in TILs which may limit the efficacy of antigen-specific TILs. Accordingly, *in vivo* ACT experiments combined with anti-PD-1 inhibitor, demonstrated the synergistic therapeutic potential of this approach as compared to single agent. Yet, it does not result in complete tumor eradication suggesting that targeting one single immune checkpoint receptor is not sufficient to drive a full anti-tumor response.
Summary/Conclusions: Combination checkpoint inhibitor approach has demonstrated synergistic potential in our ACT experimental MM model and forms the basis for a novel multi-modal immunotherapeutic combination treatment for multiple myeloma.

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ENGINEERED T CELLS TOWARDS BAFF RECEPTOR: A NOVEL STRATEGY TO EFFICIENTLY TARGET B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: B-cell Acute Lymphoblastic Leukemia (B-ALL) is most common in children (80%), but it has also a peak of incidence in adult age. Immunotherapeutic approaches targeting the CD19 molecule paved the way for the treatment of relapsed and refractory lymphoblastic leukemia, which remains a major therapeutic challenge. Recently, the emergence of relapses with CD19-epitope loss in 10-30% of treated patients has been reported[1]. This newly identified escape mechanism has been recently shown to be related to the combination of deleterious mutations and emergence of alternatively spliced RNA isoforms, as effect of selective pressure[2]. B-cell Activating Factor (BAFF) Receptor is a transmembrane protein which is fundamental for B-cell maturation and survival. Moreover, the expression of this receptor is restricted to mature B cells and, interestingly, is not present on bone marrow B-cell precursors[3]. Recent studies reported the over-expression of BAFF Receptor (BAFF-R) in various B-cell malignancies such as B-ALL, B lymphoma, chronic lymphocytic leukemia and myeloma[4,5]. In the context of B-ALL leukemic cells express both BAFF and BAFF-R suggesting the presence of an autocrine signalling loop[6]. BAFF is also expressed in bone marrow microenvironment by endothelial cells which support the proliferation and the survival of primary B-ALL blasts[4].
Aims: In the current study, we aimed to develop a chimeric antigen receptor (CAR)- mediated immunotherapeutic approach targeting the BAFF-R molecule.
Methods: We characterized the expression of BAFF-R in B-ALL primary samples. As immunotherapeutic approach to target BAFF-R molecule, we developed six anti-BAFFR.CARs that differ for the inversion of the VH and VL and the length of the spacer domain (herein long, intermediate and short according to the presence of entire CH2CH3 domain, of CH3 only or complete absence). Cytokine-induced Killer (CIK) cells, an heterogeneous populations of CD3+ effector lymphocytes with acquired NK-like cytotoxicity enriched in highly efficient cytotoxic CD3+CD56+ cells, were engineered using an improved *Sleeping Beauty* (SB) transposon platform and used as effector population.
Results: Here we showed that BAFF-R is highly expressed in B-ALL primary samples at the onset and relapse. In order to develop a chimeric antigen receptor (CAR) approach targeting BAFF-R molecule, six anti-BAFFR CAR genes that differ for the inversion of the VH and VL and the length of the spacer domain have been generated. Cytokine-induced Killer (CIK) cells, engineered using an improved *Sleeping Beauty* (SB) transposon system, stably expressed anti-BAFFR.CARs, and maintained their characteristic phenotype. Among the newly constructed CARs, the shortest VHVL anti-BAFFR.CAR exerted the highest anti-leukemic activity towards target cells, such as NALM-6, with an *in vitro* killing activity of 60%. We also evaluated later effector functions in terms of cytokine release by intracellular staining (8,9±2% of IFN-γ and 16,4±5,5% of IL-2 producing cells). Importantly, we also detected a specific cytotoxic activity towards primary B-ALL blasts (average 65,6±4,5%, n=9). Combining the INVsh.CAR with CD19.CAR we detected a superior antitumor activity towards ALL targets (78,1±6,9% and 72,2±2,9% of tumor lysis respectively *vs* NALM6 and primary B-ALL blasts) compared to single population *per se*. Furthermore, by using a sample collected from a patient relapsed with CD19 negative disease, we demonstrated the ability of the INVsh.CAR to lysate CD19-negative blasts.
Summary/Conclusions: Taken together, these findings make this receptor a safe and attractive target for a second line B-ALL immunotherapy in case of relapse after CD19-targeting therapies or for a double targeted approach. Being restricted to mature B cells, but absent on precursors and plasmablasts, our strategy could have an inferior toxicity concerning the emergence of B-cell aplasia observed in patients treated with anti-CD19 CAR-modified T cells.

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EXPLORING HUMAN TCR- AND CAR-REDIRECTED INKT CELLS FOR ADOPTIVE CELLULAR THERAPY

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Background: T cell receptor (TCR) - or chimeric antigen receptor (CAR) redirected T cells have substantially improved adoptive cellular therapy (ACT) for acute leukemias and show great promise for solid cancer. However, functional heterogeneity and advanced differentiation of *ex vivo* expanded redirected lymphocytes limits their therapeutic potential and consistent efficacy. Invariant (type I) natural killer T (iNKT) cells have been demonstrated not only to promote effector functions of dendritic cells (DC), natural killer (NK) cells and T cells but also to localize to tumors and have inherent antitumor properties. Moreover, as these cells are further restricted to the monomorphic, HLA class I-like CD1 molecule expressed only on a few cell types with limited alloreactive potential, all these features make iNKT cells as attractive alternative carriers for redirected immunotherapy.
Aims: In the current proof of concept study we therefore explored human, AML-reactive TCR- and CD19 CAR-redirected iNKT cells for their potential to induce antitumoral responses to leukemia cell lines as well as patient derived, primary AML blasts.
Methods: iNKT cells expressing the invariant TCR composed of the Vα24Jα18/Vβ11 chains were immuno-magnetically isolated from PBMC derived from adult healthy donors using Vβ11-Ab (6B11)-conjugated, anti-iNKT microbeads (Miltenyi Biotec) and expanded *in vitro* upon coculture with autologous, α-galactosyceramide (α-GalCer) loaded DC in the presence of low amounts of interleukin (IL)-2. iNKT cells were retrovirally transduced on day 6 after stimulation and selected for TCR or CAR expression utilizing a virally transduced puromycin resistance. While phenotypic analyses on iNKT markers and on the percentage of redirected cells were performed by flow cytometry functional assays such as IFN-γ ELISPOT and cytotoxicity assays were carried out using CD19$^+$ NALM-16 (pre B-ALL) and primary AML (MZ653) cells as targets.
Results: Following isolation of 0.7 - 0.8 x 10^6Vα24/Vβ11$^+$ iNKT cells from PBMC we achieved on average a 120-fold expansion 21-28 days after stimulation with GalCer loaded, irradiated autologous DC and 25 U IL-2. Additional use of lenalidomide to promote expansion as described previously had no effect. Expanded iNKT cells were mainly CD4$^+$(83%) and about 80% of cells expressed the natural killer receptor CD161 described as iNKT maturation marker but showed limited or virtually no expression of typical NK markers such as CD56 and CD16. Following retroviral transduction and selection for 6 days >80% of TCR (5B2)- and CD19 CAR-redirected iNKT cells were obtained. Subsequent functional analyses revealed that both iNKT cells expressing the AML-reactive TCR 5B2 as well as CD19-CAR iNKT cells demonstrated substantial release of IFN-γ and elicited potent antileukemic responses to AML MZ653 and NALM-16 in vitro. Studies to examine their cytotoxic potential potential *in vivo* using NSG xenograft models are currently in progress.
Summary/Conclusions: These studies demonstrate that purified human Vα24/Vββ11$^+$ iNKT cells expanded from PBMC can be successfully redirected to acute leukemia both by TCR and CAR expression. Engineered iNKT cells might therefore be promising alternative carriers for redirected ACT or beeing used in combination with redirected T cells as combined immunotherapy.

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SPECIFIC TARGETING OF ACUTE MYELOID LEUKEMIA BY THE USE OF ENGINEERED CIK (CYTOKINE-INDUCED KILLER) CELLS EXPRESSING THE ANTI-CD33 CHIMERIC ANTIGEN RECEPTOR (CAR)

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Background: Acute Myeloid Leukemia (AML) is an aggressive malignancy still associated with high relapse rates when treated with conventional chemotherapeutic and hematopoietic transplantation regimens. In search for alternative strategies, great interest has been posed on antigen-specific immunotherapies and in particular on T cells redirected with Chimeric Antigen Receptors (CARs) that have shown exciting results in cancer therapy, especially in the context of B-cell malignancies. CD33 is the only validated target in AML so far and represents a suitable antigen to be targeted with CAR-T cells, being broadly expressed on AML blasts.
Aims: The aim of the present study is to preclinically evaluate the efficacy and safety profiles of CD33 CAR redirected Cytokine Induced Killer (CIK) cells alone and in combination with standard chemotherapeutic agents.
Methods: Here we proved the feasibility of harnessing Cytokine Induced Killer (CIK) effector cells with a third generation anti-CD33.CAR through the non viral Sleeping-Beauty transposon system, starting from fresh and frozen healthy mononuclear cells (PBMCs) and also from frozen primary AML samples. The *in vitro* anti-AML activity of CD33.CAR-CIK cells is assessed by means of cytotoxicity, proliferation and cytokine production assays upon challenge with AML cell lines and primary samples. The *in vivo* efficacy of CD33.CAR CIK cells is evaluated in NSG mice transplanted with AML cell lines (MA9-NRas cells) and primary samples. Moreover, to investigate the potential benefit of CD33.CAR CIK cell immunotherapy in combination with standard-of-care treatments, xenograft chemotherapy models is exploited, by using standard AML induction therapy drugs (Ara-C and doxorubicin).
Results: CD33.CAR-CIK cells were able to induce a potent anti-leukemic activity as compared to unmanipulated CIK cells, in terms of specific killing (up to 70%), proliferation (up to 40% of $Ki67^+$CAR-CIK cells) and cytokine production (up to 30% for both IL-2 and IFN-gamma producing CAR-CIK cells) when challenged with both AML cell lines and primary leukemic cells. By treating MA9-NRas cell grafted mice with the already established "5+3" induction chemotherapy protocol, we confirmed that chemotherapy is able to significantly reduce the leukemic burden from around 20% to 0.1% in the bone marrow. Since the AML disease is not totally eradicated, this model will be therefore suitable to further investigate the efficacy of the CD33.CAR-CIK cells immunotherapy on the chemotherapy resistant/residual AML cells.
Summary/Conclusions: Having demonstrated the significant *in vitro* anti-leukemic activity of SB-modified CD33.CAR-CIK cells we next aim to assess their efficacy *in vivo*, particularly against the resistant/residual AML cells that were not eradicated by standard chemotherapy treatment. Moreover, envisaging a safer clinical translation of this immunotherapeutic approach, a transient CAR expression, by using CD33.CAR coding mRNA, is under investigation, in order to limit the potential myelotoxicity due to the long-term off-target effect on normal hematopoietic stem/myeloid progenitor cells. Finally, if successful, our results will provide the preclinical validation of CD33.CAR-CIK cell immunotherapy, supporting its development to the clinic.

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UPDATE ON THE FIRST PATIENTS WITH SEVERE HEMOGLOBINOPATHIES TREATED WITH LENTIGLOBIN GENE THERAPY

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Background: Insertion of an anti-sickling β-globin gene variant into hematopoietic stem cells (HSCs) could reduce or eliminate symptoms of severe sickle cell disease (SCD) and transfusion requirements in transfusion-dependent β-thalassemia (TDT). LentiGlobin Drug Product (DP) contains autologous CD34+ cells transduced with the BB305 lentiviral vector, which encodes a human β-globin gene containing a single point mutation (A^{T87Q}) designed to confer anti-sickling properties similar to γ-globin. We recently (ASH 2016) reported 23 months of follow-up for a patient with SCD, and 12−34 months of follow-up for 4 patients with TDT.
Aims: To evaluate the safety and efficacy of LentiGlobin gene therapy for severe hemoglobinopathies.
Methods: Patients 5-35 years old with severe SCD (*e.g.*, ≥2 acute chest syndrome episodes or ≥2 vaso-occlusive crises [VOC] in the preceding year) or TDT (≥100 mL/kg of packed red blood cells [RBCs] per year) were enrolled. After informed consent, autologous CD34+ cells were collected and transduced with the BB305 vector. Patients underwent myeloablative conditioning with busulfan prior to infusion of transduced cells. Patients were then monitored for hematologic engraftment, vector copy number (VCN), genetically engineered hemoglobin (HbA^{T87Q}) levels, and adverse events (AEs). Disease-specific assessments included transfusion requirements for TDT, or VOCs and hospitalizations for SCD.
Results: As of 9 September 2016, 1 patient with severe SCD (male; 13 years old) and 4 patients with TDT (2 male, 2 female; 16−19 years old) have received LentiGlobin DP in Study HGB-205. The median DP cell dose was 8.9 (range 5.6-13.6) $x10^6$ CD34+ cells/kg with a DP VCN of 1.2 (range 0.8−2.1) vector copies/diploid genome. Median post-infusion follow-up was 22.9 months (range 11.6-33.5). All subjects engrafted successfully with median time to neutrophil engraftment of 17 (range 14-38) days. Within patients, VCN in peripheral blood remained generally consistent from Month 3 (range 0.3−3.3 at last measurement). The toxicity profile was consistent with myeloablative conditioning with single-agent busulfan, with no ≥Grade 3 DP-related AEs or serious AEs and no evidence of clonal dominance reported to date. The patient with severe SCD who, prior to study enrollment, received regular RBC transfusions, experienced no clinical symptoms or complications of SCD in the 21 months since treatment. At Month 21, his total Hb was 13.1 g/dL, with 6.2 g/dL HbA^{T87Q} (48%) and 6.5 g/dL sickle Hb (HbS: 50%); in addition, their unconjugated bilirubin, lactate dehydrogenase and reticulocyte count had dropped by 56%, 58%, 26%, respectively, compared to screening. Of the 4 patients with TDT, 3 have β^0/β^E genotypes and 1 is homozygous for a severe β^+ mutation (IVS1 nt 110 G>A). Two of the β^0/β^E patients have completed their 2-year primary follow-up and entered a long-term follow-up study. They have been without RBC transfusions for 33 and 30 months, with total Hb of 10.9 and 13.5 g/dL, and HbA^{T87Q} of 7.7 and 10.1 g/dL, respectively. The third patient with a β^0/β^E genotype has 12 months follow-up and has not required transfusions since 4 days post-LentiGlobin DP infusion, with total Hb 11.3 g/dL and HbA^{T87Q} of 8.6 g/dL. The patient with the IVS1 genotype has 15 months of follow-up and has been free of transfusions for 11.6 months, with total Hb 8.3 g/dL and HbA^{T87Q} of 6.7 g/dL. Since September 2016, 2 more patients with severe SCD have received LentiGlobin DP.
Summary/Conclusions: Data to date from this ongoing Phase 1/2 clinical study suggest that treatment with LentiGlobin DP elicits sustained HbA^{T87Q} levels, which alleviate the clinical and biochemical effects of severe SCD and TDT, with safety consistent with myeloablative conditioning. Follow-up data on the 5 previously reported patients and early results from the 2 recently treated patients will be presented.

Indolent Non-Hodgkin lymphoma - Clinical

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A SINGLE INSTITUTIONAL EXPERIENCE OF 261 PATIENTS WITH LARGE GRANULAR LYMPHOCYTIC LEUKEMIA

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Background: Large granular lymphocytic leukemia (LGLL) is a rare clonal lymphoproliferative disorder of post-thymic T-cell or natural killer (NK)-cell lineage associated with cytopenias, splenomegaly, autoimmune disorders, and recurrent mucocutaneous infections. Treatment is dictated by the presence of these manifestations and consists of immunosuppressive therapy.
Aims: The main aim of this study is to evaluate clinical features, hematological parameters, and survival data of patients with LGLL. The secondary aim is to assess response rates and duration of response to various first line immunosuppressive therapies in LGLL.
Methods: This is a retrospective analysis of clinical and laboratory features, treatment modalities, and outcomes of LGLL patients evaluated at Moffitt Cancer Center between January 1, 1995 and May 1, 2016. Continuous and categorical variables were tested via Kruskal-Wallis ANOVA and Fisher's Exact Test, respectively. Kaplan-Meier curves were used for overall survival (OS). P-values were two-sided with significance set at <0.05.
Results: We identified 261 patients with LGLL (91.6% T-cell, 8.4% NK-cell). Median age was 66 years [21-90] and M:F ratio was 1.2:1. Median follow up was 3.07 years [0-21.88]. 42.9% of LGLL patients presented with anemia, 37.1% with neutropenia, 30.7% with thrombocytopenia, 29.1% with bicytopenia and 6.9% with pancytopenia. Transfusion dependence was noted in 20.3%, splenomegaly in 27.2% and bone marrow involvement in 69.3%. 24.9% had autoimmune diseases and 9.2% had autoimmune cytopenias. 45.6% were observed while the remainder required at least one line of therapy. 5-year and 10-year OS were 75.0% and 63.1%, respectively. There was no statistically significant difference in OS, complete response rate or duration of response based on first line agent (methotrexate, cyclophosphamide, cyclosporine A). However, there was a statistically significant improved partial response with methotrexate *versus* other therapies (p=0.01). A marginally significant association between severe anemia/transfusion dependence and poor overall response rate (p=0.075) to any immunosuppressive therapy was noted. There was no statistically significant difference in OS based on absolute LGL count. Mean number of therapies was 1.08 (range 0-6) and was higher in patients with LGL count <0.5 k/μL (p=0.0078), bone marrow involvement (p<0.0001), and splenomegaly (p<0.0001).
Summary/Conclusions: In this large retrospective study, we described the frequency of LGLL-associated manifestations and their impact on the course of LGLL. Severe anemia/transfusion dependence, lower LGL counts, bone marrow involvement, and splenomegaly were suggestive of more aggressive disease. We confirmed that there is no difference in overall survival among first line immunosuppressive therapies.

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ONGOING PHASE 1/2 STUDY OF INCB050465, A SELECTIVE PI3K-DELTA INHIBITOR, FOR THE TREATMENT OF PATIENTS WITH RELAPSED/REFRACTORY B-CELL MALIGNANCIES (CITADEL-101)

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Background: Signaling networks mediated by PI3Kδ have been implicated in proliferation, migration, and functioning of B-cells. INCB050465 is a novel, potent, and selective inhibitor of PI3Kδ (≥19,000-fold more selective for PI3Kδ *vs* other isoforms). INCB050465 demonstrated linear pharmacokinetics (PK) and achieved exposure levels several-fold greater than the IC_{90} for PI3Kδ inhibition at the recommended phase 2 dose (ASH 2016; Abstract 4195).
Aims: To evaluate INCB050465 in patients with relapsed or refractory B-cell malignancies enrolled in an ongoing phase 1/2 study (NCT02018861)
Methods: In this phase 1/2 study, eligible patients (≥18 years of age) had relapsed/refractory lymphoid B-cell malignancies (excluding Burkitt's lymphoma and precursor B-cell lymphoblastic leukemia/lymphoma), Eastern Cooperative Oncology Group performance status score ≤2 (≤1 during dose escalation), normal liver and kidney function, and had not received autologous hematopoietic stem-cell transplant (HSCT) within 3 months or allogeneic HSCT within 6 months of screening. The protocol was initiated with a single-patient cohort, treated with oral INCB050465 5mg QD. Subsequent cohorts used a 3+3 design and evaluated doses of 10–45mg QD. Based on PK/pharmacodynamics, the 20 and 30mg QD cohorts were expanded. Responses were assessed every 9 weeks using the Lugano Classification or International Working Group on Chronic Lymphocytic Lymphoma (CLL) criteria.
Results: As of the data cutoff (Nov 1, 2016), 52 patients were treated (median age, 65 years [range, 30–88]). Baseline disease subtypes included diffuse large B-cell lymphoma (DLBCL; n=14), follicular lymphoma (FL; n=10), Hodgkin lymphoma (HL; n=9), marginal zone lymphoma (MZL; n=8), CLL (n=6), and mantle cell lymphoma (MCL; n=5). Sixty-two percent (n=32) of patients had ≥3 prior systemic regimens; 31% (n=16) had prior HSCT. Median duration of therapy was 3.3 months (range, 0.6–13.4); no DLTs were identified. Sixty-seven percent of patients discontinued therapy, most commonly due to disease progression (31%) and AEs (25%). Thirty-three percent of patients had dose interruption and 4% had dose reduction. Most common nonhematologic AEs (all grade; grade ≥3) were nausea (38%; 0%), diarrhea (31%; 6%), and vomiting (25%; 0%). Grade ≥3 hematologic AEs included neutropenia (21%), lymphopenia (17%), thrombocytopenia (10%), and anemia (4%). Forty-percent of patients had serious AEs (SAEs), most frequently colitis, diarrhea, and hypotension (all n=3). One patient had grade 3 pneumonitis; none had *Pneumocystis jirovecii* pneumonia (PJP) or grade ≥2 elevated transaminase. Objective responses occurred at all doses (**Table 1**), except 5mg QD; 90% of the objective responses were observed at the 9-week disease assessment.

Table 1.

	N[a]	Objective Response, n (%)	Complete Response,[b] n	Partial Response,[b] n
NHL	31	19 (61)	10	9
DLBCL	14	5 (36)	3	2
FL	9	7 (78)	2	5
MZL	4	4 (100)	2	2
MCL	4[c]	3 (75)	3	0
CLL	6[c]	2 (33)	0	2
HL	8	1 (13)	0	1

[a]7 patients were not evaluable for response because they had not reached a postbaseline assessment as of the data cutoff date.
[b]Radiologic/metabolic.
[c]1 patient with MCL and 3 with CLL had previously received ibrutinib; among these 4 patients, 1 objective response (partial response in CLL) was observed in this study.

Summary/Conclusions: In patients with relapsed/refractory B-cell malignancies, INCB050465 demonstrated manageable toxicities with no clinically meaningful transaminitis or PJP. Objective response rates were generally high and most responses (90%) were observed at the 9-week disease assessment. Different dosing regimens/schedules, long-term safety, and disease-specific cohorts are being evaluated.

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PHASE IIIB RANDOMIZED STUDY OF LENALIDOMIDE PLUS RITUXIMAB (R2) FOLLOWED BY LENALIDOMIDE VS. RITUXIMAB MAINTENANCE IN PATIENTS WITH RELAPSED/REFRACTORY NHL: ANALYSIS OF FOLLICULAR LYMPHOMA PATIENTS

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Background: Lenalidomide is an immunomodulatory agent with direct and immune-mediated mechanisms of action, and clinical activity in indolent non-Hodgkin lymphoma (NHL). Recent studies in frontline and relapsed/refractory (R/R) iNHL show tolerability and high activity for the combination of lenalidomide plus rituximab (R^2) and support further study of R^2.
Aims: The current study evaluates the efficacy and safety of R^2 induction in patients with R/R follicular lymphoma (FL).
Methods: MAGNIFY (NCT01996865) is a Phase IIIb, multicenter, open-label study of R/R NHL patients, including grades 1-3b and transformed follicular

lymphoma (tFL). Upon informed consent, patients receive 12 cycles of R^2 induction (lenalidomide 20mg/d, 21 of 28 d; rituximab 375mg/m^2 weekly cycle 1 [d1, 8, 15, 22], then d1 of odd cycles). Responders to induction (≥SD) are randomized 1:1 to maintenance with either R^2 or rituximab alone (18 cycles); following R^2 maintenance, optional single-agent lenalidomide (10mg/d, 21 of 28 d) can be given until PD. The primary endpoint is progression-free survival (PFS).

Results: As of April 14, 2016, 106 patients with R/R FL have been enrolled, including 103 with grade 1-3a FL, 2 with tFL, and 1 unknown grade. Median age of patients with FL was 66 y (range, 41-91); most had ECOG PS of 0-1 (99%) and stage III/IV disease at study entry (80%). Patients received a median of 2 prior therapies (>2, 30%); 103 (97%) patients had received prior rituximab-containing treatment, of which 35% were rituximab refractory (defined as best response of SD/PD to rituximab/rituximab-containing regimen or a CR/PR of <6 mo after the last rituximab dose). The most common prior regimens were rituximab alone (40%), R-CHOP/R-CHOP–like (38%), and bendamustine plus rituximab (35%). Premature discontinuation of lenalidomide and/or rituximab occurred in 39 (37%) patients during the induction period, mainly due to AEs (n=20); the most common treatment-related AE leading to early discontinuation in the induction period was neutropenia in 6 patients. Four (4%) patients discontinued the study. Common grade 3/4 treatment-emergent AEs during induction in the FL safety population (n=104) were 27% neutropenia, 7% leukopenia, and 6% fatigue. At a median induction duration of 23 weeks (range, 0.4-51), 83 FL patients were evaluable for response with an overall response rate (ORR) of 65%; those who were not rituximab refractory had improved ORR compared to rituximab-refractory patients (70% *vs* 55%; Table 1). The median time to response during induction was 2.8 mo. Twenty patients have completed 12 cycles of induction and 16 proceeded to maintenance (n=6 R^2, n=10 rituximab alone). Enrollment is ongoing.

Table 1.

Table 1. Best response during R^2 induction in evaluable patients with R/R NHL

Response status, n (%)	FL* (n=83)	Not Rituximab-Refractory (n=54)	Rituximab Refractory (n=29)
ORR	54 (65)	38 (70)	16 (55)
95% CI	54%-75%	56%-82%	36%-74%
CR/CRu	31 (37)	21 (39)	10 (34)
PR	23 (28)	17 (31)	6 (21)
SD	22 (27)	13 (24)	9 (31)
PD†	7 (8)	3 (6)	4 (14)

*Includes 1 patient with tFL. †PD or death prior to completion of response evaluation.

Summary/Conclusions: R^2 induction therapy shows favorable activity and a tolerable safety profile in patients with advanced-stage, R/R FL. The study is ongoing to determine the effect of R^2 *vs* rituximab maintenance in FL patients, and updated results will be presented.

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A DOUBLE-BLIND, RANDOMIZED PHASE 3 STUDY TO COMPARE EFFICACY AND SAFETY OF CT-P10 TO INNOVATOR RITUXIMAB IN COMBINATION WITH CVP IN PATIENTS WITH PREVIOUSLY UNTREATED ADVANCED FOLLICULAR LYMPHOMA

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Background: CT-P10 is the first biosimilar of innovator rituximab (RTX), approved for all indications by the European Medicines Agency. CT-P10 has demonstrated pharmacokinetics (PK) and efficacy equivalence in patients with rheumatoid arthritis (Yoo, ACR 2016) and PK equivalence in patients with advanced follicular lymphoma (AFL) (Coiffier, ASH 2016).

Aims: This study aimed to demonstrate non-inferiority (NI) of efficacy and PK equivalence between CT-P10 and RTX in patients with newly diagnosed advanced follicular lymphoma (AFL) (NCT02162771).

Methods: A total of 140 patients were randomized in a 1:1 ratio to receive CT-P10 or RTX (375mg/m^2 intravenous) plus CVP (cyclophosphamide, vincristine, and prednisone) therapy every 3 weeks over 8 cycles. Overall response rate (ORR) based on best overall response over 24 weeks was assessed by the independent review committee, according to the 1999 International Working Group criteria.

Results: Therapeutic NI of CT-P10 to RTX has been demonstrated in terms of ORR over 8 cycles (Table 1). The ORR difference between two treatment groups was 4.3% in per-protocol (PP) population and 5.7% in intent-to-treat (ITT) population. Considering the statistical Non-Inferiority test using confidence interval (CI) approach with the exact binomial CI for the difference of ORR between two treatment groups, the lower bound of 95% CI lies on the positive side of -7% NI margin (-4.25% in PP population and -3.41% in ITT population). The pre-defined non-inferiority criterion has been met with the descriptive point estimate difference approach and the formal statistical NI test with a 5% significance level. Median number of B-cells decreased to the lower limit of quantification (LLoQ) after the 1st infusion and remained at the LLoQ over 8 cycles in both groups. Overall safety profile of CT-P10 was consistent with that of RTX (Table 2). No progressive multifocal leukoencephalopathy or Hepatitis B virus reactivation were reported in either groups. The proportion of patients with positive anti-drug antibody were similar between both groups (4.3% and 2.9%) over 24 weeks in the induction period.

Table 1. Summary of Efficacy [Number (%) of patients].

PP population	CT-P10 (N=66)	RTX (N=68)	Difference* [lower bound of 95% CI]
ORR (CR+CRu+PR)	64 (97.0)	63 (92.6)	4.3% [-4.25%]
Complete response (CR)	20 (30.3)	15 (22.1)	
Unconfirmed CR (CRu)	6 (9.1)	8 (11.8)	
Partial response (PR)	38 (57.6)	40 (58.8)	

** Difference was calculated using percentages not the round off values.*

Table 2. Summary of Treatment-emergent adverse event (TEAE) related to the study drug [Number (%) of patients].

Safety population	CT-P10 (N=70)	RTX (N=70)	P-value (Fisher's exact test)
TEAE	37 (52.9)	34 (48.6)	0.7354
Serious TEAE	6 (8.6)	4 (5.7)	0.7447
Infusion-related reaction	15 (21.4)	17 (24.3)	0.8407
Infection	6 (8.6)	9 (12.9)	0.5861

Summary/Conclusions: This study demonstrates therapeutic non-inferiority of CT-P10 to RTX combined with CVP therapy in previously untreated AFL. CT-P10 was well-tolerated and the safety profile including immunogenicity of CT-P10 was comparable to that of RTX over 8 cycles in induction period.

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DURABLE DISEASE CONTROL OF EARLY MYCOSIS FUNGOIDES PATIENTS TREATED WITH LOW-DOSE INTERFERON-ALPHA2B AND PUVA

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Background: Early stage Mycosis Fungoides (MF) has an indolent, relapsing course, with patients frequently undergoing multiple therapies. Current guidelines consider the utility of combination therapies (skin-directed therapies plus systemic biologic response modifiers) to increase the therapeutic efficacy. Recently, time to next treatment (TTNT) was applied as a new relevant measure of the durability of response of PUVA, interferon-alpha (IFN-α) and retinoids as monotherapies in early MF (Hughes et al, *Blood 2015*; Hanel et al, *AJH 2016*), but it has not been yet investigated in combination therapies.

Aims: We aimed to evaluate TTNT together with the usual time-to-event measures (OS and EFS) in the series of 89 early MF patients treated for 14 months with low-dose IFN-α2b (6-18 MU weekly) and PUVA, which was first reported in 2005 (Rupoli et al, *EJH 2005*). The follow-up was prolonged up to October 2016, in order to evaluate prospectively the regimen activity and influence on the further course of the disease.

Methods: The design, rationale, safety and efficacy results for this protocol were previously published. Clinical stages IA-IIA patients who had received no previous treatment, or had been submitted to a 4-month wash-out after systemic therapy or a 4-week wash-out after topical therapy, were included in the study. Survival curves for each efficacy endpoint were calculated according to Kaplan-Meier.

Results: Eighty-nine patients (56 men and 33 women) with a median age of 60 years (range, 17-80) were recruited. Disease stage was IA in 22 patients, IB in 55, IIA in 11, and IIB in 1 patient. The majority of patients had generalized skin disease (75% T2 *vs* 25% T1). The protocol proved to be highly effective, well tolerated and able to induce complete clearing of skin lesions in 84% of patients and an overall response in 98%. The median follow-up time was 175 months (range 4-259). Updated data showed that the median overall survival (OS) was not reached, whilst the median event-free survival (EFS) was 142 months (95% C.I. 130-153). Estimated OS rates at 1, 2, 5, 10, 15 and 20 years

were 99%, 98%, 92%, 89%, 78% and 51%; at 1, 2, 5, 10, 15 and 20 years 98%, 97%, 88%, 67%, 19%, 0%, were free from events. Median TTNT was not reached thus indicating clinical benefit with IFN-α and PUVA. Kaplan-Meier estimated rates of 97% at 1 year, and 91% at 2 years, respectively whereas 5-, 10-, 20-year TTNT remained almost unchanged with 62% of patients that still had not required further treatment.

Summary/Conclusions: There has been an ongoing debate about whether patients would benefit from adding PUVA to IFN-α in the treatment of early stage MF. We chose to initiate the combination treatment of MF as early as possible in the course of the disease to induce a permanent remission or even a *cure*. In our experience, this regimen set the realistic goal of achieve high rates of complete clearing and durable responses (median TTNT not reached) with only 38% of patients requiring a subsequent systemic treatment within 20 years. Here, we suggest a synergistic or additive effect between PUVA and IFN-α compared with either agent alone. With respect to Hughes et al, (*Blood 2015*), our combination treatment provide a longer TTNT than PUVA or IFN-α monotherapy (36.3 months and 8.7 months respectively). At 2 years, 91% of patients receiving PUVA plus IFN-α were free from further treatment as compared to 54.2% and 29.1% treated with PUVA or IFN-α monotherapy, respectively.

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PHASE 3 ALCANZA STUDY OF BRENTUXIMAB VEDOTIN (BV) OR PHYSICIAN'S CHOICE (PC) OF METHOTREXATE (MTX) OR BEXAROTENE (BEX) IN CD30-POSITIVE CUTANEOUS T-CELL LYMPHOMA (CTCL):NUMBER NEEDED TO TREAT ANALYSIS

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Background: CTCL is a generally incurable, relapsing disease associated with a significant symptom burden, including disfiguring lesions, debilitating pruritus and frequent skin infections. ALCANZA is a Phase 3 study of BV *vs* PC (MTX or Bex) for the treatment of CD30-positive (CD30+) CTCL (NCT01578499). BV was associated with significantly improved rate of objective response lasting ≥4 months (ORR4; 56% *vs* 13%; p<0.0001), longer median progression-free survival (PFS; 16.7 *vs* 3.5 months; p<0.0001), and decreased symptom burden measured by Skindex-29 (27.96 *vs* -8.62; p<0.0001), compared with PC. BV's safety profile was consistent with previous reports, with all-grade and grade 3 peripheral neuropathy of 67% and 9%, respectively. Number needed to treat (NNT), defined as the number of patients (pts) that need to be treated to prevent one outcome event relative to the comparator therapy, is an effective method to assess the benefit-risk of BV in a clinically relevant manner. NNT values of 3–28 have been previously reported, at various time points, in hematologic malignancies (multiple myeloma, B-cell non-Hodgkin lymphoma) to prevent one disease progression event or death. Data from the Phase 3 AETHERA study demonstrated that, at various time points, and dependent on risk group, one in 3–8 Hodgkin lymphoma pts treated with BV consolidation therapy post-autologous stem cell transplant will benefit by avoiding disease progression/death, compared with placebo.

Aims: To determine the NNT with BV to avoid one additional event of disease progression or death compared with PC in the ALCANZA trial.

Methods: The NNT with BV was calculated as the inverse of the absolute risk reduction (ARR); ARR was the PFS event rate per independent review facility (IRF) assessment in the PC arm minus the event rate in the BV arm. PFS was defined as the time from randomization until progressive disease/death due to any cause, counting all events despite two or more missed visits or starting of subsequent anticancer therapy (European Medicines Agency [EMA] criteria). ALCANZA recruited adults (≥18 years) with previously treated CD30+ mycosis fungoides or primary cutaneous anaplastic large cell lymphoma. Pts were randomized 1:1 to receive BV 1.8mg/kg IV, once every 3 weeks, for up to 16 three-week cycles, or PC of MTX 5–50mg PO, once weekly, or Bex 300mg/m² (target dose) PO, once daily, for up to 48 weeks. All pts gave informed consent.

Results: The intent-to-treat (ITT) population comprised 128 pts (median age 60 yrs [range 22–83]; 55% male) who received BV (n=64) or PC (n=64). Fewer PFS events per IRF assessment per EMA criteria were experienced by pts in the BV arm *vs* PC arm (Table). The NNT with BV to prevent a disease progression/death ranged from 2.00 (95% CI 1.59, 3) to 3.76 pts (95% CI 2.5, 8.44) over 24 months (Table). At 24 months, the NNT to prevent a disease progression/death was 3.37 pts (95% CI 2.26, 7.67).

Table 1. NNT analysis per IRF assessment of PFS in the ALCANZA ITT population.

Month	Number of PFS events per IRF analysis		NNT	95% CI
	BV (n=64)	PC (n=64)		
3	7	24	3.76	2.5, 8.44
6	11	42	2.06	1.63, 3.13
9	15	47	2.00	1.59, 3
12	19	47	2.29	1.75, 3.73
15	21	47	2.46	1.84, 4.24
18	29	47	3.56	2.33, 8.99
21	29	48	3.37	2.25, 7.83
24	31	50	3.37	2.26, 7.67

Summary/Conclusions: ALCANZA data suggest that, at various time points, one in every 2–4 pts treated with BV will benefit by avoiding disease progression/death. This further demonstrates BV's clinical benefit in CD30+ CTCL pts requiring systemic therapy. This is, to our knowledge, the first report of an NNT analysis for a treatment in the CTCL setting.

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PRIMARY OCULAR ADNEXAL LYMPHOMA OF ALL HISTOLOGIC SUBTYPES: SURVIVAL OUTCOMES AND RISK FACTORS IN LARGE COHORT OF PATIENTS AND LONG-TERM FOLLOW-UP

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Background: Although the recent reports show that interest in ocular adnexal lymphomas (OAL) and their biologic and clinical characteristics have been increased, the most OAL-related clinical study is still limited in the small number with insufficient follow-up period, result in retrospective studies with non-reproducible. Moreover, because the majority of OAL were in the low-grade histologic subtypes as primary ocular adnexal MALT (mucosa-associated lymphoid tissue) lymphoma, there is few comparative analysis study of all histologic subtypes in OAL patients especially for non-MALT type OAL in large cohort OAL.

Aims: So our purposes of this study were to identify a correlation between histopathological diagnosis and significant parameters associated with clinical outcomes of patients with OAL in patients with diverse histologic subtypes.

Methods: We evaluated the consecutive 207 primary OAL patients who diagnosed at Catholic University Lymphoma Group (CULG) of Catholic Bone Marrow Center, Seoul between January 2004 to April 2015. Clinical information and parameters were gathered from the electronic medical records such as geographic status, complete blood count (CBC) with blood chemistry, the status of BM involvement, primary therapeutic modalities, response to initial therapy, and treatment-related complications with survival outcomes.

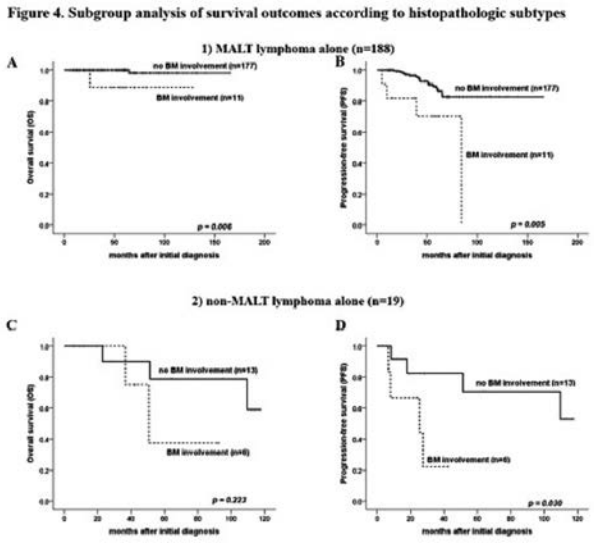

Figure 1.

Results: In OAL of all histologic subtypes, 10-year lymphoma-specific OS and PFS were 89.3% and 71.0% respectively. 182 patients achieved CR (87.9%). CR rate according to primary therapy was 90.4% (n=103) in T1N0M0, 95.2% (n=40) in T2N0M0, 100% (n=7) in T3N0M0, 83.3% (n=5) in T4N0M0, and 71.1% (n=27) in TxN1-4M0/ TxNxM1. Multivariate analysis in OAL of all histologic subtypes showed that the risk factors-associated PFS were positivity of BM involvement and non-MALT lymphoma subtype (hazard ratio; HR=5.98, *p* <0.001 and HR=2.96, *p*=0.025, respectively), the risk factors-related OS was only non-MALT lymphoma subtype (HR=9.18, *p*=0.013). Then, subgroup analysis

according to histopathologic subtypes, BM involvement alone was regarded as a statistically significant factor in the group of MALT lymphoma (HR=3.99, *p*=0.013) and there were no statistically significant factors in the group of non-MALT lymphoma. Although there were no risk factors with statistical significance, the BM involvement and advanced TNM stage showed a trend toward statistical significance about affecting to the failure of PFS (BM involvement of HR 5.19, *p*=0.054 and advanced TNM stage of HR 3.06, *p*=0.056). The median time-to-progression (TTP) was from 3 to 3.5 years after initial therapy in relapse or dead patients (range from 4.6 to 109.6 months).
Summary/Conclusions: Our study confirmed that OAL of all histologic subtypes also represented the indolent nature and localized behavior with favorable survival outcomes. Although BM involved OAL consisted of a small number, it was associated with poor survival outcomes. Also, relapse and lymphoma-related mortality had long-term delayed TTP, so we suggested that BM biopsy might be a necessary study for initial staging at least in all OAL and long-term follow-up is required for patients with all histologic type of OAL.

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CLONAL B-CELL LYMPHOCYTOSIS OF MARGINAL ZONE ORIGIN (CBL-MZ): A PROSPECTIVE REGISTRATIONAL STUDY ON 96 CASES

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Background: Clonal B-cell lymphocytosis of marginal zone origin (CBL-MZ) has been recognized as a provisional entity in the WHO classification. Despite diagnostic similarities with SMZL, the exact relation between them has not been established yet. AIM: To describe the main features of CBL-MZ and to further elucidate its relation to SMZL.
Aims: To describe the main features of CBL-MZ and to further elucidate its relation to SMZL.
Methods: 96 CBL-MZ were analyzed. Staging at diagnosis included CBCs, blood morphology and immunophenotype, biochemistry, viral test for hepatitis C and B, serum immunoglobulin levels and immunofixation as well as whole body CT scan. BM biopsies were available in 78 cases which were studied with the following panel of moAbs: CD20, DBA44, CD23, CD5, CD25, CD38, CD27, s/cIgM/D, TCL-1, MNDA, T-bet and IRTA-1. Gastroscopy with multiple biopsies was performed in 56 cases. FISH analysis for del7q was done in 13 pts, and detection for *MYD88* mutation in 60.

Table 1.

Table. Main disease features of 96 CBL-MZ pts

Parameter	# of cases	%
Age (median)(range)	70 (38-87)	
Male sex	47	49
ALC (median)	5098/μL	
Circulating Clonal B-cells (median)	2880/μL	
Paraproteinemia	41/87	47
Del7q	1/13	8
MYD-88	11/60	18
% BM infiltration (median)(range)	30(10-85)	
Clinical course in 90 pts		
Stable	55	61
ALC increase	18	20
Splenomegaly	5	6
Increase of paraproteinemia	6	7
Cytopenias due to extensive BM infiltration	5	6
Lymphadenopathy	1	1
Autoimmune thrombocytopenia	1	1
Regression of lymphocytosis	2	2
2ry neoplasm	4	4
Treatment due to disease progression	9	10
Outcome	2	2
• Toxic death	1	1
• Death from 2ry neo	1	1

Results: A synoptic presentation of the main characteristics of CBL-MZ is given in the table. The median age was 70 y without sex predilection. By definition, no case presented with cytopenia, lymphadenopathy, splenomegaly or any other organ involvement. Median ALC and clonal B-cell counts were 5098/μL and 2880/μL, respectively. 47% had paraproteinemia, mainly of the IgM type. H.pylori (+) gastritis was evident in 30%. Hp eradication had no influence on the lymphocyte counts. The percentage of BM infiltration was highly variable, ranging from 10% to 85%, with an intrasinusoidal pattern in 31%. TCL-1, T-bet, IRTA-1, and MNDA were invariably negative. *MYD-88* mutation was detected in 18% and was significantly associated with IgM paraproteinemia. 6 cases were lost to follow-up. At a median follow-up time of 41 months, the majority of the cases had no disease progression (90%): 61% had stable CBCs, 20% solely an increase in ALCs and 7% an increase in paraproteinemia only, while in 2% lymphocytosis regressed. A total of 9 (10%) pts progressed and required treatment: 5/9 due to cytopenias caused by extensive BM infiltration without splenomegaly, 1 due to bulky splenomegaly,1 due to lymphadenopathy,1 developed autoimmune thrombocytopenia, while in one due to high IgM levels in a MYD-88(-) case. A total of 5 (6%) pts developed splenomegaly after a median time of 78 mos (48-151).
Summary/Conclusions: After a median follow up time of 4y we demonstrated that CBL-MZ, although displaying many diagnostic similarities with SMZL, it rarely evolves to it. Most cases remain stable, while few develop cytopenias due to an extensive BM infiltration. These latter cases apparently represent a distinct MZL category which requires further investigation.

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SAFETY OF SUBCUTANEOUS ADMINISTRATION OF RITUXIMAB DURING THE FIRST-LINE TREATMENT OF PATIENTS WITH NON-HODGKIN LYMPHOMA: THE MABRELLA STUDY

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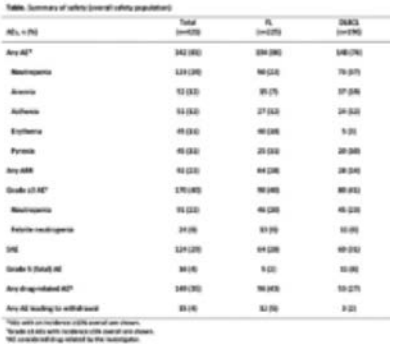
Background: Intravenous (IV) rituximab is the mainstay of treatment for CD20+ B-cell non-Hodgkin lymphoma (NHL). A subcutaneous (SC) formulation of rituximab has been approved in Europe and other countries that reduces healthcare resource burden and improves patient (pt) satisfaction and convenience compared with rituximab IV. MabRella is a global umbrella study comprising three local open-label, single-arm, Phase IIIb studies of rituximab SC, which share a core protocol and primary endpoint but have flexibility for exploratory endpoints (NCT01889069; NCT01987505; NCT02406092). Data from participating countries are pooled for predefined global analyses.
Aims: To evaluate the safety of first-line (1L) rituximab SC in follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL) with a focus on administration-related reactions (ARRs).
Methods: Eligible pts were aged 18–80 years with grade 1–3a FL/DLBCL and ECOG performance status ≤3. All pts had received ≥1 full dose of rituximab IV as 1L induction/maintenance before study entry, and were expected to receive ≥4 additional induction cycles (FL/DLBCL) or ≥6 additional maintenance cycles (FL). Informed consent was obtained. For induction, pts received rituximab SC 1400mg every cycle (14, 21 or 28 days) for 4–7 cycles, plus standard chemotherapy. FL pts undergoing maintenance treatment received single-agent rituximab SC 1400mg every 2 months for 6–12 cycles. The primary endpoint was incidence of ARRs, *i.e.* all adverse events (AEs) occurring within 24 hours of administration, considered related to study drug by the investigator. Secondary endpoints included grade ≥3 AEs and serious AEs (SAEs). The safety analysis included all pts who received ≥1 dose of study treatment. Safety data were not collected for rituximab IV, as pts entered the trial after switching to SC. Updated data are presented (data cut-off February 7, 2017).

Table 1.



Results: The safety population comprised 421 pts: 160 Italy; 140 Spain; 121 North Africa (Tunisia, Morocco and Algeria). Median age was 58 years (range 19–80); 49% of pts were male; 225 pts had FL and 196 had DLBCL. Of the pts with FL, 97 completed ≥1 cycle of rituximab SC induction (45 completed 7 cycles) and 204 completed ≥1 cycle of maintenance (175 completed 6 cycles;

73 completed 12 cycles). Among DLBCL pts, 99 completed 7 cycles of induction. Overall, 342 pts (81%) experienced an AE during treatment (Table). Common AEs (≥10% of pts) were neutropenia, anemia, asthenia, erythema and pyrexia. ARRs were observed in 92 pts (22%); the most common (≥2% of pts) were erythema (10%), injection-site erythema (4%), neutropenia (3%) and leukopenia (2%). Grade ≥3 AEs occurring in ≥5% of pts were neutropenia (22%) and febrile neutropenia (6%). Grade ≥3 ARRs were observed in 4 pts (1%). SAEs were reported in 29% of pts (7% drug-related). Of 16 grade 5 (fatal) AEs, 2 were considered related to treatment (*Klebsiella* infection and sepsis). The safety profile of rituximab SC was generally comparable between FL and DLBCL, although neutropenia and anemia (any grade) were more prevalent in DLBCL pts, and erythema (any grade) was more common in FL pts. The AE profile in FL pts was similar during induction and maintenance, but the intensity and seriousness of events was lower during maintenance.
Summary/Conclusions: In line with previous reports, rituximab SC is well tolerated during the 1L treatment of pts with NHL. The safety profile of 1L rituximab SC is similar to reports for IV administration, with the exception of an expected increase in ARRs, the majority of which are mild or moderate in intensity, and resolve spontaneously.

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REAL-WORLD EXPERIENCE WITH RITUXIMAB-FLUDARABINE (RF) AND DEXAMETHASONE, RITUXIMAB, CYCLOPHOSPHAMIDE (DRC) IN WALDENSTROM MACROGLOBULINEMIA : A RETROSPECTIVE STUDY FROM 163 PATIENTS

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Background: symptomatic Waldenstrom macroglobulinemia (WM) may be managed with various regimens. After the 8th IWWM congress, experts recommended the use of rituximab-based regimens. Two phase II trials supported DRC (n=72, 1L) and RF (n=43, one-third having received chlorambucil alone, +/- steroids/rituximab before), with published long-term follow up of 95 and 40 months, respectively. Mature data from larger cohorts confirming trials' results in real-life practice are lacking.
Aims: Mature data from larger cohorts confirming trials' results in real-life practice are lacking. Indeed, outside clinical trials, patients are older and experience potentially more long term side effects.
Methods: We report data from a retrospective study in 163 symptomatic WM treated as first-line treatment (or second-line after chlorambucil, n=47) with RF (n=56) or DRC (n=108) between January 01, 2005 and december 31, 2015.
Results: Median follow up for the entire cohort is 5 years, median age at diagnosis 68.6y and at therapy 71.2y, 75% being above 65y at treatment. Significant differences between DRC/RF cohorts were: median age 74/64y, high IPSS score 63%/26%, B2M>3mg/l 74%/56%. *DRC cohort:* median PFS/Time To Next Therapy and Overall Survival were 33mo, 45.8mo and 78% at 5 years, respectively. Dose reductions>20% had no impact on these outcomes, but age>65y and anemia<11.5g/dl decreased PFS. Previous CLB therapy increased the risk for delayed toxicities (infections 39% *vs* 16%, myelodysplasia 13% *vs* 3.8%), but not second cancers including Richter transformation. IPSS scoring system predicted PFS and OS with good accuracy. *RF cohort:* median PFS/Time To Next Therapy and Overall Survival were 53mo, 65mo and 90% at 5 years, respectively. Previous CLB had no impact on outcomes, but dose reductions>20% adversely impacted TTNT. IPSS scoring system did not improve prognostication. Long-term follow-up: 22% of patients had second solid cancers. RF significantly increased the risk of Richter, and CLB exposure the risk of myelodysplasia. Second PFS upon salvage (PFS2) was available in 72 patients: 47 DRC (PFS2 47mo), and 25 RF (PFS2 66mo), not significantly different. Only two parameters decreased the duration of PFS2 with immunochemotherapy: anemia and B2M>3mg/l, suggesting future trials should focus on this subgroup to challenge standard R-based regimens with ibrutinib.
Summary/Conclusions: We conclude that clinical trials results of DRC and RF are reproduced in our real-life cohort despite older ages, and high IPSS scores. Long-term toxicities are also seen, at similar rates and second cancers monitoring should be part of physician's practice in these WM patients.

Infectious diseases, supportive care

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MICAFUNGIN *VERSUS* LIPOSOMAL AMPHOTERICIN B FOR EMPIRICAL ANTIFUNGAL THERAPY IN FEBRILE NEUTROPENIC PATIENTS WITH HEMATOLOGICAL MALIGNANCIES: A RANDOMIZED CONTROLLED TRIAL

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Background: Invasive fungal infections (IFIs) incur significant morbidity and mortality in neutropenic patients with hematological malignancies (HEM) after chemotherapy. The risk for these infections is related to the intensity and duration of neutropenia, and varies from 2% to 40%. Mortality rates associated with documented IFIs are considerable, reportedly ranging from 30% to 60%. Empirical antifungal therapy is the standard care for neutropenic patients with HEM, who remain febrile despite broad-spectrum antibacterial treatment. Several antifungal agents including voriconazole (VRCZ) or liposomal amphotericin B (L-AMB) have been studied as empirical therapy for febrile neutropenia (FN). However, limited data are available concerning the efficacy and safety of micafungin (MCFG) in FN patients with HEM.
Aims: We conducted a randomized, cooperative group, open-label trial comparing MCFG (150mg once daily) with L- AMB (2.5mg/kg once daily) as first-line empirical antifungal treatment for FN patients with persistent fever of HEM.
Methods: 138 hospitalized FN patients with persistent fever of HEM (AML 78, APL 4, ALL 13, MDS (RAEB) 7, NHL 28, MM 5, other hematological malignancy 3 cases) were randomized to each drug group (MCFG, 72; L-AMB, 66). The efficacy end point was a favorable overall response, as determined by a five-component end point according to the criteria of Walsh et al (N Engl J Med 2004; 351: 1391).
Results: At the time of enrolment, there were no significant differences in the demographics or baseline characteristics between the two groups. The mean treatment duration for MCFG and L-AMB was 13.8 and 16.4 days, respectively. The efficacy rates of MCFG and L-AMB were not significantly different (38/72 cases (52.8%) *vs* 26/66 cases (39.4%), p=0.115*), evaluated based on: (1) successful treatment of baseline fungal infection (3/4 cases (75.0%) *vs* 0/1 case (0%), p=0.170*), (2) absence of breakthrough fungal infection (65/72 cases (90.3%) *vs* 65/66 cases (98.5%), p=0.112*), (3) survival for ≥7 days after study completion (66/72 cases (91.7%) *vs* 59/66cases (89.4%), p=0.855*), (4) absence of premature study drug discontinuation due to poor efficacy or drug-related adverse events (54/72 cases (75.0%) *vs* 47/66 cases (71.2%), p=0.615*), and (5) resolution of fever during neutropenia (45/72 cases (62.5%) *vs* 33/66 cases (50.0%), p=0.258*). However, discontinuation due to drug-related adverse events occurred less frequently in the MCFG group (1/72 cases (1.4%) *vs* 9/66 cases (13.6%), p=0.006*). In safety evaluation, adverse events of creatinine increase and hypokalemia were less often in the MCFG group than in the L-AMB group (6/72 cases (8.3%) *vs* 19/66 cases (28.8%), P=0.001*, 14/72 cases (19.4%) *vs* 34/66 cases (51.5%), P=0.0001*). *: Chi square test.
Summary/Conclusions: MCFG was as effective as L-AMB, and better tolerated than L-AMB as an empirical antifungal therapy in FN patients with HEM.

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ANTIFUNGAL DRUGS INFLUENCE NEUTROPHIL EFFECTOR FUNCTIONS IN VITRO AND MODULATE PULMONARY DAMAGE IN INVASIVE ASPERGILLOSIS

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Background: Antifungal agents like azoles, echinocandins or polyenes substantially contribute to reduced morbidity and improved survival of high risk patients in hematology. However, besides their well-known antifungal activity there is a growing body of evidence for immunomodulatory side effects on different effector cells of the immune system.

Aims: The aim of our study is to clarify the immunomodulatory capacity of different antifungal drugs on the effector functions of polymorphonuclear neutrophils (PMN) and on the clinical course of invasive pulmonary aspergillosis (IPA).
Methods: Firstly, isolated PMN from healthy donors were preincubated with different antifungals *in vitro*. Here, we used the azoles fluconazole (FLU), voriconazole (VOR), posaconazole (POS), and isavuconazole (ISA), as well as the echinocandins caspofungin (CAS) and micafungin (MIC), and the polyenes amphotericin b (AmB) and liposomal amphotericin b (LAmB). Furthermore, PMN were simultaneously stimulated with lipopolysaccharides (LPS) or zymosan. Afterwards, PMN were analyzed by flow cytometry regarding activation, degranulation, and phagocytosis. Additionally, a dichlorofluorescein assay was used to detect reactive oxygen species (ROS). IL-8 synthesis was measured by enzyme-linked immunosorbent assay (ELISA). Secondly, a murine model was used to investigate the influence of MIC and POS on the clinical course of IPA *in vivo*. Therefore, mice were treated with antifungals and inoculated intratracheally with A. fumigatus conidia. Afterwards, mice were analyzed concerning fungal burden and pulmonary damage (albumin ELISA) with neutropenic animals serving as controls.
Results: *In vitro*, pretreatment with POS lead to enhanced activation (CD62L: 44% +/- 8 *vs* 13 +/- 2, *; mean +/- SEM; p value ≤0.05 considered to be significant [*]), increased degranulation, and intensified generation of ROS (26880 rfu +/- 2834 *vs* 8528 +/- 161, *), whereas zymosan triggered IL-8 synthesis was reduced by trend. In contrast, ISA pretreated PMN showed decreased expression of activation markers. Moreover, ISA impaired degranulation and LPS triggered generation of ROS (6980 rfu +/- 1338 *vs* 28730 +/- 6893, *). FLU and VOR did not show a significant influence on PMN effector functions *in vitro*. MIC pretreatment resulted in enhanced expression of activation marker CD62L but reduced expression of CD11b, and decreased degranulation. Additionally, phagocytosis (27% +/- 4 *vs* 44 +/- 1, LPS, *) as well as generation of ROS (22660 rfu +/- 3286 *vs* 41190 +/- 2584, zymosan, *), and IL-8 synthesis were substantially impaired. CAS showed an increased phagocytosis (75% +/- 6 *vs* 44 +/- 5, LPS, *), whereas degranulation and LPS triggered generation of ROS were reduced by trend. Pretreatment with conventional AmB resulted in activation of almost all effector functions besides impaired phagocytosis (43% +/- 3 *vs* 59 +/- 3, LPS, *). In contrast, LAmB did not significantly alter any effector functions. Regarding IPA, treatment with POS resulted in reduced fungal burden as expected but lead to reduced albumin concentration in BAL (111 ng/ml +/- 46 *vs* 380 +/- 31, *) indicating a decreased pulmonary damage. Despite significant influence on PMN effector functions *in vitro*, MIC did not affect clinical course IPA *in vivo*.
Summary/Conclusions: AmB and POS induce PMN activation, whereas ISA and MIC inhibit PMN effector functions *in vitro.* CAS shows variable modification on PMN. Possibly independent from its antifungal effects, POS reduces pulmonary damage in mice suffering from IPA *in vivo*. Further studies are needed to distinguish the obviously multidimensional immunomodulatory effects of different antifungal agents and to clarify their relevance in clinical practice.

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CHARACTERISTICS AND OUTCOME OF PULMONARY INFILTRATES IN ACUTE LEUKEMIA CLASSIFIED ACCORDING TO EORTC/MSG CRITERIA OF INVASIVE FUNGAL INFECTION: A PROSPECTIVE STUDY BY THE RETE EMATOLOGICA LOMBARDA

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Background: In acute leukemia (AL) patients (pts) pulmonary infections may be severe and worsen the final outcome of AL. They have been recently shown to adversely affect the outcome of bloodstream infections (BSI) in AL pts (Cattaneo et al, 2016). The radiologic characteristics of PI belong, according to the EORTC/MSG Study Group, to the diagnostic criteria of a pulmonary invasive fungal infection (IFI).
Aims: In order to better define the clinical and prognostic significance of PI in AL pts in a real-life setting, we have analyzed all PI diagnosed during consecutive febrile/infectious episodes developing over a 26 months period in pts admitted to 9 hematological institutions within the Rete Ematologica Lombarda (REL) network.
Methods: From Dec-12 to Jan-14, all febrile/infectious episodes were recorded and data concerning PI extracted. PI were classified as *specific* and *aspecific* for IFI according to radiologic criteria.
Results: During 1086 episodes, 256 PI were diagnosed in 195 AL pts (M/F 124/71; median age 60y; AML/ALL 163/32). PI incidence was similar during induction and relapse (28.8% and 29%, respectively), but significantly lower in complete remission (14.2%, p<0.0001). Overall, PI were detected in 57% of cases during AML induction/reinduction and in 44.5% during posaconazole prophylaxis. Posaconazole was not responsible for a decreased sensitivity of serum galactomannan (GM), which was positive in 18.4% and in 18.6% pts with *specific* PI receiving posaconazole or not, respectively. *Aspecific* PI were observed in 157 cases (61.3%). In the remaining 99 cases (38.3%) the *specific* radiologic criteria for suspecting IFI were met, but in 70 of them (27.3%) just in the context of a diagnosis of possible (poss) IFI. Probable/proven (prob/prov) IFI criteria were met in 29 PI (11.3%). The characteristics of the three subgroups of PI are listed in Tab 1. Prob/prov IFI PI were associated with lack of posaconazole prophylaxis in comparison with poss IFI (72.4% *vs* 57.1%, p=0.0074). *Aspecific* PI did not differ from poss IFI except for their lower frequency during neutropenia, particularly if ≥15d (80.3% *vs* 92.9%, p=0.0164, and 56.1% *vs* 80%, p=0.0005, respectively), and higher frequency in patients on Fluoroquinolone (Fq) prophylaxis (57.3% *vs* 22.9%, p<0.0001). Multivariate analysis confirmed that *aspecific* PI were less frequent during prolonged neutropenia (HR 0.382, IC 0.189-0.772), and poss IFI during Fq prophylaxis (0.344, 0.159-0.742). All but two *specific* PI were treated with systemic antimold therapy. Thirty-day mortality was observed in 41 cases (16%). It was similar for *aspecific* and poss IFI (15.9% and 10%), but significantly higher in prob/prov IFI (31.3%, p=0.0192). Multivariate analysis confirmed a prob/prov IFI (3.277, 1.243-8.644) predictive for death, as well as relapsed/refractory AL (2.45,1.092-5.498) and BSI (2.383, 1.056-5.377).

Table 1.

	ASPECIFIC PI	POSS IFI	PROB/PROV IFI
	157 (%)	70 (%)	29 (%)
AML	85,3	84,3	79,3
Age>60	50,3	41,4	58,6
M sex	61,8	60	79,3
Induction	45,9	45,7	51,7
CR	24,8	15,7	20,7
Rel/Refr	29,3	38,5	27,6
PMN<500	80,3	92,9	89,7
Npenia≥15g	56,1	80	72,4
Fq Y	57,3	22,9	44,8
Posaconazole N	51,5	57,1	72,4
BSI Y	31,8	42,8	41,4
Death	15,9	10	31

Summary/Conclusions: Among PI occurring in AL patients IFI could be suspected according to EORTC/MSG Study Group criteria in nearly 40% of cases but only 11% of PI met the criteria for prob/prov IFI. Posaconazole did not affect serum GM sensitivity and was protective against prob/prov PI, which had a higher risk of death. On the other hand, PI associated with a diagnosis of poss IFI had a similar outcome compared to aspecific PI, although they occurred more frequently during neutropenia and outside from Fq prophylaxis. These findings may be relevant in the context of a reevaluation of the criteria for suspecting IFI in AL patients with PI as well as for a more appropriate antimicrobial stewardship.

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ANTIFUNGAL PROPHYLAXIS WITH CD101 IN IMMUNOSUPPRESSED MOUSE MODELS OF CANDIDIASIS, ASPERGILLOSIS, AND PNEUMOCYSTIS PNEUMONIA (PCP)

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Background: Fungal infections continue to carry high morbidity and mortality. Disease- and treatment-related immunosuppression in patients with hematological diseases increase the risk of opportunistic infection caused by *Candida* spp., *Aspergillus* spp., and *Pneumocystis* spp., and antifungal prophylaxis is an important consideration. Agents currently used for prophylaxis, voriconazole and TMP/SMX, carry safety and tolerability concerns. CD101 is a novel echinocandin in phase 2 clinical development that has demonstrated preclinical efficacy in treatment of invasive fungal infections and has physical and pharmacokinetic attributes that enable once-weekly IV dosing and subcutaneous (SC) administration.
Aims: To evaluate CD101 as antifungal prophylaxis in neutropenic mouse models of candidiasis, aspergillosis, or PCP.
Methods: Candidiasis model: ICR mice (5/grp) were rendered neutropenic by cyclophosphamide (cpm) on day -4 (150mg/kg) and day -1 (100mg/kg) and challenged (day 0) with *Candida albicans* ATCC SC5314 (IV, 100 µL, 10^5 CFU/mouse). One dose of CD101 5, 10, or 20mg/kg SC was given prior to challenge on day -5, -3, or -1. Kidneys were removed for CFU enumeration 24 h postchallenge. **Aspergillosis model**: ICR mice (6/grp) were rendered neutropenic by cpm on days -3 (6mg/mouse), +1 and +4 (2mg/mouse) and then challenged (day 0) with *Aspergillus fumigatus* ATCC 13073 (IV, 100µL, 10^4 CFU/mouse). One dose of CD101 5, 10, or 20mg/kg SC was given prior to

challenge on day -5, -3, or -1. Survival was monitored for 14 days. **PCP model**: C3H/HeN mice (10/grp) were immunosuppressed by dexamethasone (4mg/L) in acidified drinking water and inoculated with *Pneumocystis murina* (intranasally, 2 x 10^6/50 µL). CD101 0.2, 2, or 20mg/kg intraperitoneally was given at the time of inoculation and 1x or 3x/wk for 6 wks. TMP/SMX 50/250mg/kg/3x/wk was used as positive control. At 6 wks, lungs were processed for quantification of trophic and asci (cyst) forms of *P. murina*.
Results: Candidiasis: Kidney CFU decreased with higher doses of CD101 and shorter times between prophylaxis and challenge. At 20mg/kg, there was complete clearance of CFU burden regardless of treatment day in all animals except one (prophylaxis on day -3). There was complete clearance in all animals given 10mg/kg on days -3 and -1 and significant decreases in CFU in those given 5mg/kg on days -3 and -1. **Aspergillosis**: Survival rates significantly increased following CD101 5, 10, and 20mg/kg prophylaxis on day -5, -3 or -1 compared with vehicle. Prophylaxis closer to challenge increased the rate of survival in the 5mg/kg group. All animals given higher doses survived regardless of day of prophylaxis. **PCP**: Trophic nuclei counts were significantly reduced *versus* untreated controls in all CD101 groups except 0.2mg/kg/1x/wk, and efficacy in 3 different CD101 groups was comparable to TMP/SMX (no nuclei observed microscopically). Asci counts also were significantly reduced in all CD101 groups *versus* untreated controls. There was no difference in efficacy between TMP/SMX and CD101 in all but the lowest dose group (0.2mg/kg/1x/wk), with no asci observed microscopically.
Summary/Conclusions: CD101, a novel echinocandin, was protective against fungal challenge in immunosuppressed mouse models of candidiasis, aspergillosis, and PCP. These data suggest that CD101 may provide benefit as antifungal prophylaxis in patients with hematological diseases at risk for infection. The efficacy of SC-administered CD101 demonstrated in the candidiasis and aspergillosis models suggests potential utility in the outpatient setting for treatment or prophylaxis.

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SURGICAL MANAGEMENT OF INVASIVE FUNGAL INFECTIONS IN ADULT LEUKAEMIA PATIENTS–EXPERIENCE FROM A LARGE TERTIARY CENTRE IN SOUTH-EAST ASIA

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Background: Invasive fungal infections (IFI) are a major cause of morbidity and mortality in patients undergoing chemotherapy or stem cell transplantation for acute leukaemias. Though optimised antifungal therapy might be effective, in selected patients, surgical interventions might be an useful tool both for diagnostic and therapeutic reasons. However due to the nature of the disease and circumstances, prospective data of Surgical interventions in these situations is very difficult and the evidence is usually from small cohorts often from single centers.
Aims: The purpose of this study is to report our single center experience of surgical interventions for IFI in acute leukaemia patients.

Table 1.

Number of surgical procedures	20 (in 19 patients)
Organs involved	11 isolated pulmonary/pleural, 3 disseminated, 2 Sino-nasal, 2 cardio-pulmonary and 1multiple organ (lung and spleen) involvement
Types of Surgery	2 – Thoracotomy with decortication 5 – VATS (4 upper lobe and 1 lower lobe wedge resection) 6 – Open thoracotomies - 1 middle and upper lobe and 2 upper lobe wedge resections, 1 each upper and lower lobectomies, 1 pericardial window 4 – Endoscopic sinus surgeries(FESS) 1 – Splenectomy 1 – Abscess drainage and debridement 1 – Multiple procedures (arthrotomy for pus drainage and radial reaming) – also had FESS
Proven IFI	Aspergillus –5, Fusarium –2, Mucor –1, Unknown Molds –5 Candida – 1, Dual – Mold in Lung and Candida in blood – 1
Confirmation of Diagnosis	Histology alone – 7 Histology and culture – 5 Culture alone – 2 1 – Aspergillus PCR
Complications	Pleural effusions – 2 Pneumothorax – 4 (1patient with extensive subcutaneous emphysema) Hydropneumothorax - 1
Antifungal Treatment	Monotherapy – 10 (1 Caspofungin,6 Liposomal amphotericin, 3 Azoles) Combination therapy – 4 (2 Liposomal amphotericin + azoles, 1 Caspofungin + Voriconazole, 1 Anidulafungin + Voriconazole) Tandem therapies – 3 (Caspofungin → Liposomal amphotericin, Caspofungin → Voriconazole, Liposomal amphotericin → Voriconazole) Info not available – 2 patients

Methods: A retrospective review of our Hospital's Leukaemia database (IRB approved) was made for clinical characteristics and outcomes in surgically managed IFI patients diagnosed between Jan 2005 and Dec 2015. IFI was defined by EORTC/MSG 2008 criteria.
Results: Among 795 acute leukaemia patients diagnosed during this period, we found 19 patients with IFI who had undergone surgical interventions (15 proven, 1 probable and 3 possible IFI). The details of the IFI, surgical interventions, antifungal treatments and perioperative complications are summarized in Table 1. Most commonly performed surgical intervention was either open thoracotomy or video assisted thoracoscopic surgery for wedge resection or lobectomy. Nine of the 15 proven IFI patients had overall benefit from the procedure through optimization of antifungal therapy with MIC/sensitivities, arrest of aspergilloma related massive bleeding and/or complete resolution of the IFI allowing further chemotherapy or transplantation. Of these, 7 patients were alive and well at the time of data collection and 2 had died. Among the survivors, the mean duration of the survival post-surgery was 57.7months (range 9–118.3 months). The 2 patients who died also had benefitted from the procedure and had survived for 6.5 and 47 months post-surgery but both succumbed to septic events unrelated to the IFI during subsequent chemotherapy. Of the remaining 6 patients (out of the 15 proven IFI), 3 had temporary clinical and/or radiological improvement only but succumbed 2 to 6 months post-surgery due to unrelated septic events, 2 died due to progression of the IFI despite treatment and 1 lacked information to draw any conclusions. The patient with probable IFI diagnosed during induction was able to proceed with further chemotherapy post-surgery but succumbed to CNS relapse of leukaemia 8 months later. Of the 3 patients with possible IFI, 2 were able to proceed with transplantation and 1 with chemotherapy post-surgery, but all the 3 patients succumbed to leukaemia and/or unrelated septic events.
Summary/Conclusions: Major surgical interventions are feasible in selected leukaemia patients with IFI. In carefully selected patients they can yield valuable information to guide anti-fungal therapy or enable therapeutic outcomes allowing patients to proceed with curative chemotherapy and stem cell transplantation.

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INFECTIONS IN MULTIPLE MYELOMA ARE FREQUENT AND PREDOMINANTLY CAUSED BY BACTERIA: RESULTS OF A 12-YEAR SURVEY FROM A SINGLE CENTER

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Background: The outcome of patients with multiple myeloma (MM) has improved dramatically in the past years, mainly due to a better control of the disease. However, it is not clear what influence this has on treatment- or disease-related complications like infections. Recent data even suggested an increased rate of infections in patients with MM, possibly associated with the use of novel drugs.
Aims: To determine the rate and the type of infections in MM patients undergoing treatment and to evaluate possible disease- or treatment-related risk-factors.
Methods: All patients with MM treated at our institution between 2003 and 2014 were included in this retrospective analysis after approval by the institutional review board. Data on age, sex, diagnosis, comorbidities, treatment modalities, and infectious complications were recorded. Each type of therapy (*e.g.* high-dose therapy *versus* conventional therapy) defined a patient-case (duration per patient-case: beginning of therapy until the beginning of another type of therapy) and infections were recorded per case. To determine risk-factors, generalized estimating equations comparing cases were used.
Results: Four-hundred seventy-nine patients (male: 272, 57%) accounted for 1690 cases (median number of cases per patient 3, range 1-15). At presentation in our institution, median age was 62 (35-89) years, and most patients had advanced disease (Stage III according to Salmon-Durie classification in 364 patients, 76%) and an IgG-paraprotein (255 patients, 53%). Type of therapy given were as follows: 534 (32%) conventional long-term chemotherapy, 514 (30%) induction-type chemotherapy, 237 (14%) chemotherapy for stem-cell mobilisation, 310 (18%) high-dose melphalan with stem-cell transplantation and 95 (6%) supportive care only. One-hundred sixty-six patients (35%) with 295 patient cases never experienced an infection including 25 cases with high-dose melphalan. However, the majority of patients experienced at least one episode of infection throughout their treatment, accounting for 773 infections in 627 patient cases (37% of all patient cases). Most (559, 72%) infections were of bacterial origin including 156 cases with pneumonia (9% of all patient cases). Herpes zoster was noted in 37 patient cases. Relapse (OR 1.9, 95% CI 1.5-2.5, $p<0.001$) and high-dose chemotherapy (OR 11.3, 95% CI 8.4-15.3, $p<0.001$) were associated with a higher risk of infection whereas time of treatment (2003-2008 *versus* 2009-2014) or use of novel drugs did not influence the rate of infection.
Summary/Conclusions: More than 60% of MM patients experience at least one episode of infection during their course of treatment. These infections are mostly of bacterial origin and strongly associated with high-dose chemotherapy or relapse. Novel drugs do not seem to influence the rate of infection. Unfortu-

nately, despite the general improvement in the care of patients with MM, no difference in the rate of infections could be detected in recent years.

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HUMAN L-FICOLIN POLYMORPHISMS CONTRIBUTE TO SUSCEPTIBILITY TO INFECTIONS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: In neutropenic patients with acute myeloid leukemia (AML) bacterial infections and sepsis are a leading cause of mortality. Several studies propose a contribution of individual single nucleotide polymorphisms (SNPs) of the innate immune system to the course of infections. Human ficolins represent recognition molecules of the lectin complement pathway whereas especially ficolin-2 (L-ficolin) is emerging as an important component of the lectin pathway in the circulation. Ficolins share structural and functional characteristics with C1q from the classical pathway of the complement that acts with Pentraxin 3 (PTX3) that helps the innate immune system targeting pathogens like bacteria or viruses. In the context of hematopoietic stem cell transplantation polymorphisms of PTX3 have been identified as an individual risk factor for developing pulmonary aspergillosis.
Aims: We sought to investigate the impact of L-ficolin and PTX3 SNPs on the occurrence of infectious events such as sepsis and pneumonia, including invasive fungal disease (IFD), in 186 adult patients with newly diagnosed AML following anthracycline-based induction chemotherapy. In addition to our studies on membrane receptors**,** this work represents an important extension on soluble molecules of the innate immune system and their potential implication on infections.
Methods: Genotyping of L-ficolin and PTX3 SNPs (rs17514136, rs17549193, rs1800450 and rs3816527, rs2305619, rs1840680) was performed by TaqMan assay. Multiple logistic regression analyses were applied to evaluate the association between the polymorphisms and the occurrence of infectious events.
Results: Two L-ficolins SNPs were identified as risk factors for developing sepsis and/or pneumonia. Patients harboring $rs17514136_{GG/AG\ or\ GG}$ (n=100 or 22) revealed a significantly higher risk for developing sepsis (odds ratio (OR): 1.88; 95% confidence interval (CI): 1.01–3.37, p=0.039) or pneumonia (OR: 2.79; 95% CI: 1.1–6.9, p=0.033). A similar risk profile could be demonstrated for patients carrying $rs17549193_{TT/CT\ or\ TT}$. No association was found between SNPs of the PTX3 gene and the analysed infectious events.
Summary/Conclusions: To our best knowledge, this study represents the first analysis demonstrating that polymorphisms of human L-ficolin (rs7309123, rs17549193) represent an independent risk factor of developing sepsis and/or pneumonia in patients with AML undergoing induction chemotherapy. Interestingly, no association of PTX3 SNPs and infectious events such as IFD was found in this non-transplant setting. In conlusion, a genetic risk profile based on membrane bound and soluble molecules of the innate immune system might be helpful in identifying patients prone for infectious events.

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PREDICTIVE FACTORS OF RESPONSE TO EPOETIN THETA IN CHEMOTHERAPY-INDUCED ANEMIA: A FRENCH MULTICENTER OBSERVATIONAL STUDY (PIVOINE)

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Background: Whereas erythropoiesis stimulating agents (ESA) are indicated in the management of chemotherapy-induced anemia (CIA), their use in clinical practice is a matter of controversy with regards to some meta-analyses, opinions released by regulatory institutions and available international guidelines. However, supportive care is an area of high importance in onco-hematology thus justifying to study the use of ESA in cancer patients in the real-life setting.
Aims: The PIVOINE study aims to provide a better knowledge about the use of epoetin theta for the treatment of CIA in onco-hematology in the real-life setting and to identify predictive factors of response to ESA.
Methods: Multicenter, observational, prospective study conducted with 136 oncologists or onco-hematologists on adult patients suffering from non-myeloid malignant tumors, treated with chemotherapy and initiating epoetin theta treatment according to standard medical practice.
Results: From November 2014 to October 2015, 1379 evaluable patients were followed in the study (mean age 68.3 ± 11.3 years, 47.2% men). Overall, 21.8% of patients presented with hematological malignancies, 19.9% with digestive tumors, 18.2% with lung cancer and 40.1% with other solid tumors. The majority had a good performance status (75.2% ECOG 0-1). More than 90% of patients had never received ESA prior to enrolment in this study and 45.2% benefited from first-line chemotherapy. Median Hb level at inclusion was 9.7 g/dL. Epoetin theta was initiated at a weekly dose of 20 000 IU for 76.1% of patients and 12.3% of patients benefited from dose adaptation during follow-up, mainly dose increase (90.5%). Overall, 18.5% of patients received blood transfusion during the study. Five-hundred and sixty-three patients (45.2%) achieved complete response (CR) (i.e. Hb level increased by at least 2 g/dL) within 12 weeks after epoetin theta initiation. According to Kaplan-Meier analysis, the probability of CR was 12.7% at 4 weeks, 35.8% at 8 weeks and 52.4% at 12 weeks. Multivariate analysis showed that the lower the Hb level at baseline, the greater the chance of complete response (OR 0.4 IC95% [0.335;0.478]). Moreover, good performance status (ECOG 0 or 1), hematological malignancies (vs. solid tumor) and the absence of blood transfusion are independent predictive factors for complete response (OR 1.577 IC95% [1.186;2.098], OR 1.946 IC95% [1.459;2.597], OR 1. 969 IC95% [1.411;2.747] respectively). Overall, only 27 patients (2%) experienced treatment-related adverse events, 2 of them (0.1%) presenting with a serious one (non fatal pulmonary embolism).
Summary/Conclusions: The PIVOINE study confirms that the response rate to epoetin theta varies considerably among patients treated similarly. This observational study conducted on a large population could help targeting the patients that could positively benefit from such treatment to prevent CIA, mainly patients with hematological malignancy, with good performance status and with low initial Hb level. The safety results confirmed the safety profile of epoetin theta.

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TIMING OF DEFIBROTIDE INITIATION POST-DIAGNOSIS OF HEPATIC VENO-OCCLUSIVE DISEASE/SINUSOIDAL OBSTRUCTION SYNDROME AFTER PRIMARY CHEMOTHERAPY: EXPLORATORY ANALYSIS OF AN EXPANDED-ACCESS PROTOCOL

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Background: Hepatic VOD/SOS, which may be unpredictable and potentially life-threatening, is typically considered a complication of hematopoietic stem cell transplantation (HSCT); however, VOD/SOS can occur after chemotherapy without HSCT. VOD/SOS with multi-organ dysfunction (MOD) may be associated with >80% mortality. Defibrotide is approved to treat severe hepatic VOD/SOS post-HSCT in the European Union and to treat hepatic VOD/SOS with renal or pulmonary dysfunction post-HSCT in the United States.
Aims: To perform an exploratory *post hoc* analysis of the impact of timing of initiation of defibrotide after VOD/SOS diagnosis in patients developing VOD/SOS after primary chemotherapy without HSCT (off label).
Methods: In an expanded-access protocol for patients with VOD/SOS post-HSCT or chemotherapy, with or without MOD (renal and/or pulmonary dysfunction), defibrotide 25mg/kg/d (4 divided doses of 6.25mg/kg) was given a recommended ≥21 days after patients provided informed consent. In this post-chemotherapy subgroup, survival was analyzed *post hoc* from the day VOD/SOS was diagnosed (days 0–30 after start of chemotherapy) through follow-up, which was collected for 100 days post-chemotherapy. For these exploratory analyses, survival rates in the post-chemotherapy subgroup were examined by time from VOD/SOS diagnosis to start of defibrotide for (1) all patients before/after days 1, 2, 3, 4, 7, and 14, using Fisher's exact test and (2) patients starting defibrotide on a particular day: 0, 1, 2, 3, 4, 5, 6, 7, 8–14, and ≥15, by Cochran-Armitage test for trend across days. Causes of treatment delay were not assessed.
Results: In the final dataset, 137 patients developed VOD/SOS after primary chemotherapy. Of these, 87 patients (41 with MOD) developed VOD/SOS by day 30 after the start of chemotherapy. In the latter group, 79.3% (69/87) were aged ≤16 years. In 26.4% (23/87) of post-chemotherapy patients, defibrotide was started the day of diagnosis; in 89.7% (78/87), by Day 7. In the population-wide analysis of initiation before/after days 1, 2, 3, 4, 7, and 14 post-diagnosis in both the overall group and MOD subgroup (**Figure**), earlier initiation was associated with higher Day +100 survival rates for all days, which was significant at a number of timepoints. The trend test for particular initiation days

also showed a significant trend over time for higher Day +100 survival with earlier defibrotide initiation post-diagnosis in both the overall group and MOD subgroup (P<.05). In the overall post-chemotherapy population, adverse events (AEs) and serious AEs occurred in 66% and 40% of patients, respectively. Aside from multi-organ failure, the most common AE of any severity was hypotension (9.5%). Possibly related AEs lead to discontinuation in 7.3%; most common was gastric hemorrhage (3.7%).

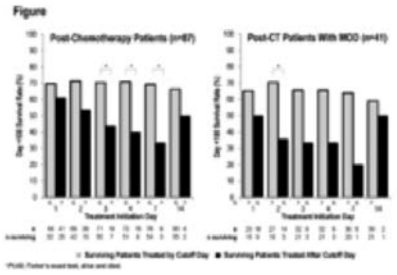

Figure 1.

Summary/Conclusions: In this exploratory analysis of final study data in the subgroup of patients developing VOD/SOS after chemotherapy, earlier defibrotide initiation post-VOD/SOS diagnosis was associated with improved Day +100 survival, confirmed by the Cochran-Armitage test (P<.05), even in the small MOD subgroup. This time-dependent relationship was consistent with that found in the HSCT subgroup from this study. No specific day appears to provide a clinically meaningful cutoff for better Day +100 survival, suggesting that later intervention retains value if treatment must be delayed.

Support: Jazz Pharmaceuticals

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ADAMTS-13 REGULATES NEUTROPHIL RECRUITMENT IN A MOUSE MODEL OF INVASIVE PULMONARY ASPERGILLOSIS

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Background: Von Willebrand factor (VWF) is produced as multimers of various sizes and is secreted as an acute phase protein during inflammation. The main mechanism regulating the size and prothrombotic activity of VWF is the specific proteolytic activity of ADAMTS-13 (a disintegrin and metalloprotease with ThromboSpondin type 1 repeats-13) which is diminished under several pathological conditions.
Aims: To determine the relevance of this regulatory pathway for the innate inflammatory response by polymorphonuclear neutrophils (PMN), we employed a mouse model of invasive pulmonary aspergillosis (IPA) where PMN functionality is crucial for fungal clearance and survival.
Methods: IPA was induced by intratracheal application of *Aspergillus fumigatus* (*A. f.*) conidia in wildtype (129/Sv/Pas) or ADAMTS-13 deficient (*Adamts13*$^{-/-}$) mice, and VWF deficient (*Vwf*$^{-/-}$) mice or respective controls (B6). Some mice were sacrificed 24 h after infection. Fungal load was assessed as colony forming units (CFU) after plating and culturing lung homogenates on Sabouraud agar plates. For histological analysis paraffin sections of the lungs were stained with H&E, mouse complement component C3d and VWF antibody. Broncho alveolar lavage fluid (BALF) was analyzed for cell count (bead-based by flow cytometry or by an animal blood counter), ELISA was performed for albumin amount and cytokines were analyzed by a multiplex assay. Bone marrow-derived PMN were isolated by magnetic cell sorting using biotin labeled Ly6G/C specific antibody. PMN functions were analyzed for degranulation, oxidative burst activity and CD62L shedding by flow cytometry. Fungal killing of PMN *in vitro* was assessed by a XTT assay. Chemotactic properties of *A.f.*-activated and control serum from wildtype and knock-out mice was evaluated by migration of purified human PMN, isolated by dextran sedimentation and Histopaque® centrifugation, in a transwell assay.
Results: While infected neutropenic mice developed lethal IPA, all wildtype mice survived the infection. Interestingly, *Adamts13*$^{-/-}$ mice displayed more severe signs of disease with a lethal course in about 24% of the animals. Examination of the lungs revealed a higher fungal burden along with increased signs of acute lung injury and levels of pro-inflammatory cytokines in ADAMTS-13 deficiency. Histology sections demonstrated a more pronounced perivascular leukocyte infiltration in support of a dysregulated inflammatory response in *Adamts13*$^{-/-}$ mice. Importantly, we observed no general defect in the activation of neutrophil effector functions in response to conidia or hyphae *in vitro*. Furthermore, innate inflammatory response to IPA was not altered in VWF deficient (*Vwf*$^{-/-}$) mice compared to wildtype (B6) control.
Summary/Conclusions: Therefore, we conclude that the proteolytic regulation of VWF by ADAMTS-13 or ADAMTS-13 by itself is an important mechanism to control PMN recruitment in acute inflammatory processes, such as fungal pneumonias.

Myelodysplastic syndromes - Biology

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IDENTIFICATION OF THE SPECIFIC HEMATOPOIETIC STEM CELL POPULATIONS RESPONSIBLE FOR FAILURE TO HYPOMETHYLATING AGENTS IN MYELODYSPLASTIC SYNDROMES

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Background: Myelodysplastic syndromes (MDS) are hematopoietic disorders characterized by the ineffective production of mature blood cells of one or more lineages and by the risk of evolution to acute myeloid leukemia. The current standard of care for MDS patients is the treatment with hypomethylating agents (HMA); however, response to drugs from this family occurs in just about half of the patients and is accompanied by high rates of therapy failure. Failure to HMA in MDS is a poorly understood process associated to increased risk of disease progression and to a dismal prognosis and cannot be, thus far, predicted or prevented.
Aims: Given that MDS are stem cell disorders, our aim was the identification and molecular characterization of the specific hematopoietic stem/progenitor cell (HSPC) population in which the relapse-driver clones arise. This is an essential step for the development of effective monitoring and early intervention protocols for HMA failure.

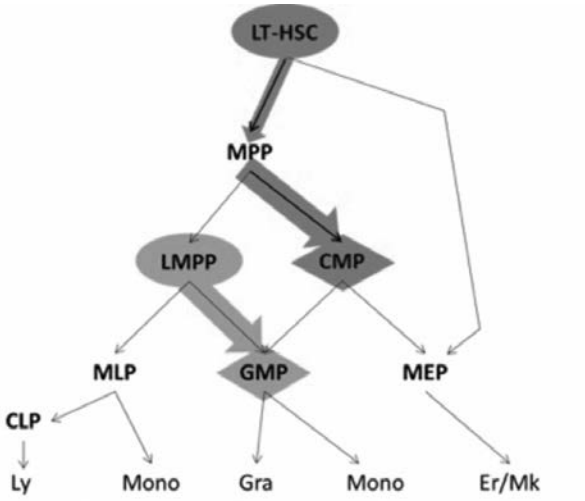

Figure 1. Working model of hematopoietic abnormalities in MDS. Colored arrows indicate differentiation steps that seem to be favored at the expense of others in each MDS subtype. Red represents CMP pattern MDS; blue represents GMP pattern MDS. Diamonds indicate the myeloid progenitor population that is predominant in each subtype. Ellipses indicate the populations that expand during HMA failure with blast progression.

Figure 1.

Methods: Using flow cytometry immunophenotyping, we quantitatively analyzed the different cell subpopulations within the $CD34^+CD38^-$ and $CD34^+CD38^+$ HSPC compartments in 122 sequential MDS bone marrow samples obtained from 93 patients at different stages of HMA treatment.
Results: In line with earlier reports suggesting the presence of alterations in myeloid progenitor frequencies in MDS, our flow cytometry data stratified untreated patient samples in two groups representative of two abnormal differentiation patterns, which were independent of the IPSS risk classification. The "CMP pattern" group (12 samples, 34%) was characterized by an increased frequency of the common myeloid progenitors (CMP) (2.6-fold; $p<10^{-4}$), whereas the "GMP pattern" group (23 samples, 66%) was characterized by the increased frequency of the granulo-monocytic progenitors (GMP) (1.7-fold; $p<10^{-3}$) within the HPC compartment, when compared to those from healthy individuals. Importantly, these two patterns were not caused by the expansion of the aforementioned populations, but by the depletion of other progenitors within the same compartment, which points to the existence of selective differentiation defects in upstream HSC or multipotent progenitors (MPP). For instance, decreased numbers of long-term (LT)-HSC (-5.1-fold, p=0.001) and increased counts of the GMP precursors, lymphoid-primed multipotent progenitors (LMPP) (4.7-fold, p=0.016), were observed in GMP pattern patients but not in CMP pattern patients. These data suggest that each abnormal differentiation pattern arises from defects in different HSC populations and has a differential impact in the number and functionality of downstream progenitor cells (Figure 1). In agreement, a deeper immunophenotypic analysis of recently defined HPC functional fractions showed decreased erythroid and megakaryocytic potential in CMP (-2-fold each, $p<0.05$) and megakaryocytic-erythroid progenitor (MEP) populations (-3.8-fold erythroid, -7.6-fold megakaryocytic; p=0.07, p=0.04, respectively) from CMP pattern patients but not in GMP pattern patients. HSPC frequency-monitoring of 69 samples collected from 36 patients throughout therapy showed persistence of both abnormal differentiation patterns even during clinical remission. Furthermore, specific HSC populations were differentially expanded upon HMA failure with leukemic progression in the two groups of patients. In CMP pattern MDS, LT-HSC frequency significantly increased after relapse (10.4-fold; $p<10^{-4}$), whereas the LMPP frequency sharply increased (8-fold; $p<10^{-4}$) in GMP pattern patients. The fact that a proliferative switch occurred in different HSC subpopulations confirmed that the two subgroups are distinct entities with different hierarchical origins.
Summary/Conclusions: Overall, our data provide evidence of the existence of biologically different MDS subtypes which are caused by separate differentiation defects and progress through the expansion of characteristic HSC populations.

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FUNCTIONAL STUDY ON THE COOPERATION OF ASXL1 AND RUNX1 MUTATIONS FOR LEUKEMIC TRANSFORMATION

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Background: Our previous studies showed that *RUNX1* and *ASXL1* mutations were frequently co-existed in chronic myelomonocytic leukemia (CMML) (EHA 2015) and clonal evolution of *RUNX1* and/or *ASXL1* occurred most frequently in chronic myeloid leukemia (CML) with myeloid blastic crisis (EHA 2016). The molecular pathogenesis of cooperation of *RUNX1* and *ASXL1* mutations has not been reported yet.
Aims: We aimed to determine the functional role of collaborative association of *RUNX1* and *ASXL1* mutations for secondary acute myeloid leukemia (sAML) transformation.
Methods: For *in vitro* study, we overexpressed *RUNX1*-WT/MT (R135T) in K562 cells which harboring *ASXL1*-MT (Y591X) and co-expressed with *ASXL1*-WT/MT (R693X) in murine 32D cells. After stable expression, functional properties were examined by using immunoblot, co-immunoprecipitation, quantitative RT-PCR, flow cytometry, cell proliferation, colony formation and gene expression microarray analyses. C57BL/6 mice were used for bone marrow transplantation (BMT) experiments for *in vivo* study.
Results: We found that *RUNX1*-MT augmented cell proliferation, colony formation, *HOXA* gene expression and inhibited megakaryocytic differentiation in *ASXL1*-MT K562 cells compared to *RUNX1*-WT or empty vector control. The cooperation of *RUNX1* and *ASXL1* mutations or the knocked down of *ASXL1* cooperating with *RUNX1*-MT inhibited apoptosis and impaired differentiation in 32D cells. Nine months post BMT mice with the combined *RUNX1* and *ASXL1* mutations, but not *RUNX1*-MT or *ASXL1*-MT alone, developed disease characterized by marked splenomegaly, hepatomegaly, and leukocytosis with a shorter latency. We found that RUNX1-MT stabilized hypoxia-inducible factor 1α (HIF1-α) and increased its target gene expression such as *ID1* (inhibitor of DNA binding 1). Clinical samples analyses showed that *ID1* expression increased in both *RUNX1*-MT and *ASXL1*-MT or the combined mutations of *RUNX1* and *ASXL1* compared to control samples. We also examined the impact of *RUNX1* and *ASXL1* mutations on sAML-free survival of 104 Patients with CMML in whom 11 had co-occurrence of *RUNX1* and *ASXL*1, 39 had either mutated *ASXL1* or *RUNX1* and 54 patients were negative for both mutations. We found that patients carrying co-existed mutations had a shorter sAML-free-survival (median 16.1 months, 95% CI 0.0-60.1 months) than those carrying either mutated gene alone (median 23.0 months, 95% CI 17.8-28.2 months) or negative for both mutated genes (median not reached, 59.2% ± 8.8%% at 5 years) (*P*=0.023).
Summary/Conclusions: The present study demonstrated that clinical and functional evidence for a collaborative association of RUNX1-MT and ASXL1-MT for sAML transformation. We identified HIF-1α targeting a new pathway which may be critical for leukemic progression of *RUNX1*/*ASXL1*-mutated myeloid malignancies.

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A NOVEL MASS SPECTROMETRY METHOD REVEALS THE INTRACELLULAR PHARMACOKINETICS OF AZACYTIDINE THERAPY IN VIVO

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Background: The cytidine analog 5'-Azacitidine (AZA, Fig. A), a DNA demethylating agent, is the primary drug for the treatment of high-risk Myelodysplastic Syndrome (MDS) and Chronic Myelomonocytic Leukaemia (CMML), and response is associated with improved survival benefits. However, only ~50% of treated patients will ever respond to AZA and the molecular basis for poor response is poorly understood. It is unclear whether non-responders to therapy have different rates of AZA uptake into their cells and/or AZA incorporation into nucleic acids compared to AZA responders, nor whether these might relate to DNA methylation *in vivo*.
Aims: We aimed to develop an analytical method capable of simultaneously detecting all the subcellular fractions of AZA (Fig B) within the bone marrows of patients undergoing AZA therapy, while also assessing DNA and RNA methylation levels. This would provide the most comprehensive snapshot of the intracellular pharmacokinetics of AZA therapy *in vivo* as a first step towards better understanding AZA resistance.
Methods: We have developed a new method utilising mass spectrometry to accurately quantify all the different subcellular fractions of AZA within the same sample (Fig C). Using an Orbitrap mass spectrometer with very high mass resolution, we have achieved the first mass separation of DAC and AZA from all naturally occurring isotopes of deoxycytidine and cytidine respectively (a difference of less than 1 Da), thus enabling accurate quantification. We utilised subcellular fractionation to obtain purified quantities of DNA- and RNA-incorporated nucleotides, as well as free unincorporated nucleotides present in the cytoplasm. We developed a reduction reaction to reduce the spontaneous hydrolysis of AZA and DAC, thereby greatly improving the sensitivity of detection.
Results: Using our new method, we report for the first time direct simultaneous quantification of: (1.) DNA-incorporated DAC, (2.) intracellular, free DAC, (3.) methyl deoxycytidine in DNA, (4.) RNA-incorporated AZA, (5.) intracellular, free AZA, and (6.) methyl cytidine in RNA within the same sample. We demonstrate an inverse correlation between the amount of DAC incorporated into DNA and DNA methylation. However, no such correlation was observed between AZA incorporation and RNA demethylation (Fig D). The sensitivity and resolution of our method also enabled, for the first time, a comprehensive survey of the total intracellular pharmacokinetics of AZA *in vivo* in patients undergoing a standard cycle of treatment. We discovered that the bone marrow cells of AZA responders (n=4) incorporated more DAC into DNA compared to non-responders (n=4). DAC incorporation was also inversely proportional to DNA methylation levels, with greater DNA demethylation observed in the responders compared to non-responders. Furthermore, we observed two patterns in AZA non-responders, with DAC-incorporation and DNA demethylation occurring in some individuals (n=2), while other non-responders (n=2) showed low or no DAC incorporation and no DNA demethylation (Fig E). Our method also enabled us to directly prove that low DAC incorporation was not a result insufficient AZA accumulation intracellularly, as cytoplasmic measurements of unincorporated AZA and DAC were highest in the non-responders with the lowest levels of DNA-incorporated DAC. Additionally, in these non-responders, there was also concomitant increase in AZA incorporation into RNA.

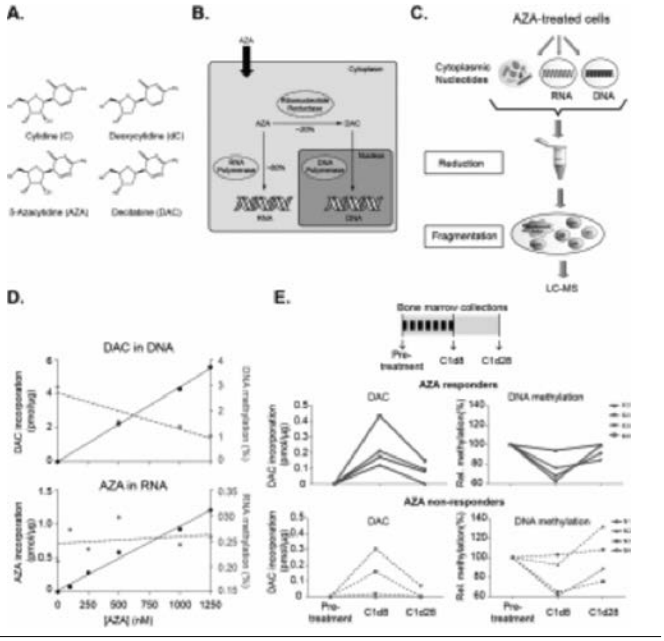

Figure 1.

Summary/Conclusions: We have developed a new method that has enabled the first comprehensive analysis of the intracellular pharmacokinetics of AZA therapy *in vivo*. Our results have revealed that while AZA responders incorporated AZA efficiently into DNA, leading to DNA demethylation, there were two modes of primary AZA resistance: in some non-responders, low levels of AZA incorporation into DNA likely derives from cell cycle quiescence, resulting in low amounts of DNA demethylation. However, in other non-responders who showed DAC incorporation into DNA and demethyltion, resistance arises from as-yet-unknown mechanisms not connected with AZA metabolism.

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CLONAL EVOLUTION OF STAG2 AND NRAS DURING PROGRESSION FROM MDS TO SAML ASSESSED BY WHOLE-EXOME AND TARGETED-DEEP SEQUENCING

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Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematological disorders at high risk of progression to acute myeloid leukemia (sAML). Due to recent high-throughput sequencing studies, the mutational dynamics and clonal evolution underlying disease progression have just begun to be understood. However, large longitudinal sequencing genomic studies are still required.
Aims: To analyze the relationship between the dynamics of gene mutations and cell pathways they are involved in with the progression from MDS to sAML in order to study the mechanisms underlying disease evolution.
Methods: Sixty-eight serially collected samples from 34 MDS/CMML patients evolving to sAML were studied by a combination of whole-exome sequencing (WES) and targeted-deep sequencing (TDS). Each patient was studied at two different time-points: at the time of diagnosis (MDS/CMML stage) and after sAML progression (disease evolution, leukemic phase). At initial presentation of the disease, diagnoses were as follows: 18 RAEB-1/2, 9 RCMD and 7 CMML. Initially, WES was carried out on 40 diagnosis/progression-matched samples. Driver mutations were identified, after variant calling by a standardized bioinformatics pipeline, by using the novel tool "Cancer Genome Interpreter" (https://www.cancergenomeinterpreter.org). Secondly, in order to validate mutations and precise variant allele frequencies (VAFs) estimation, TDS using a custom MDS/AML-related capture enrichment panel (Illumina ®) of 117 genes was performed in 30 out of 40 of the initial cohort. Moreover, a total of 28 paired-samples from a cohort of 14 patients were analyzed by TDS.
Results: Combining both WES and TDS approaches, a total of 143 mutations in 50 different genes were identified at the sAML stage, with most of them (118 mutations) already present at the MDS stage, at clonal or subclonal levels.
The most recurrently mutated genes were *SRSF2* (41%), *TET2* (41%), *STAG2* (28%), *SF3B1* (21%), *ASXL1* (21%), *TP53* (21%) and *NRAS* (21%). However, it should be noted that 68% genes were mutated only in less than 10% of the patients, highlighting the great heterogeneity that exists in the mechanisms of disease evolution. To study the mutational dynamics during disease progression we compared VAFs of mutations detected at both time-points (sAML to MDS/CMML stage) in each patient. We identified 4 different clonal dynamics: mutations that were initially present but increased VAF (type-1), decreased (type-2), were newly acquired (type-3) or persisted with similar allelic burden (type-4) at sAML stage. Interestingly, most of type-1 mutations were detected in *STAG2* gene. Thus, mutational burden of *STAG2* were markedly increased (6/8 patients) at sAML progression. Moreover, type-3 mutations, only detected at the sAML-stage, were predominantly identified in *FLT3* (3/4) and *NRAS* (5/6). Conversely, type-4 mutations were present in MDS-related genes such as *SRSF2* (9/12), *SF3B1* (3/6) and *TET2* (8/12). Most of mutations in these genes showed no changes during progression to sAML.
Summary/Conclusions: Progression from MDS to sAML could be explained by different mutational processes, as well as by the occurrence of unique and complex changes in the clonal architecture of the disease during the evolution. Mutations in genes such as *STAG2*, *FLT3* or *NRAS* could play an important role during disease progression.

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PROGRESSION OF MDS TO AML FEATURES GAIN OF SINGLE DRIVER MUTATIONS WITH CONSEQUENT CHANGES IN CLONAL COMPOSITION AND OCCURRENCE OF MULTIPLE CLONES WITH MUTATIONS IN IDENTICAL GENES

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Background: Progression of myelodysplastic syndromes (MDS) to acute myeloid leukemia (AML) associates with acquisition of genetic aberrations. Similar aberrations may occur in the development of primary AML, particularly in the context of a clonal hematopoiesis of indeterminate potential. Thus, in-depth knowledge of the genetics and clonal composition of MDS and paired AML samples allows insights into MDS progression in particular and AML development in general.
Aims: Here, we assessed mutations in serial samples of patients with MDS and progression to AML by next generation (NGS) and single-cell sequencing to identify mutations and clonal changes associated with AML development.
Methods: Mononuclear cells from 21 bone marrow (BM) samples of 8 patients with MDS and progression to AML were studied for mutations by an NGS panel (Agilent HaloPlex, Illumina MiSeq) comprising 98 genes relevant in hematologic neoplasms, and for copy number variations (Affymetrix CytoScan HD). All AMLs had normal karyotype, except one with del(5q). Samples were collected during MDS, at AML diagnosis and under treatment. Variants were verified by Sanger- and pyrosequencing or fragment analysis in BM and CD3+ cells (germline). Clonal assignment of variants was verified by single-cell mutation analysis using a Single-Cell Printer.
Results: Applying predefined criteria and verifying variants by orthogonal methods in blasts and CD3+ cells, a median of 3 variants (range, 1-6) in the MDS and 4 (range, 1-6) in the AML samples were deemed pathogenic. During MDS, all patients except one had mutations in genes involved in RNA-splicing (*SF3B1*, *SRSF2, ZRSR2, U2AF1*) or epigenetic regulation (*TET2, IDH1, DNMT3A*, *ASXL1, EZH2*). Additional mutations existed in *FLT3, NRAS, PTEN, STAG2, CEBPA, RUNX1* or *WT1*. Subclonal mutations (i.e. variant allele frequency (VAF) <10%) were present in only two MDS samples. Towards AML, patients acquired a median of 1 (range, 0-2) new mutation in *FLT3, CSF3R, KRAS, NRAS, PHF6*, *IDH1* or *WT1*. The VAF shifts from MDS to AML indicated cooperativity of mutations on clonal outgrowth, *e.g.* gain of *CSF3R* p.T618I was accompanied by a chromosome 19q-loss resulting in hemizygosity of a preexisting *CEBPA* mutation; or acquisition of a *FLT3*-TKD mutation was associated with outgrowth of a *RUNX1* mutation. Changes in mutations or VAFs also occurred under treatment, *e.g.* in one patient, AML progressed under decitabine treatment by gaining two distinct *FLT3* mutations. In another patient, who achieved complete remission after induction chemotherapy, but relapsed with MDS, which again progressed to AML, mutations were lost or gained, while a *STAG2* mutation was detectable at all time. Interestingly, identical genes were recurrently mutated in different clones within single patients, *e.g.* progression to AML associated with acquisition of a *WT1* mutation in an *NRAS* mutated MDS clone and with the generation of further subclones harboring distinct combinations of different *WT1* and *NRAS* mutations. The co-occurrence of the specific *WT1* and *NRAS* mutations in the different clones was demonstrated by mutation analyses of 72 single patient cells.
Summary/Conclusions: Mutations in MDS are few in number, but enriched in genes involved in RNA-splicing or epigenetic regulation; gain of single driver mutations leads to clonal outgrowth and thus, AML. Subsequent treatment can change the mutational and clonal profile. Mutations in identical genes occur in different clones, as confirmed by single-cell analyses; this suggests a fertile ground (*e.g.* microenvironment) for such mutations in a patient and may lead to (a therapeutically exploitable) competition of clones.

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PRECLINICAL MODELING OF MYELODYSPLASTIC SYNDROMES

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Background: Myelodysplastic syndromes are a heterogeneous group of clonal hematopoietic stem cell disorders with diverse phenotypes, characterized by ineffective hematopoiesis and bone marrow morphological dysplasia with varying risk of leukemic transformation. Over the last decade, there has been significant progress in understanding the pathogenesis underlying the MDS. Notably, patient derived xenograft (PDX) models offer the most advanced preclinical opportunity to capture the complexities of this myeloid malignancy. A number of different animal models have been proposed but the more promising to date are the NSG and the NSG-S (humanized with SCF, GM-CSF and IL-3).
Aims: Here we have used bone marrow cells from 39 MDS patients, covering all risk groups, to generate a preclinical *in vivo* and *in vitro* model, which could be used to study clonal evolution and test targeted therapies.
Methods: We have used NSG and NSG-SGM3 mice to assess the scid-repopulating capacity of the MDS stem cells in presence or absence of mesenchymal stromal cells (MSCs). Moreover we have developed an *in vitro* 2D co-culture system as an alternative/complementary tool to *in vivo studies.*
Results: Our data showed promising results with the injection of mononuclear cells obtained from patient BM, however the co-injection of mesenchymal stromal cells (MSCs) did not improve the level of engraftment. To address the question of the becoming of MSCs once injected, we tracked them back into the mice BM and showed that they disappeared after a week of engraftment. With a 2D *in vitro* system, we showed that we could co-culture CD34$^+$ cells from MDS patient BM, on auto- and allo-genic MSCs, over 4 weeks with a fold expansion ranging from 50 to 600 times. More importantly those cells conserved their clonal architecture and chromosomal aberrations.

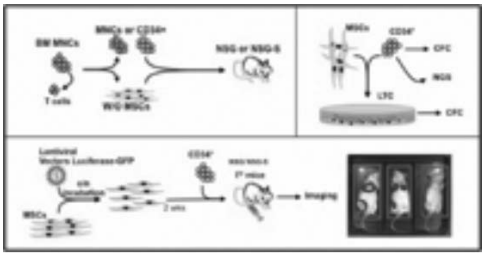

Figure 1.

Summary/Conclusions: Although the *in vivo* model cannot be replaced, the low level of engraftment of most of the patients is a limit in the study of MDS. Here we have demonstrated the value of the 2D co-culture system using MSCs (or murine MS5) as an alternative model to study MDS. This *ex vivo* culture system, which lasts for only 4 weeks and requires low number of human CD34$^+$ cells, provides a robust preclinical assessment model to test therapeutic effects of different drugs and other approaches on the MDS clonality and autologous MSCs prior to treatment of MDS patients.

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MYELODYSPLASTIC SYNDROMES WITH IRON OVERLOAD ARE CHARACTERIZED BY A SWITCH FROM OXIDATIVE PHOSPHORYLATION TO GLYCOLYSIS AND THIS DEFECT IS PARTIALLY RESTORED BY IRON CHELATION. A FISM STUDY

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Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of diseases characterized by a clonal and ineffective hematopoiesis, as well as the tendency to develop iron overload, mainly due to red blood cell transfusions. Iron overload has been described to increase ROS production and progressively worsen hematopoiesis. In mitochondria, iron is a fundamental component of cytochromes belonging to the oxidative phosphorylation (OXPHOS), which is considered the main source of cellular energy. Mitochondria are also the main site of ROS production." In this regard, cancer energetic metabolism is an emerging issue that could represent an attracting therapeutic target.
Aims: The aim of the study was to investigate the energetic metabolism in MDS patients and to understand the impact of iron overload on the energy production.
Methods: We selected 37 samples from patients with MDS with or w/o iron overload (7 RA, 5 RARS, 9 RCMD, 4 RAEB-I, 2 RAEB II and 10 s-AML). In addition we analyzed 86 samples from healthy subjects stratified according to age (20-103 years) and 4 samples from beta thalassemia with iron overload. In all these samples, we evaluated the ATP/AMP ratio, as marker of energy status, the OXPHOS activity, in term of oxygen consumption and ATP synthesis, the lactate dehydrogenase (LDH) activity, as marker of anaerobic glycolysis, and malondialdehyde (MDA), as marker of lipid peroxidation. The same parameters have been analyzed also after iron chelation with deferasirox (DFX) and after incubation of the cells with DFX and DFO
Results: Our study clearly demonstrated that mitochondrial function is altered in MDS, leading to a strong energetic defect and an increase in oxidative stress, far beyond the expected paraphysiological decrease resulting from ageing. The OXPHOS efficiency is highly reduced in MDS compared to controls, determining an impairment of the ATP/AMP ratio, which is 2.4 in young controls, 0.75 in elderly controls and it is 0.2 in b-thalassemia and MDS patients. By contrast, LDH activity increased in the MDS patients (6mU/mg) with respect

the controls (88 mU/mg), suggesting an attempt to compensate the energy unbalance with the increment of anaerobic glycolysis. MDA level, which reflects the lipid peroxidation, is 1mM in young subjects, 9mM in elderly subjects, 9mM in b-thalassemia and 15mM in MDS. In vitro iron chelation partially restored this abnormalities in MDS patients: ATP/AMP ration increases from 0.2 to 0.6 in MDS and b-thalassemia, by contrast it is reduced in healthy subjects from 2.4 to 1.6. Anaerobic glycolysis is reduced after DFX incubation, in fact LDH decrease from 88 to 77 in MDS. By contrast, in healthy samples the iron chelation determined a reduction of OXPHOS activity, with a consequent impairment of ATP/AMP ratio and an increment of anaerobic glycolysis flux. Lipid peroxidation is significantly reduced of 28% with DFX and 23% with DFO (p value <0.001 for both). Similar reduction is observed in b-thalassemia. By contrast MDA levels increased in healthy subjects incubated with DFX. Curiously, all these abnormalities are more pronounced in MDS with IOL compared to MDS w/o IOL and are significantly worse in MDS without IOL compared to elderly normal subjects. Finally, *in vivo* treatment of patients with DFX reproduces similar findings as *in vitro* incubation.

Summary/Conclusions: In summary OXPHOS activity and the energetic status are highly impaired in MDS compared to elderly subjects. MDS cells used O2 to produce ROS instead of ATP. This is typical of ageing but is significantly increased in MDS compared to elderly controls and it is further increased by IOL. DFX is able to restore mitochondrial activity and ATP production in all the patients analyzed after *in vivo* or *in vitro* treatment.

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V-SET AND IMMUNOGLOBULIN DOMAIN-CONTAINING 4 (VSIG4) EXPRESSED ON MONOCYTES INCLUDING TUMOR-ASSOCIATED MACROPHAGES SUPPRESSED ANTITUMOR IMMUNE RESPONSES IN MYELODYSPLASTIC SYNDROMES

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Background: In myelodysplastic syndromes (MDS), blast cells increase with clonal proliferation during disease progression, while immune cells in the bone marrow (BM) microenvironment become less efficient. The V-set and immunoglobulin domain-containing 4 (VSIG4) molecule is a new B7 family-related protein and strong negative regulator of T-cell proliferation. However, the role of VSIG4 in tumors including hematological malignancies remains unknown.

Aims: We investigated the expression and functions of VSIG4 in MDS.

Methods: 1) Peripheral blood (PB) and BM samples were obtained from 39 patients with acute leukemia transformed from MDS (AL-MDS, N=21), MDS (N=13), and chronic myelomonocytic leukemia (CMML, N=5) and from healthy controls (N=14). The expression of VSIG4 in mononuclear cells (MCs) from the samples and MDS cell lines (F-36P and SKM-1) was analyzed with real-time PCR and flow cytometry (FCM). 2) After cultivation with BM stromal HS-5 cells, its culture supernatants (HS-5 sup.), immunomodulatory drugs (lenalidomide [LEN] and pomalidomide [POM]), and anti-MDS agents (cytarabine and azacitidine), VSIG4 expression on cells was determined using FCM. 3) The proliferative potential was examined by BrdU incorporation using FCM and the MTT assay. 4) The cytotoxic activity of natural killer (NK) cell line NK-92MI against target cells was determined by measuring lactate dehydrogenase activity in culture supernatants. The production of IFN-γ from donor T cells was measured in the cell culture supernatants using ELISA.

Results: 1) VSIG4 mRNA expression in PBMCs from patients with AL-MDS was significantly higher than in those from controls. Cell-surface VSIG4 expression on CD14+CD11b+ monocytes from MDS and AL-MDS patients was higher than in those from controls, but VSIG4 expression was not detected on CD34+ blasts. In CD14+CD11b+ monocytes of MDS and AL-MDS patients, VSIG4 was strongly expressed on CD68+CD206+ tumor-associated macrophages (TAMs). Furthermore, the expression levels of VSIG4 on CD14+ monoblasts from CMML patients were significantly upregulated in comparison with those from controls. 2) Two MDS cell lines expressed both VSIG4 mRNA and its cell-surface protein. VSIG4 expression on MDS cell lines, and on monocytes and monoblasts from MDS and CMML patients, respectively, was significantly upregulated by co-cultivation with HS-5 sup, LEN, and POM, but not with cytarabine or azacitidine. 3) VISG4+ MDS cells had higher proliferative potential than VISG4– cells, and the proliferation of VSIG4+ cells was suppressed by VSIG4-specific small interfering RNA. 4) LEN-treated K562 cells, which induced high VSIG4 expression, were more resistant to NK-mediated cytotoxicity than untreated K562 cells. The cytotoxic activity of NK cells was inversely correlated with the expression levels of VSIG4 on target cells. CD45-mediated ADCC activity of NK92-MI cells against LEN-treated MDS cells was inhibited in comparison with untreated cells. Moreover, the production of IFN-γ from donor T cells co-cultivated with LEN-treated MDS cells was suppressed compared with control cells.

Summary/Conclusions: Our study demonstrated that VSIG4 was highly expressed on monocytes including TAMs in MDS and AL-MDS patients, and on monoblasts in CMML patients. VSIG4-expressing monocytes and monoblasts may suppress antitumor immune responses and be associated with disease progression in MDS and CMML. The results will allow us to elucidate the function of VSIG4 in MDS pathophysiology and lead to the development of new immunotherapy.

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TRANSCRIPTOME ASSESSMENT OF DNA REPAIR GENES IN CHRONIC MYELOMONOCYTIC LEUKEMIA: SYNTHETIC LETHALITY TARGETS

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Background: Though genetic instability is a hallmark of myeloid disorders, the lack of recurrent somatic mutations, inarguably pathogenic, in the DNA repair machinery have precluded a predominant interest in this pathway. However, the recent discovery of non classical leukaemogenesis by splicing defects, the paramount influence of epigenetic anomalies, and the development of of unbiased high-throughput sequencing approaches oblige us to revisit those routes in blood cancers.

Aims: To perform improved massive RNA-seq in chronic myelomonocytic leukemia (CMML) samples to identify neoplasm-specific targets for a synthetic lethality therapeutic aproach. To validate the candidates through a direct strategy in an extended cohort of CMML, myelodisplastic sydromes (MDS) and acute myeloid leukemia (AML) patients.

Methods: We performed enhanced RNA-seq on 27 CMML bone marrrow samples at diagnosis and 10 healthy BM samples We validated those DEGs by RT-PCR in an extension cohort of 73 additional CMML patients and assessed their potential singular pattern in this disease by analyzing 80 MDS and 90 AML patients. We further chose 13 of the diferentially expressed genes for validation and characterization through the myeloid spectrum based on clinical considerations: i) druggable oncogenes found to be highly overexpressed in our cmml patients or allowing for the inhibition of an specific DNA repair pathway (i.e. XPA, XRCC4, MSH4): ii) oncogenes infra-expressed in our cohort but with inhibitory molecules already being tested in myeloid neoplasms (i.e. PARP1).Global pattern of DNA repair gene expression was compared with MDS and AML MILE study data.

Results: Of 27 CMML patients and 10 healthy donors, the expression of 18 genes was significantly different between the two groups (p-value<0.05) with 6 genes up-regulated and 12 genes down-regulated in CMML patients compared with donors. Defects on genes predominantly unique to a single strand breaks repair pathway included: NEIL1 and OGG1 in base excision repair (BER), XPA and MSS19 in nucleotide excision repair (NER) and MSH4 in Mismatch Repair (MMR). Only XRRC4 overexpression was found as a significant defect in genes associated exclusively to double strand break repairs. We found different directions of misregulation in our DNA repair genes candidates in CMML when we validated them by RT-PCR in an extended cohort of 73 CMML, 80 MDS and 90 AML patients (i.e. PARP1 is down-regulated in our CMML and up-regulated in our complex-karyotype AML subset). To validate this, we extracted the DNA repair transcriptional components from a large dataset of 206 MDS, 47 complex karyotype AML (AMLcx) and 73 healthy donors bone marrow from the MILE study. Surprisingly, MDS misregulation was characterized for a predominance of upregulated genes (14 out of the 20 misregulated targets) while AMLcx showed a global defect with a predominance of downregulation (37 out of 50 misregulated targets). Of note, some genes showed opposite sense of misregulation according to the myeloid disorder: TDP1 was upregulated in CMML cases and downregulated in AMLcx, and viceversa for BAP1. CDK1 and EXO1 were upregulated in MDS cases and the opposite effect was found among AML cases.

Summary/Conclusions: Using an unbiased and massive DNA repair transcriptome assessment, we have identified a series of candidate targets for a synthetic lethality approach in CMML. In addition, the different sense of misregulation of these and other targets within the myeloid diseases, some of them already being targeted in the clinical trial setting, emphasize the need of a neoplasm-personalized test of DNA repair modulators.

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DIFFERENTIAL DIAGNOSIS BETWEEN MYELODYSPLASTIC SYNDROMES AND NON-CLONAL CYTOPENIAS BY FLOW CYTOMETRY ANALYSIS USING A MYELOID MATURATION DATABASE

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Background: The diagnosis of myelodysplastic syndromes (MDS) is based

on cytomorphological characteristics, but it remains a challenge in some patients who do not fulfil diagnostic criteria. Flow cytometry (FC) immunophenotyping can be in an important tool for MDS diagnosis, but a lack of standardisation and subjectivity of the analysis hinder its applicability.
Aims: To develop a methodology for FC immunophenotyping that allows us to stablish the differential diagnosis between MDS patients and non-clonal cytopenias using a myeloid maturation database.
Methods: Bone marrow samples from 55 MDS patients, and 51 controls with cytopenias of several origins (immune disease, hypersplenism, drug toxicity) were analysed by FC. We elaborated a Myeloid Maturation Database using the *Infinicyt*® v1.7 software (*Cytognos*, Spain). From all bone marrow controls, we merged files stained with a 4-colour combination (CD16-FITC/CD13-PE/ CD45pCP/ CD11bAPC). We selected myeloid population from the merged file and drew a maturation path. We obtained a maturation diagram that displays the fluorescence intensity of each parameter measured along the maturation stages. Then, for patients and controls, we obtained the fluorescence intensities whose median values exceeded ±2SD range in comparison with the stored database values (Figure 1). We elaborated a score, considering the relevant changes in fluorescence intensities (deviations) in the four markers analysed (CD16, CD13, CD45, CD11b) and in the four maturation stages, with a punctuation from 0 to 16.
Results: We found a mean of 1.9 deviations (fluorescence intensities values exceeded ±2SD) in controls, and a mean of 4.5 deviations in patients. Our test resulted reliable for differential diagnosis between controls and patients (curve ROC analysis, AUC=0.748; *p= 0.016*). We found that with a cut-off of 4.5 deviations, we obtained a high specificity in the diagnosis of MDS (100%) but a low sensitivity (45%). With a high suspicion of MDS (specificity 90%), we can consider patients with scores above 3.5, thus achieving higher sensitivity (59%). Additionally, the number of immunophenotyping changes correlated well with prognostic risk. We confirmed that the higher the risk, the greater impact on deviations from the normal pattern (average of 3.7 at low risk, 4.5 at intermediate risk; 6.8 at high risk) (Figure 2).

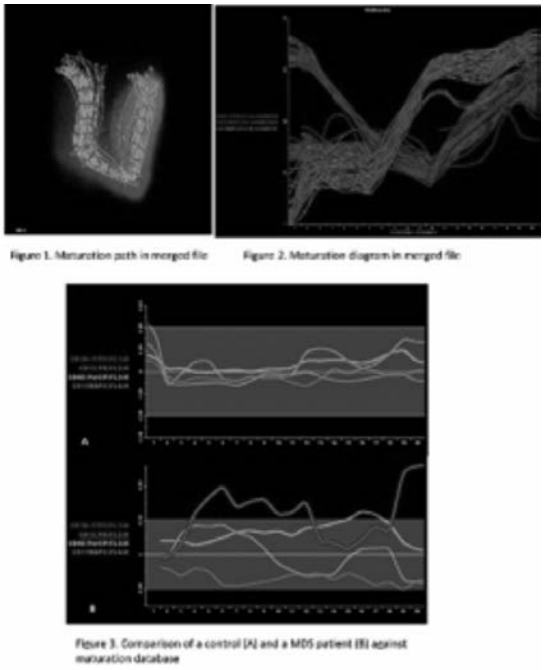

Figure 1.

Summary/Conclusions: The maturation database (using the maturation analysis from *Infinicyt*® software) was useful to discriminate between MDS patients and non-clonal cytopenias, proving to be a reliable diagnostic test, also with prognostic implications. The application of this database as a diagnostic tool has the advantage that the result is independent of the observer. Inclusion of more myeloid markers and incorporation of erythroid parameters could increase sensibility in differential diagnosis.

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A PHASE IB STUDY EVALUATING THE SAFETY AND CLINICAL ACTIVITY OF ATEZOLIZUMAB ALONE AND IN COMBINATION WITH AZACITIDINE IN PATIENTS WITH RELAPSED OR REFRACTORY MYELODYSPLASTIC SYNDROMES

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Background: Treatment options are limited and prognosis is poor for patients (pts) with relapsed or refractory myelodysplastic syndromes (R/R MDS), those who relapse after or fail to achieve complete response with hypomethylating agents (HMAs). Atezolizumab (atezo) is a humanized IgG1 monoclonal antibody that binds programmed death-ligand 1 (PD-L1), disrupting the immune activation checkpoint. The role of PD-L1 blockade in hematologic disorders is not yet established. Immune evasion via the PD-L1/PD-1 pathway may play a role in MDS resistance to HMA treatment (Yang et al. *Leukemia.* 2014). Upregulation of PD-L1 expression seen in pts during and after HMA treatment suggests that atezo could provide benefit in HMA-exposed pts as a single agent or in combination with an HMA.
Aims: To determine the safety and clinical activity of atezo alone and in combination with azacitidine (aza) in an open-label Phase Ib clinical trial (NCT02508870).
Methods: Pts with R/R MDS were eligible for Cohorts A and B, excluding those with prior solid organ or allogeneic hematopoietic cell transplant. Pts in Cohort A received atezo 1200mg intravenously (IV) on day 1 of each 21-day cycle for up to 17 cycles. Pts in Cohort B received aza 75mg/m^2 on days 1 through 7 and atezo 840mg IV on days 8 and 22 of each 28-day cycle for 6 cycles, followed by single-agent atezo 1200mg IV on day 1 of each 21-day cycle for up to 8 cycles. Primary endpoints were safety and tolerability. Secondary endpoints included overall response rate, time to AML progression, PFS, OS and changes in transfusion rate. Blood and bone marrow samples were collected for PK evaluation and biomarker analysis.
Results: At the time of data cutoff, 10 pts in Cohort A and 6 pts in Cohort B were safety evaluable. The median age was 76 years (range, 63-89 years); 4 of 16 pts (25%) had ≥2 previous lines of therapy. All pts were previously exposed to aza, and 2 of 16 pts (13%) were also exposed to decitabine. 15 of 16 pts were refractory to prior therapy. All pts experienced ≥1 treatment-emergent adverse event (AE). The most common Grade 3-4 AEs were febrile neutropenia (31%) and decrease in neutrophil count (25%). One pt died on study of an unknown cause. The median duration of treatment was 101 days (range, 70-275 days) in Cohort A and 92.5 days (range, 39-144 days) in Cohort B. Four of 10 pts in Cohort A and 3 of 6 pts in Cohort B remained on study as of 16 Sep 2016. All 10 pts in Cohort A and 5 of 6 pts in Cohort B were evaluable for response. No pt achieved an objective response; 6 of 10 pts (60%) in Cohort A and 3 of 5 pts (60%) in Cohort B had stable disease. Compared with pretreatment, a trend toward decreased RBC transfusions was observed in pts in Cohort A, especially in those who remained on therapy beyond 12 weeks. Preliminary PK analysis showed that exposure to atezo as a single agent or in combination with aza was comparable to historical data in pts with solid tumors.
Summary/Conclusions: Early evaluation of atezo alone and in combination with aza suggests that the safety profile is consistent with that expected in the study population. Additionally, the duration of therapy observed is encouraging in this R/R population with no standard-of-care options. Updated safety, efficacy and survival data will be presented. Emerging correlative biomarker information will also be discussed.

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EPIGENETIC DRUG TREATMENT GLOBALLY INDUCES CRYPTIC TRANSCRIPTION START SITES ENCODED IN LONG TERMINAL REPEATS (LTRS)

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Background: Epigenetic drugs are currently used for the treatment of several hematologic malignancies, but their pharmacological mechanism remains poorly understood. For DNA methyltransferase and histone deacetylase inhibitors (DNMTi and HDACi) several mechanisms of action have been proposed, mainly based on candidate gene approaches. However, less is known about their genome-wide transcriptional and epigenomic consequences.
Aims: To investigate the effects of epigenetic treatment on transcription and chromatin, we profiled genome wide transcription start sites (TSS) activities and associated chromatin changes following the treatment with inhibitors against DNMTs, HDACs, or both.
Methods: Genome wide analysis of transcription start sites (TSS) (Cap analysis of gene expression (CAGE) sequencing), methylation status (whole-genome bisulfite sequencing) and chromatin dynamics (Chromatin-immunoprecipitation (ChIP) sequencing) were done by using a previously described reporter cell line model. Functional assays were used to investigate the mechanisms of LTR reactivation, a neuroblastoma mouse xenograft model to confirm the LTR reactivation in vivo.
Results: Following the treatment with inhibitors against DNMTs, HDACs, or both, we observed the activation of thousands of cryptic, currently non-annotated transcription start sites (treatment-induced non-annotated transcripts, TINATS). These TINATs arose most commonly from LTR12 elements, particularly LTR12C (ca. 50% of all TINATs). The resulting transcripts frequently splice into protein-coding exons and encode truncated or chimeric open reading frames which translated into currently uncharacterized protein isoforms with predicted abnormal functions or immunogenic potential, the last one based on their foreign sequence and capability of being presented on MHC-class I molecules. TINAT transcription after DNMTi coincided with DNA hypomethylation and gain in H3K4me3, H3K9ac, and H3K27ac, and further activating histone marks, while HDACi specifically induced a subset of TINATs in association with H2AK9ac, H3K14ac, and H3K23ac. Despite this mechanistic difference, both inhibitors convergently induced transcription from identical sites since TINATs are encoded in solitary long-terminal repeats of the endogenous retrovirus-9 family, epigenetically repressed in virtually all normal cells. Moreover, we found a consensus GATA2 binding motif which strongly distinguished LTR12Cs with TINATs from LTR12Cs without TINATs, supporting that GATA2 is likely the upstream transcription factor responsible for TINAT activation. Knock-down of GATA2 resulted in a reduced LTR12C expression despite epigenetic drug treatment. Overexpression of LTRs in our cell line model showed reduced cell viability in 3 out of 10 TINAT candidates. The reactivation of LTR12C elements upon epigenetic drug treatment could be confirmed in other malignant cell lines as well. Importantly, treatment with several chemotherapeutic agents did not affect LTR12C transcript levels, suggesting that their induction is a specific effect of epigenetic modulation rather than a general consequence of cellular stress. Additionally, we measured the transcription of LTR12C transcripts after SAHA treatment in a neuroblastoma mouse xenograft model, thereby confirming LTR12C induction *in vivo*.
Summary/Conclusions: DNMTi and/or HDACi induce *de novo* transcription of LTRs (LTR12 family), resulting in numerous fusion transcripts that encode novel protein isoforms which partly have the potential to influence cell proliferation, might explain the priming effect of epigenetic therapy and will be further investigated regarding their role as potential marker for epigenetic treatment response. Other future experiments will include proteomic approaches combined with T-cell cytotoxicity assays to further shed light on the interaction between epigenetic and immune therapy and the role of ERV-derived antigen presentation.

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LYMPHOPENIA IS AN INDEPENDENT RISK-FACTOR IN PATIENTS WITH LOW-RISK MDS ACCORDING TO THE IPSS-R

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Background: Lymphopenia is associated with an increased mortality in different medical conditions, including solid tumours and lymphoma. Little is known about its prognostic impact in myelodysplastic syndromes (MDS).
Aims: To clarify the prognostic impact of lymphopenia in MDS in addition to the Revised International Prognostic Scoring System (IPSS-R)
Methods: The Düsseldorf MDS-registry was searched for patients with a complete differential blood count at diagnosis. Patients having received allografting or with an absolute lymphocyte count >5.0 G/l were excluded. The influence of the absolute lymphocyte count at diagnosis on overall survival was determined by Kaplan-Meier analysis. Multivariate Cox regression analyses were performed.
Results: 2035 patients (RA n=182, RCMD n=978, RARS n=170, MDSdel5q n=92, RAEB-1/2 n=613) with a median follow-up of 23 months (mo) were identified. Data were sufficient for IPSS-R calculation in 651 patients. The mean absolute lymphocyte count (ALC) in the whole population was 1402/µl (95% *CI* 1368-1437, range 0.12-4972) with no significant differences between the IPSS-R groups (very low-risk [n=77] mean 1471/µl, low-risk [n=255] mean 1406/µl, intermediate-risk [n=154] mean 1244/µl, high-risk [n=96] mean 1419/µl, very-high risk [n=69] mean 1255/µl, p=0.067). 688 patients (34%) were lymphopenic (ALC < 1000/µl) with a significantly shorter survival (median 33.7 *versus* 26.4 months, Log Rank p < 0.001). After stratification according to IPSS-R, survival of lymphopenic patients was not significantly different in the very-low, intermediate or (very) high risk group. Within the low risk group the survival difference was of borderline significance (median 67 *vs* 47 months, Log Rank p=0.1, Breslow p=0.039). With an ALC above the first quartile of the whole population (850/µl) as discriminator, the survival difference between lymphopenic and non-lymphopenic patients within the IPSS-R low-risk group reached statistical significance (survival median 67.4 versus 43.0 months, Log Rank p= 0.002). This was not the case in the other IPSS-R subgroups. In multivariable analyses, an ALC <850/µl retained its independent prognostic value for the IPSS-R low risk group after inclusion into a Cox regression model together with age <>70 and LDH <> normal value (240 U/l) (p= 0.039). Patients with an ALC <850/µl had significantly lower platelet (median 97 *versus* 150 G/l, p<0.001) and neutrophile (median 1478 *versus* 1971/µl, p <0.001) counts but similar haemoglobin levels (median 9.1 *versus* 9.4 g/dl, p=0.052).
Summary/Conclusions: An absolute lymphocyte count < 850/µl is an independent risk factor in patients with low risk MDS according to the IPSS-R. Whether lymphopenia in MDS is a direct consequence of the underlying haematopoietic stem cell defects or arises from immune-modulating stimuli related to the disease or to other host conditions remains to be elucidated. The lower levels of platelets and neutrophils in lymphopenic patients observed in our cohort point towards an association of lymphopenia with marrow insufficiency. In addition, further studies with larger patient cohorts are necessary to define the lymphocyte count most suitable for prognostication.

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IMPACT OF MARROW COMPLETE RESPONSE IN THE NATURAL HISTORY OF PATIENTS WITH MYELODYSPLASTIC SYNDROMES (MDS) AND CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML) TREATED WITH HYPOMETHYLATING AGENTS

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Background: The concept of marrow complete response (mCR) was included for the first time in the 2006 International Working Group (IWG) response criteria in MDS. mCR is defined as a reduction to ≤5% myeloblasts and decrease by ≥50% compared to baseline with persistent cytopenias. However, their inclusion in the response criteria remains controversial as it is not known how it affects the natural history of MDS.
Aims: The aim of our study was to describe the impact of mCR in survival outcomes in patients with MDS treated with hypomethylating agents (HMA).
Methods: We retrospectively reviewed 713 patients diagnosed with MDS or CMML and treated with frontline HMA between 2004 and 2015 at a single institution. Clinical and demographic data were obtained from an electronic database. Response was assessed by modified 2006 IWG criteria. Statistical analyses were performed with the IBM SPSS Statistics 23.0 software. All tests were 2-sided with significance set at p<0.05.
Results: 444 (62.3%) patients from the initial cohort achieved at least hematologic improvement (HI) as best response and were included in the analysis. 162 (37%) patients were female. Median age at diagnosis was 68 years (range 17-91). Following the 2016 WHO classification: 30 patients (7%) were MDS-SLD, 50 (11%) MDS-MLD, 20 (5%) MDS-RS, 230 (52%) MDS-EB, 10 (2%) MDS-U and 104 (23%) CMML. According to the International Prognostic Scoring System (IPSS), 37 patients (8%) belonged to the low risk group, 176 (40%) to the intermediate-1 risk group, 198 (45%) to the intermediate-2 risk group, and 31 (7%) to the high risk group. 200 (45%) patients received azacitidine-based therapies and 244 (55%) decitabine-based therapies. Responses included: 238 (33% of the total population) complete responses (CR), 61 (9%) mCR, 2 (<1%) partial responses (PR) and 143 (20%) stable disease (SD). HI was observed in 410 (58% of the total population) of the patients. The median time to response was 3 cycles (range 1-24). Median overall survival (OS) since the

start of treatment was 21 months (95%CI=19-24): CR: 25 months (CI95%=20-30); PR: 27 months; mCR: 20 months (CI95%=20-30); and SD: 17 months (CI95%=14-19) (p=0.006). We compared OS between mCR *vs* CR (p=0.193, HR 0.796 [95%CI=0.765-1.122]), mCR *vs* PR (p=0.572; HR =0.564 [95%CI=0.078-4.105]) or mCR *vs* SD (p=0.243; HR=1.242 [95%CI=0.863-1.788]), without any statistical difference (Fig. 1A). Median progression-free survival (PFS) was 14 months (95%CI=13-16): CR: 16 months (CI95%=13-21); PR: 11 months; mCR: 10 months (CI95%=5-15); and SD: 10 months (CI95%=9-12) (p=0.013). No statistical differences were observed between PFS in patients who achieved mCR *vs* PD (p=0.410; HR 1.816 [95%CI=0.439-7.512]) and SD (p=0.7743; HR 1.059 [95%CI=0.752-1.491], but PFS was increased in those patients who achieve CR when compared to mCR (p=0.013; HR 0.665 [95%CI=0.482-0.918]) (Fig. 1B).

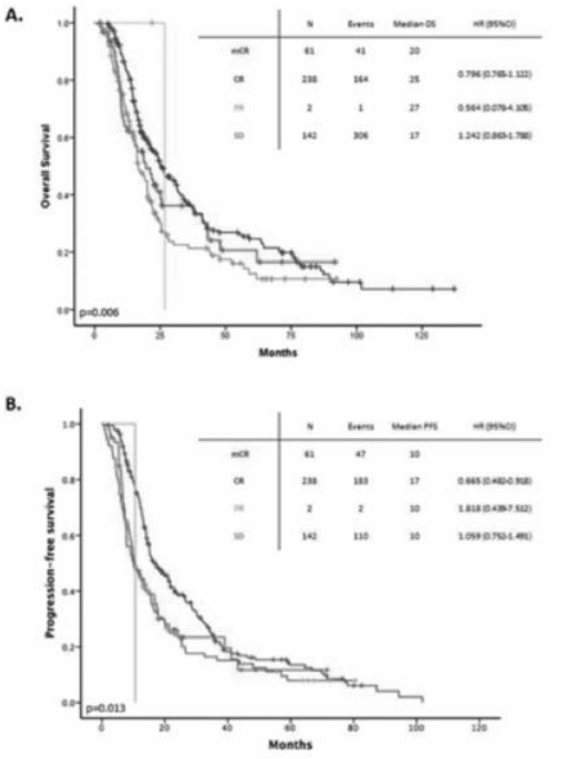

Figure 1.

Summary/Conclusions: Although mCR and CR result in the same OS, PFS is increased in patients achieving CR when compared with mCR. These data indicate that mCR should be considered as a valid endpoint in clinical trials.

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LUSPATERCEPT INCREASES HEMOGLOBIN AND REDUCES TRANSFUSION BURDEN IN PATIENTS WITH LOWER-RISK MYELODYSPLASTIC SYNDROMES (MDS): LONG-TERM RESULTS FROM PHASE 2 PACE-MDS STUDY

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Background: Management of anemia is a common therapeutic challenge in patients (pts) with MDS. Luspatercept (ACE-536), a fusion protein containing modified activin receptor type IIB, is being developed for treatment of anemia in lower-risk MDS. Luspatercept binds to select TGF-β superfamily ligands (such as GDF11) reducing aberrant Smad2/3 signaling and promoting late-stage erythroid differentiation and increased hemoglobin (Hgb) levels (Suragani R, Nat Med, 2014; Attie K, Am J Hematol, 2014).
Aims: This ongoing, phase 2, multicenter, open-label study followed by a long-term extension (ext) study evaluates the effects of luspatercept in pts with lower-risk MDS. Endpoints include long-term safety and tolerability, erythroid response (IWG HI-E), RBC transfusion independence (RBC-TI, ≥8 weeks), duration of HI-E, pharmacodynamic and iron metabolism biomarkers, and pt-reported quality of life (QoL).
Methods: Inclusion criteria: MDS IPSS low or int-1, age ≥18 yr, Hgb <10 g/dL (if <4U RBC/8 weeks), no prior HMA, and no current lenalidomide or erythropoiesis-stimulating agent (ESA). The dose-escalation phase of the study is completed. An expansion cohort of up to 56 patients was added to this phase 2 study to evaluate response to luspatercept in pts who would not qualify for the phase 3 MEDALIST trial (for regularly transfused ring-sideroblast positive [RS(+)] patients with EPO >200 U/L). These include pts with low transfusion burden (LTB, <4U RBC/8 weeks) and either 1) RS(+) (≥15% in bone marrow) with baseline EPO ≤200 U/L or 2) RS(-) and any EPO level. RS(-) pts were allowed up to 6U RBC/8 weeks. Pts are treated every 3 weeks subcutaneously for up to 5 doses (titration up to 1.75mg/kg) in the base study (NCT01749514) and are then eligible for long-term treatment up to 5 additional years (NCT02268383).
Results: Data (as of 09Sept2016) were available for 73 base and 42 ext study pts. 32 base and 22 ext pts were LTB; 41 base and 20 ext pts were high transfusion burden (HTB, ≥4U RBC/8 weeks). Median (range) age (yr) was 72 (27-90), 53% pts had prior ESA, 51% pts had baseline EPO <200 U/L. Median (range) Hgb (g/dL) for LTB pts was 8.6 (6.4-10.1). Median (range) RBC transfusion burden (U/8 weeks) for HTB pts was 6 (4-18). 71% base and 86% ext pts were RS(+). IWG HI-E response rates for pts treated with ≥0.75mg/kg in the base and ext studies, respectively, were 62% (18/29) and 83% (19/23) for RS(+) pts with EPO <200 U/L and 46% (5/11) and 88% (7/8) for RS(+) pts with EPO 200-500 U/L. RBC-TI rates for pts treated with ≥0.75mg/kg in the base and ext studies, respectively, were 68% (13/19) and 71% (10/14) for RS(+) pts with EPO <200 U/L and 33% (3/9) and 60% (3/5) for RS(+) pts with EPO 200-500 U/L. Preliminary RS(-) response rates (IWG HI-E and RBC-TI) by subgroup will also be presented at the meeting. Luspatercept was well tolerated, with related grade 3/serious adverse events (in 3 pts) as of 28Nov2016 of blast cell count increase, myalgia, and worsening of general condition. The most common related AEs (≥2 pts) were diarrhea, fatigue, headache, hypertension, arthralgia, bone pain, injection site erythema, myalgia, and peripheral edema.
Summary/Conclusions: Lower-risk MDS patients treated long-term with luspatercept demonstrated robust and sustained increases in Hgb and decreases in transfusion burden and a high rate of RBC-TI. A Phase 3 study of luspatercept in regularly-transfused RS(+) patients with lower-risk MDS according to IPSS-R is ongoing (MEDALIST study; NCT02631070).

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RATE AND CAUSES OF 5-AZACYTIDINE DISCONTINUATION AND SUBSEQUENT THERAPEUTIC OPTIONS IN 418 MDS PATIENTS FROM THE ITALIAN MDS REGISTRY OF FONDAZIONE ITALIANA SINDROMI MIELODISPLASTICHE (FISM)

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Background: Azacytidine (AZA) is the current standard of care for patients with high-risk myelodysplastic syndrome (MDS) in Europe. AZA has shown a

survival advantage when compared with conventional therapies and has also shown activity in IPSS lower-risk patients. However, about 40% of patients do not respond and most patients loose response within 2 years. Treatment options for MDS patients failing hypomethylating agents therapy are scarce and overall survival (OS) is extremely short.

Aims: Objectives of this study were to describe in a cohort of real life MDS patients treated with AZA, the reasons causing treatment discontinuation, and to evaluate the clinical outcome after the end of AZA therapy.

Methods: Unselected patients recorded in the MDS Registry of Fondazione Italiana Sindromi Mielodisplastiche (FISM) and treated with AZA from January 2009 to June 2014 were considered for the analysis. All types of conventional published schedules of AZA were allowed. Clinical response, cause of discontinuation, salvage treatments and OS from discontinuation of AZA were the major end points.

Results: Between January 2009 to June 2014 1799 newly diagnosed MDS patients were enrolled in the Registry, and 418 received AZA; 269 as 1st line treatment (64%), 115 as 2nd line treatment (28%), and 34 as a line ≥3rd (8%). Median age was 73 years (range 18-91); 260 patients (62%) were male. WHO diagnosis was RA or RARS (n=27, 6%), RCMD with or without RS (n=62, 15%) AREB-1 (n=126, 30%), AREB 2 (n=189, 45%), other subtypes (n=15, 4%). At start of AZA therapy IPSS score was low in 14 (3.4%), int-1 in 97 (23.2%), int-2 in 183 (43.8%), high in 67 patients (16%), and not available in 57 patients (13.6%). Patients received a median of 7 courses of treatment (range 1-63). Seventy-three % of the whole cohort (418 pts) were alive at 1 year from beginning of AZA therapy and median OS was 23 months, (25 for IPSS lower-risk MDS and 21 for IPSS higher risk MDS). OS after discontinuation of AZA was 8 months. Clinical responses according to IWG criteria were available in 344/418 patients (82%): 45 (13%) patients achieved a complete hematological response, 77 (22%), a partial response, 86 (25%) had stable disease while 136 (40%) did not respond. Response was achieved after a median of 6 cycles. After a median follow up of 16 months (range 7-35) in 37 (9%) patients AZA therapy was still ongoing while in 381 (91%) the treatment has been discontinued. Interruption of treatment was due to loss of response in 59 (16%) patients, AML evolution in 154 (40%), death in 43 (11%), toxicity or poor compliance in 39 (10%), allogeneic transplant (HSCT) in 12 (3%), other reasons in 22 (6%), not reported in 52 patients (14%). Of the 381 patients who discontinued AZA, 15 (4%)were managed with intensive AML-like chemotherapy, 22 (6%), received an allogeneic HSCT, 27 (7%) low-dose chemotherapy (7%), 22 (6%) erythroid stimulating agents,18 (5%) other treatments and 277(72%) patients no further treatment or only supportive therapy.

Summary/Conclusions: Our data confirm that AZA therapy is effective for MDS patients, both with higher and lower IPSS risk disease. Response rate is consistent with what previously reported, with a median OS of 23 months. Interestingly, at 16 months, 91 % of patients had discontinued treatment, either for progression or loss of response and only in 10% of cases for reported toxicity. Only 28% of patients received any kind of salvage therapy and overall survival after AZA discontinuation was poor (8 months).

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COMBINATION OF DEEP PHENOTYPING AND TARGETED NEXT GENERATION SEQUENCING AS A DIAGNOSTIC TOOL IN CHILDREN WITH SUSPECTED MDS

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Background: Paediatric Myelodysplastic Syndromes (MDS) are a rare and heterogeneous group of disorders distinct from adult MDS. They may present with symptomatic anaemia, life threating infection or evolving leukaemia; however, they may also present as unexplained cytopenias or with multisystem disease of unclear aetiology. Diagnosis can represent a huge challenge for clinicians, even in highly specialised centres and this can delay the delivery of the most appropriate treatment. Hence an accurate diagnosis is crucial in selecting the most appropriate management, including surveillance and follow up.

Aims: To devise a clinical grade diagnostic targeted NGS panel and combine the results with extensive clinical phenotypic information to obtain a diagnosis in children referred with suspected MDS.

Methods: Children (0- 18yrs) were referred from 14 UK centres with a diagnosis of suspected MDS and/or sustained cytopenias with morphological features of myelodysplasia. Extensive phenotypic information including family history, detailed clinical examination and disease course details were collected and captured on an online database using the Human Phenomiser tool. A customised targeted NGS panel was designed using the Illumina design studio containing 32 genes, 916 amplicons and 301 exons; selected through literature reports and well described mutations in Paediatric MDS and potential overlap Bone Marrow failure syndromes (BMFS). Coverage of each base within target regions was assessed for every sample on each sequencing run using Covermi software. Library preparation was performed using an Illumina Truseq Custom Amplicon panel, followed by sequencing on an Illumina MiSeq. Data analysis was performed using our established bioinformatic pipelines (Hamblin A: Blood 2014 124:2373)

Results: In total 59 patients (females= 29, males 30) have been screened and 3 subgroups identified based on the original suspected clinician diagnosis at presentation: MPN/JMML (n= 15), *de novo* MDS (n=9) and idiopathic cytopenias of undetermined significance, (ICUS) with some features of dysplasia (n= 35). Mutations were detected in 24/59 patients (40%, Table 1). Of these, NGS results confirmed the original clinical diagnosis in 15 cases (62.5%); established the diagnosis for the first time in 6 cases (25%); and led to a change in diagnosis (from autoimmune neutropenia to Shwachman-Diamond Syndrome) in 1 case leading to a significant change in patient management. In two already known cases, it allowed monitoring of the disease molecular signature. As expected, *RAS* pathway mutations were common in the JMML/MPN (100%) and *de novo* MDS patient subgroups (33%). Additional mutations in epigenetic modifiers, splicesome mutations as well as second *RAS* pathway hits were also detected in 40% of JMML patients and in one case within the *de novo* MDS group; this finding was associated with poor outcome. Within the heterogenous ICUS patient group, pathogenic mutations were identified in 5/35 (14.3%) cases with BMFS genes (*SBDS, ELANE, TP53*). In contrast to the other MDS/MPN cases, in this group, no *RAS* pathway mutations were detected.

Table 1.

	JMML	MDS (RC, RAEB, RAEB-T)	ICUS
Number	15	9	35
Age(range, yrs)	0-21	0-21	0-21
Cytogenetics	Normal (n=13) Mon 7 (n=1) Other (n=1)	Normal (n= 4) Mon 7 (n=2) Other (n=3)	Normal (n=31) Mon 7(n=3) Other (n=1)
Mutations identified	15	3	5
Mutations in cellular pathways			
Signalling			
KRAS	3	1	0
NRAS	2	1	0
CBL	2	0	0
PTPN11	5	0	0
NF1	4	0	0
RNAsplicing			
SRSF2	2*	0	0
Transcription			
RUNX1	0	0	0
TP53	0	0	2
GATA1	0	1*	0
GATA2	0	1	0
DNA Methylation			
TET2	1*	0	0
Histone Modification			
ASXL1	2*	0	0
EZH2	0	1*	0
Bone Marrow Failure genes			
ELANE	0	0	1
SBDS	0	0	2

Mon 7=Monosomy 7
* additional/secondary mutations

Summary/Conclusions: Targeted NGS together with detailed phenotyping is a useful tool for the diagnosis of suspected MDS and unexplained cytopenias in children, with 40% of patient showing a disease-associated mutations. Results were available within 6-8 weeks in most cases enabling both rapid initial diagnosis and, in some cases, appropriate molecular markers for monitoring of clonal evolution and response to therapy. For the children who remain without a clinical diagnosis, whole genome sequencing (WGS) may identify pathogenic mutations and this is currently underway.

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OUTCOMES IN PATIENTS ALLOCATED TO NO-ASCT BASED ON DEPTH OF RESPONSE: INITIAL RESULTS OF A PHASE 2 TRIAL ASSESSING THE IMPACT OF MINIMAL RESIDUAL DISEASE (MRD) IN PATIENTS WITH DEFERRED ASCT (PADIMAC)

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Background: The role of autologous stem cell transplantation (ASCT) as first line therapy for newly diagnosed (ND) patients with multiple myeloma (MM) remains under evaluation given the deep responses to novel induction regimens. Outcomes for those not proceeding to ASCT following induction remain unclear, likely to be influenced by genetic risk and response depth. This study was designed to evaluate a stratified approach to ASCT, investigating if patients in CR/VGPR to induction may safely be assigned to delayed ASCT.
Aims: This single arm phase 2 clinical trial conducted at 13 UK sites aimed to determine the progression free survival (PFS) for patients who achieved ≥VGPR to induction therapy with no further treatment. Here we report the primary endpoint, PFS at 2 years in the patients not proceeding to ASCT, and the influence of MRD status on PFS.
Methods: NDMM patients eligible for ASCT received PAD (bortezomib 1.3mg/m2 IV or SC days 1, 4, 8, 11; doxorubicin 9mg/m^2 days 1-4, dexamethasone 40mg days 1-4 (and days 8-11 and 15-18 for cycle 1 only)) for 4-6 cycles. Those achieving <PR were off protocol; all others had PBSCH followed by restaging including MRD assessment on bone marrow using multi-parameter flow cytometry. Those in PR were stratified to ASCT (no maintenance) whereas those achieving ≥VGPR stopped treatment. Responses were assessed at 100 days post PBSCH (including MRD), and at monthly intervals for up to 2 years. High risk disease was defined by the presence of one or more adverse FISH lesions (t(4;14), t(14;16), t(14;20), del(17p13), +1q21).
Results: Between April 2011 and January 2014 153 patients were enrolled (median age 55, range 28-71 years), 139 (91%) received 4-6 cycles of PAD. The majority (88.2%) received SC bortezomib, 18 (11.8%) received at least 1 cycle IV. FISH data was available for 132 patients, 89 (67.4%) patients were standard and 43 (32.6%) adverse risk. 51 (33.6%) patients were ISS I, 67 (44.1%) ISS II and 34 (22.4%) ISS III. The overall response rate to PAD was 82.4% (≥VGPR: 41.2%). Responses were similar irrespective of ISS or genetic risk (standard: ≥VGPR 37.5%, PR 40.9%, adverse: ≥VGPR 53.5%, PR 34.9%). Post-PBSCH, 63 (41.2%) patients achieved ≥VGPR, and 44 (28.8%) patients achieved PR of whom 36 proceeded to ASCT. After a median follow-up of 45.4 months from registration, median overall PFS was 22.5m (95% CI: 18.1-25.3). For those who achieved ≥VGPR, median PFS from PBSCH was 8.9m (95% CI: 4.6-13.3) and 25.7m (95% CI: 13.7-37.6) for MRD+ (N=25) and MRD- (N=16) patients at D100 post-PBSCH respectively, 2y-PFS 28.0% (95% CI: 10.4-45.6) and 56.3% (95% CI: 32.0-80.6) respectively. PR patients proceeding to ASCT had a median PFS of 17.2m (95% CI: 14.2-20.2) and 23.1m (95% CI: 16.8-29.4) for those who were MRD+ (N=20) and MRD- (N=7) at D100 respectively, 2y-PFS 15.0% (95% CI: 0-30.7) and 42.9% (95% CI: 6.2-79.6) respectively.
Summary/Conclusions: This is the first study to report outcomes of patients stratified to ASCT by depth of response. The overall PFS for the study is shorter than other published trials, most likely due to the inferior outcome for MRD+ patients not proceeding to ASCT. The median PFS for ≥VGPR patients who are MRD- and stopped therapy was similar to that in PR patients achieving MRD- status post-ASCT. The PFS for ASCT was relatively short, reflecting selection of those only achieving PR. Response rate alone is not sufficient to identify patients who would benefit from ASCT and use of MRD to stratify treatment is now being investigated.

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PROPORTION AND COMPOSITION OF BONE MARROW LYMPHOCYTE POOL AT BASELINE CORRELATES WITH OUTCOME IN MM PATIENTS AFTER RVD AND ASCT

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Background: In multiple myeloma (MM) the interactions between malignant plasma cells and the bone marrow (BM) microenvironment are important for treatment outcome. There is limited data on the effects of lenalidomide (LEN) on the BM immune profile and its therapeutic predictive value. The FMG-MM02 study (NCT01790737) was designed to explore the response to LEN, bortezomib and dexamethasone (RVD) induction, followed by a single autologous stem cell transplantation (ASCT) and LEN maintenance as a first-line therapy for MM patients (n=80). The primary endpoint was achievement of an immunophenotypic remission. Here we report the results of one of the secondary endpoints; composition of lymphocyte subsets at baseline and during LEN maintenance.
Aims: The aim of this study was to assess the proportion of different lymphocyte subsets at baseline and after ASCT and correlate lymphocyte composition with patient outcome.
Methods: Flow cytometry (FC) panel included antibodies against CD38, CD138, CD45, CD19, CD56, CD27, CD28, CD81, CD117, intracytoplasmic kappa and lambda. Sequential analyses (at baseline, after induction and at 3, 9 and 16 months after ASCT) were performed in 37/80 patients who achieved at least near complete remission (nCR) and/or minimal residual disease (MRD) negativity by FC. In addition to MRD the samples of these patients were analyzed for mature B-cells (CD19+/CD45++), B-cell progenitors (CD19+/CD81++/CD38+/CD45+), B-cell blasts (CD19+/CD81++/CD38+/CD45dim), NK/T-cells (CD45++/CD56+), T-cells (CD45++ lymphocytes other than B-cells and T/NK-cells) and CD38+ activated T-cells as calculated per total bone marrow nucleated cells (TNC). The results were compared between two different response cohorts: the good cohort (n=26) defined by persistent stringent complete remission/FC negativity, or PCR negativity (≤ 0.006%) and the control cohort (n=11), who only achieved nCR or had early relapse within one year of ASCT.
Results: At baseline, markers for disease burden, such as the percentage of myeloma cells in BM or paraprotein levels did not differ between the response cohorts. No differences were noted in R-ISS or ISS risk stratification either, but there were more IMWG high risk patients in the control cohort (p=0.048). The median percentage of total lymphocytes (15.4 *vs* 11.9; good *vs* control), T-cells (9.6 *vs* 7.8), CD19+ B-cells (1.6 *vs* 1.3), and CD38+ T-cells (0.9 *vs* 0.5) in BM were all higher in the good response cohort at baseline. In particular, the median proportion of mature B cells in BM was significantly higher in the best cohort at baseline (1.32 *vs* 0.91; p=0.02), after induction (0.27 *vs* 0.13; p=0.002) and 16 months after ASCT (1.73 *vs* 0.56; p=0.008) (*Figure)*.

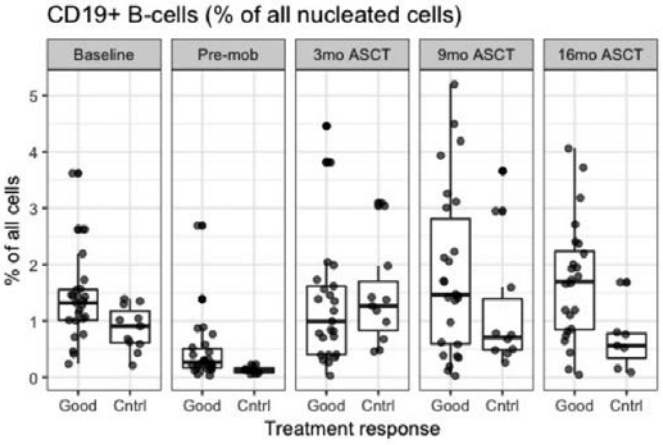

Figure 1.

Summary/Conclusions: Composition of the BM lymphocyte pool at treatment baseline may have an influence on treatment outcome in multiple myeloma. More detailed subtyping of the lymphocyte phenotypes is ongoing and may reveal potential predictive biomarkers for immunomodulatory drugs such as lenalidomide and checkpoint inhibitors.

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VALUE OF THE 18F-FDG PET-CT IN THE IDENTIFYING BONE INVOLVEMENT EITHER AT DIAGNOSIS OR DURING FOLLOW-UP OF PATIENTS AFFECTED BY MULTIPLE MYELOMA

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Background: Lytic lesions occur in the majority of patients with multiple myeloma (MM) and represent one of the criteria for starting therapy. In the past, whole-body X-ray (WBX) represented the method of choice for detecting skeleton abnormalities; today, magnetic resonance imaging (MRI), positron emission tomography (PET) and computed tomography (CT) have been adopted for their higher power in detecting extra-medullary localizations and their higher sensitivity. Nevertheless, which technique would be really the best one is still matter of debate.
Aims: Our single-center retrospective study was designed to compare PET-CT with other imaging techniques (WBX, vertebral column CT and MRI) at the diagnosis and during the follow-up of MM patients. Finally, we assessed a possible predictive/prognostic role of the PET-CT in terms of quality of response and survival.
Methods: We enrolled 160 patients with diagnosed symptomatic (N=149) or smoldering multiple myeloma (N=11) observed at the AOUP, Pisa, Italy, between January 1996 and December 2015. Eighty-three were male and 77 female; the median age was 70 years (range, 28-85), and half of them presented with low ISS risk score. Forty-five subjects were not eligible to high-dose therapy; 64% of them received bortezomib- and 23% melphalan-based regimens. Patients eligible to high-dose therapy received VAD, TAD or VTD and then one (88%) or two (12%) autologous transplants. At the relapse, lenalidomide (57%) or anthracyclines (40%) were administered.
Results: Overall, we compared 160 PET-CT, 233 WBX, 106 CT, and 85 MRI exams. At diagnosis, PET-CT allowed detecting skeletal involvement in 18% of cases negative by WBX, in 37% of those CT-negative, and in 10% of those MRI-negative. Sensitivity of PET-CT was superimposable to that of MRI (90%), and higher than that of WBX (60%) and CT (73%). Nevertheless, the specificity was lower for PET-CT and MRI (40%) in respect of CT (51%) and WBX (71%). Analogously to that observed at diagnosis, PET-CT during follow-up showed distinct advantages in terms of sensitivity compared to X-rays (83% *vs* 60%, respectively). In contrast, PET-CT sensitivity was comparable to that of CT and MRI. As at diagnosis, the specificity was higher for WBX (70%) than for CT, RM and PET-CT (40% for all of these). When PET-CT was correlated to the quality of response, it was significant only in the not transplanted cohort (>PR rate in PET-negative cases=67% *vs* 23% in the PET-positive group; p=0.016). Nevertheless, PET-CT positivity either at diagnosis or during follow-up did not impact on long-term OS and PFS.
Summary/Conclusions: Our study showed that PET-CT and MRI would represent the techniques of choice in the assessment of bone involvement in MM patients in view of their high and comparable sensitivity. Moreover, PET-CT gives the possibility of a "whole body" analysis in exchange for higher "biologic" cost.

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INITIAL PHASE 2 RESULTS OF IBRUTINIB COMBINED WITH BORTEZOMIB/DEXAMETHASONE IN PREVIOUSLY TREATED PATIENTS WITH MULTIPLE MYELOMA

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Background: Bruton's tyrosine kinase (BTK) is overexpressed in, and has been implicated in the growth and survival of multiple myeloma (MM) cells, providing a rationale for evaluating BTK inhibitors in MM (Yang *Cancer Res* 2015; Tai *Blood* 2012). Yang 2015 demonstrated that BTK overexpression (OE) contributes to blunted responses in MM cells when treated with widely used MM drugs (ie, bortezomib [BTZ], etoposide and doxorubicin). Increased activity of the ABC transporter efflux pump and expression of the ABCB1 transporter protein was seen in BTK OE cells, and subsequent inhibition led to a restoration of BTZ sensitivity in these cells. Ibrutinib (ibr), a first-in-class, once-daily oral inhibitor of BTK, is approved in the US and EU for the treatment of various B-cell malignancies. In the EU, regimens containing BTZ and corticosteroids are a standard of care in MM treatment. A previous study of ibr+carfilzomib±dexamethasone (dex) demonstrated an ORR of 58% in relapsed/refractory MM patients (pts) with a median of 3 prior therapies and 88% refractory to their most recent therapy (Chari ASH 2015), warranting further investigation of ibr with proteasome inhibitors.
Aims: To evaluate safety and efficacy of combination ibr+BTZ/dex in previously treated MM pts.
Methods: In this phase 2, open-label, multicenter, European study (PCYC-1139), eligible pts received 1-3 prior therapies and demonstrated disease progression on or following the most recent therapy. Prior BTZ use was permitted provided pts were sensitive (ie, no progression ≤60 days after having achieved minimal response or better). All pts provided informed consent. For cycles 1-8 (21-day cycles), pts received ibr 840mg once daily with BTZ 1.3mg/m^2 subcutaneously twice weekly (Days 1, 4, 8, 11) and dex 20mg on day of and after BTZ. For cycles 9-12 (42-day cycles), BTZ was dosed weekly (Days 1, 8, 22, 29). The primary endpoint was PFS with secondary endpoints including safety, ORR, PFS at landmark points, duration of response, and time to progression (TTP).
Results: As of November 21, 2016, 20 pts were enrolled (Table). Median age was 68.5 years (range, 49-86). Median number of prior therapies was 1, with 50% refractory to the most recent therapy and 70% previously exposed to BTZ. Gene expression profiling (GEP) in initial pts indicated high-risk GEP in 35% of pts. Virtual fluorescent *in situ* hybridization identified 40% of pts with high-risk cytogenetics. Median treatment duration was 2.1 months (range, 0.5-3.7). All pts experienced at least one treatment-emergent adverse event (AE) of any grade. The most common all-grade nonhematologic AEs occurring in >15% (>3 pts) were diarrhea (50%), upper respiratory tract infection (30%), and asthenia, peripheral edema, hypocalcemia and hypokalemia (20% each). The most common Grade ≥3 AEs occurring in ≥10% (>1 pt) were thrombocytopenia (25%), asthenia and pneumonia (15% each), and hyponatremia, abnormal hepatic function, infection, and bone pain (10% each). Three deaths were reported (sudden death in a pt with cardiac history, pneumonia, and myocardial infarction). With early follow-up, 19 pts are evaluable for response with an ORR of 47%, including MR or better in 68%. Updated data will be presented.

Table 1.

Characteristic	Patients (N=20)
Median age, y (range)	68.5 (49-86)
Median time from diagnosis, y (range)	3.1 (1.2, 10.9)
Male, n (%)	10 (50)
ECOG PS 0/1/2, n (%)	11/8/1 (55/40/5)
ISS stage at baseline, n (%)	
I/II/III	6/7/5 (30/35/25)
Disease status to last treatment, n (%)	
Relapsed	9 (45)
Relapsed and refractory*	10 (50)
Unknown	1 (5)
Median prior therapies, n (range)	1 (1-3)
Autologous stem cell transplant, n (%)	11 (55)
Prior BTZ, n (%)	14 (70)
Genomic Profile	
Gene Expression risk score, n	
Low/High	13/7
Molecular subtype, n	
CD1 (Cyclin-D1)	1
CD2 (Cyclin-D2)	5
HY (Hyperdiploidy)	4
LB (Low Bone-Disease)	4
MF (MAF-associated)	0
MS (MMSET)	2
PR (Proliferation)	4

ECOG PS, Eastern Cooperative Oncology Group performance status; FISH, fluorescent in situ hybridization; ISS, International Staging System. *Refractory defined as either no response or progression on or within 60 days of last treatment

Summary/Conclusions: The initial data indicate promising clinical potential for the combination of ibr+BTZ+dex. Treatment was generally well tolerated without any unexpected safety signals noted for the combination. The preliminary ORR of 47% after a minimum 2 treatment cycles is encouraging with further follow-up needed.

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PROGNOSTIC SIGNIFICANCE OF CLONAL CIRCULATING PLASMA CELLS BY MULTI-PARAMETRIC FLOW CYTOMETRY IN PATIENTS WITH LIGHT CHAIN AMYLOIDOSIS UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background: Presence of circulating plasma cells (cPCs) prior to autologous stem cell transplant (ASCT) is an adverse prognostic factor in patients with multiple myeloma (MM). Prognostic value of cPCs prior to ASCT in patients with light chain amyloidosis (AL) is not known.
Aims: The aim of our study was to evaluate if the presence of cPCs by multiparametric flow cytometry (MFC) prior to ASCT is prognostic in patients with AL.
Methods: We retrospectively analyzed 130 patients diagnosed from 2008–2015 with AL who had cPCs analyzed by MFC prior to ASCT, and categorized them as follows: a) Group 1: patients proceeding directly to ASCT without induction therapy and b) Group 2: patients who received induction therapy before ASCT.

Results: There were 78 patients in Group 1 and 52 patients in Group 2. Patients in Group 2 had higher baseline dFLC, bone marrow plasma cells (BMPC), Mayo stage and were more likely to have active MM compared to patients in Group 1. Table 1 lists baseline characteristics of the patients in Groups 1 and 2. Patients in Group 1 had higher rate of renal involvement. cPCs were detectable in 22% (n=28) of patients at the time of ASCT. More patients in Group 1 had detectable cPCs than in Group 2 (31% *vs* 8%; p=0.002), likely due to clearance of cPCs with treatment. Data on cPCs at diagnosis in the induction group was available in 14 patients, of whom 57% (n=8) had detectable cPCs *vs* 31% in the direct ASCT group (p=0.06). 6 of the 8 (75%) patients cleared cPCs with induction therapy. There were no significant differences in patients who had detectable and undetectable cPCs before transplant, including organ involvement, baseline dFLC, BMPC, and Mayo Stage (data not shown).

In Group 2, both progression free survival (PFS) (10.5 months *vs* 58 months, p <0.0001) and overall survival (OS) (16 months *vs* not reached, p <0.0001) were worse in patients who had detectable cPCs compared to those without cPCs (Figure 1). This difference was not seen in Group 1 (OS: not reached *vs* 98 months, p=0.96; PFS 43 *vs* 52 months, p=0.74). In multivariate analysis, adjusting for Mayo Stage and induction chemotherapy, there was a trend towards worse OS in patients with detectable cPCs (p=0.06).

Table 1.

	Induction Group (n=52)	Direct ASCT group (n=78)	P value
Median age (interquartile range [IQR])	60 years (55-67)	61 years (55-67)	0.6
Sex, males	65% (n=34)	64% (n=50)	0.88
Involved light chain (Lambda)	51% (n=26)	72% (n=54)	**0.04**
Median dFLC at diagnosis (IQR)	49 mg/dL (20-166)	13 mg/dL (5-53)	**<0.0001**
Median BMPC (IQR)	20% (10-49)	10% (5-10)	**<0.0001**
Cardiac involvement	49% (n=25)	47% (n=37)	0.86
Renal involvement	50% (n=25)	68% (n=52)	0.05
Liver involvement	8% (n=4)	4% (n=3)	0.3
Mayo Stage - 1	19% (n=8, N=43)	52% (n=40, N=77)	**0.002**
Mayo Stage - 2	49% (n=21)	29% (n=22)	
Mayo Stage - 3	19% (n=8)	7% (n=5)	
Mayo Stage - 4	14% (=6)	13% (n=10)	
Active Myeloma	48% (n=25)	4% (n=3)	**<0.0001**

Figure 1
Patients receiving induction chemotherapy before ASCT

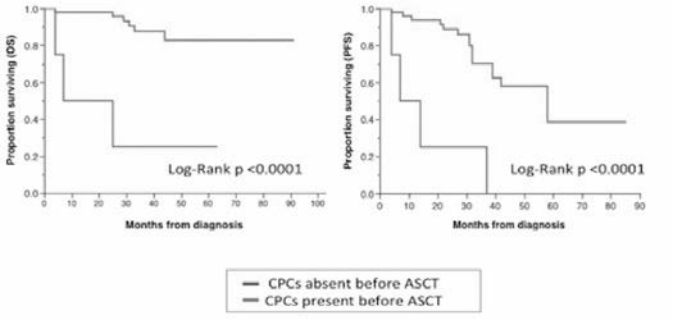

Figure 1.

Summary/Conclusions: cPCs are cleared after induction treatment in majority of AL patients. Patients who have detectable cPCs prior to proceeding to ASCT after induction have worse PFS and OS than patients without cPCs. On the other hand, presence of cPCs was not found be an adverse prognostic factor in patients proceeding directly to ASCT. This may be due otherwise excellent prognosis in this group, with absence of other high-risk features that are seen in patients who require induction. A limitation of our study is lack of data on cPCs at diagnosis in all patients who received induction therapy.

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RENAL IMPAIRMENT IN MYELOMA - PATIENT CHARACTERISTICS, TREATMENT MODALITIES, STEM CELL TRANSPLANT & OUTCOMES FROM THE AUSTRALIAN AND NEW ZEALAND MYELOMA REGISTRY

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Background: Renal impairment (RI) is a poor prognostic factor in multiple myeloma (MM). Analysis of disease characteristics, therapy & outcomes can improve treatment & prognosis.

Aims: To assess (1) characteristics of patients with RI at diagnosis - severity of RI, age, risk factors, high risk features, stage, disease manifestations & performance status, and (2) treatment including induction therapy & autologous stem cell transplant (ASCT) and outcomes.

Methods: Data from newly diagnosed MM patients enrolled in the Australian and New Zealand Myeloma Registry from 1 Feb 2013 to 31 Dec 2016 were analysed.

Results: Of 867 patients, 775 had eGFR available at diagnosis: 34% (267/775) had eGFR <60ml/min (22% at 30-60ml/min; 6% at 15-30ml/min; 6% at <15 ml/min). Mean age of patients with RI (<60 ml/min) was 72 *vs* 64 yrs without RI. Diabetes mellitus (DM), a major cause of chronic kidney disease (CKD), was more prevalent in patients with RI: 17% of patients with eGFR <30 ml/min compared with 8% >30ml/min. Patients with RI (<30ml/min) and DM had a similar response to first-line therapy compared to RI without DM (≥PR, 75% *vs* 82%, p=0.56), with no difference in OS (26 *vs* 37 mths, p=0.68) or PFS (24 mths, p=0.82). High risk features of FISH (del17p, t(4:14), t(14;16), amp1q21, del13q) & high LDH were more prevalent in RI (55% vs 46%, p=0.02), as was advanced stage (ISS III) (66% *vs* 12% p<0.001). Anemia was more prevalent in RI (44% *vs* 14 %, p<0.001), but bone lesions were less prevalent (52% *vs* 65% p=0.001). There was no difference in ECOG performance status. Most patients (87%) received Bortezomib-based therapy in first line (81% RI *vs* 91% no RI, p<0.001), with no difference with or without ASCT. Response rates (≥PR) were the same in patients with eGFR <60ml/min compared with normal renal function (84% *vs* 85%, p=0.87). PFS & OS decreased with reduction in eGFR (Fig 1). However, patients with eGFR<15ml/min had better OS & PFS compared with eGFR 15-30ml/min; dialysis in eGFR <15ml/min may be a factor. Using age 70 yrs as a common age limit for ASCT, we analysed the effect of ASCT in patients <70 yrs with & without RI. While a smaller proportion of RI patients received ASCT (21% *vs* 79%, p<0.07), it was performed at all levels of renal function including eGFR <15ml/min. In patients with eGFR <60ml/min, those who received ASCT had a longer PFS (HR 0.37, 95%CI 0.16-0.88, p=0.03) & OS (HR 0.28, 95%CI 0.08-1.01, p=0.05) compared with no ASCT. The improvement was also seen in severe RI (<30ml/min), with a longer PFS (HR 0.21, 95%CI 0.05-0.86, p=0.03) & OS (HR 0.10, 95%CI 0.01-0.82, p=0.03) with ASCT.

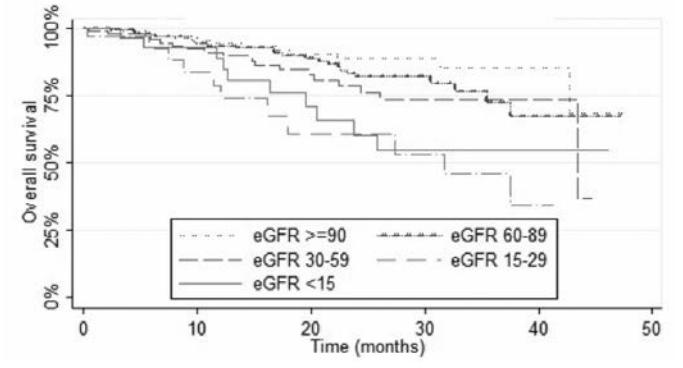

Figure 1.

Summary/Conclusions: RI occurred in one-third of newly diagnosed MM. DM, an underlying risk factor for CKD, was more common in RI patients, but not associated with a difference in outcome. Advanced stage & high risk features were more prevalent in RI patients, but bone disease was less common. RI patients had a shorter PFS and OS, overall correlating with eGFR. However patients with eGFR <15ml/min had a better OS than 15-30 ml/min, for which dialysis may be a factor. In transplant-eligible patients assessed by age <70 yrs, ASCT was performed in 21% of RI patients, at all levels of renal function. Patients with RI who underwent ASCT had a superior PFS and OS than those who did not have ASCT, including those with severe RI (eGFR <30ml/min), supporting the benefit of ASCT in MM patients with RI.

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VENETOCLAX AS TARGETED THERAPY FOR RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background: Venetoclax (VEN), an orally available selective small-molecule

BCL-2 inhibitor, induces cell death in multiple myeloma (MM) cells, particularly those with the t(11;14) translocation.
Aims: The objectives of the study are to evaluate safety, PK, recommended phase two dose, and preliminary efficacy of VEN monotherapy in relapsed/refractory (R/R) MM.
Methods: Patients (pts) with relapsed/refractory (R/R) MM received VEN monotherapy in this phase 1 study. Daily VEN was given at 300–1200mg in dose escalation cohorts and 1200mg in the safety expansion. Pts with disease progression (PD) on VEN monotherapy could receive VEN plus dexamethasone and remain on study.
Results: As of 19Aug2016, 66 pts were enrolled. Median age was 63 years and 30 (46%) pts had t(11;14). Median number of prior therapies was 5 (range: 1–15); 46 (70%) pts were refractory to bortezomib, 20 (30%) to carfilzomib, 51 (77%) to lenalidomide, 35 (53%) to pomalidomide, and 52 (79%) were refractory to the last prior therapy. Median time on VEN monotherapy was 2.5 months (range: 0.2–23); 17 pts received VEN plus dexamethasone after PD for a median of 1.4 months (range: 0.1-13). Fifty-five (83%) pts discontinued, with 41 due to PD. Common adverse events (AEs) were nausea (47%), diarrhea (36%), vomiting (21%) and grade 3/4 hematologic toxicities [thrombocytopenia (32%), neutropenia (27%), anemia (23%), leukopenia (23%)]. Common serious AEs were pneumonia (8%), sepsis (5%), cough, hypotension, pain, and pyrexia (3% each). There were no events of TLS. Six deaths were reported due to PD, and 1 each due to lung disorder and brain hemorrhage following trauma. Overall response rate (ORR) for all pts on VEN monotherapy was 21% (14/66); 10 (15%) achieved very good partial response (VGPR) or better [2 stringent complete response (sCR), 3 CR, 5 VGPR]. For all pts, median time to progression (TTP) and duration of response (DoR) were 2.6 and 9.7 months, respectively. A clear difference in responses was seen among pts with t(11;14) *vs* without [ORR, 40% *vs* 6%; ≥VGPR, 27% *vs* 6%]. For pts with t(11;14), median TTP was 6.6 months [vs 1.9 months for pts without t(11;14)] and median DoR was 9.7 months. A high *BCL2:BCL2L1* (BCL-X_L) gene expression ratio was observed in 10/44 (23%) baseline tumor samples, enriched in pts with t(11;14) compared with non-t(11;14) (38% *vs* 5%) and associated with clinical response; 80% (8/10) of pts [all t(11;14)] with a high *BCL2:BCL2L1* ratio achieved ≥PR with a median TTP of 11.5 months. Among pts with t(11;14) who were refractory to the last therapy, ORR was 42% (11/26); for t(11;14) pts refractory to both bortezomib and lenalidomide, or who were refractory to bortezomib, carfilzomib, lenalidomide, and pomalidomide, ORR was 40% (8/20) and 50% (3/6), respectively. No difference was seen in ORR for t(11;14) pts with high-risk del(17p) *versus* those without the deletion [40% (2/5) *vs* 40% (10/25)].
Summary/Conclusions: VEN has an acceptable safety profile with promising single-agent anti-myeloma activity in pts with R/R MM positive for t(11;14) who failed multiple prior lines of therapy.

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AN OPEN-LABEL, PHASE 1B STUDY (MMY1001) OF DARATUMUMAB COMBINED WITH CARFILZOMIB, LENALIDOMIDE, AND DEXAMETHASONE (KRD) IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA (MM)

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Background: The combination of daratumumab with standard of care regimens has demonstrated significantly prolonged progression-free survival (PFS), deeper responses, and a manageable safety profile *versus* standard of care alone in patients with relapsed or refractory MM.
Aims: To determine the tolerability and efficacy of daratumumab in combination with KRd in patients with newly diagnosed MM.
Methods: This open-label, phase 1b study enrolled patients with newly diagnosed MM regardless of transplantation eligibility. Patients received daratumumab 16mg/kg qw for Cycles 1-2, q2w for Cycles 3-6, and q4w thereafter, with all patients receiving the first dose split over 2 days. Carfilzomib was given on Days 1, 8, and 15 of each 28-day cycle (20mg/m^2 on Cycle 1 Day 1, 36 or 70mg/m^2 thereafter, based on tolerability of first dose) for up to 13 cycles or elective discontinuation for autologous stem cell transplantation (ASCT). Lenalidomide 25mg was administered on Days 1-21, and dexamethasone 20-40mg per week. The primary endpoint of the study was tolerability. Overall response rate (ORR; defined as partial response or better) was a major secondary endpoint.
Results: A total of 22 patients were enrolled in the study; median (range) patient age was 60 (34-74) years, and 95% had an ECOG score of ≤1. At a median (range) follow-up duration of 7.4 (4.0-9.3) months, the majority of patients remained on treatment and had received a median of 8 (1-10) treatment cycles. Six patients discontinued treatment, 1 due to an adverse event (AE; pulmonary embolism), 1 due to progressive disease, and 4 due to "other" (all ASCT). The dose of carfilzomib was increased to 70mg/m^2 in 19 patients by Cycle 1 Day 15. Serious treatment-emergent AEs (TEAEs) occurred in 46% of patients, 14% and 18% of which were at least possibly related to daratumumab or carfilzomib, respectively. Eighteen (82%) patients had a grade 3/4 TEAE, most commonly (>10%) lymphopenia (50%) and neutropenia (23%). One (5%) patient experienced a grade 3 cardiac TEAE (congestive heart failure) which resolved; the patient was able to resume study treatment at a reduced carfilzomib dose. There were no grade 5 TEAEs. Six (27%) patients had daratumumab-associated infusion-related reactions, and all were grade ≤2 in severity. ORR with daratumumab in combination with KRd was 100% among the 21 response-evaluable patients: 18 (86%) patients achieved a very good partial response or better and 1 patient (5%) achieved a complete response. Median duration of response has not been reached. With only 1 PFS event having occurred at the time of follow-up, 6-month PFS rate was 100%, and median PFS has not been reached.
Summary/Conclusions: Daratumumab in combination with KRd was well tolerated, with an overall safety profile consistent with previous reports for KRd. No additional toxicity occurred with the addition of daratumumab, and deep and durable responses were achieved. Preliminary results from this ongoing study support continued investigation of daratumumab plus KRd as a first-line treatment regimen for patients with newly diagnosed MM. Updated data based on longer follow-up will be presented.

P677

GENE EXPRESSION CLASSIFIER EMC92/SKY92 AND REVISED ISS ROBUSTLY IDENTIFY HIGH-RISK MULTIPLE MYELOMA IN ELDERLY PATIENTS OF THE HOVON-87/NMSG-18 STUDY

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Background: Multiple myeloma (MM) affects mostly elderly people with a median age of 69 years at diagnosis, with 35-40% of patients older than 75. Overall survival (OS) is variable: of patients aged 66-79, 9% survive less than 3 months and 23% survive longer than 10 years.
Recently the revised ISS (rISS) has been proposed as a prognostic marker that incorporates ISS, FISH and LDH. Another marker, the SKY92 prognostic gene classifier (published as the EMC92 classifier) was developed in younger, transplant eligible multiple myeloma (MM) patients who were included in the HOVON-65/GMMG-HD4 trial. The SKY92 classifier was thoroughly validated in eight independent cohorts, at the time of its initial publication, and since.
Aims: Here, we validated the SKY92 gene expression classifier and rISS in elderly, non-transplant eligible patients included in the HOVON-87/NMSG-18 trial (Zweegman *et al.* Blood 2016;127(9):1109-1116).
Methods: In this trial, melphalan, prednisone, thalidomide (MPT) plus thalidomide maintenance was compared with melphalan, prednisone, lenalidomide (MPR) plus lenalidomide maintenance. The MMprofiler™ CE IVD assay was used to obtain SKY92 scores, classifying a patient as high-risk or standard-risk. In addition, the international staging system, LDH, FISH and rISS were analyzed.
Results: The 178 patients in the analysis for which enough bone marrow was available to perform GEP, had a median age of 73 years. At the time of data analysis, the median follow up was 34 months. The SKY92 classifier identified 25 of 178 patients as high-risk (14%). The median OS for the 25 patients classified as SKY92 high-risk was shorter than the median OS of standard-risk patients: SKY92 high-risk 21 months *versus* SKY92 standard-risk 53 months (hazard ratio (HR)=3.0, 95% confidence interval (CI)=1.7-5.3; p <.001; Figure 1a). The proportion of patients with stage rISS-III was 8% which is comparable to the 10% identified in the initial report of the rISS. Interestingly, the proportion of SKY92 high-risk patients is larger (14%), whereas the median OS associated with these patients is shorter (21 *vs* 25 months). The SKY92 classifier performed better compared to the rISS as high-risk marker for OS. The 2-year OS rate was 48% in the SKY92 high-risk patients *versus* 58% for rISS-III. (Figure 1). The 2-year progression free survival (PFS) rate was similar for SKY92 high-risk and rISS-III (16% and 17%, respectively). In the multivariate analysis, SKY92, rISS and deletion of 13q were independently associated with OS. Inde-

pendent association with PFS was found for SKY92, rISS, deletion of 13q and t(11;14) (Table 1). A bidirectional stepwise selection procedure was applied excluding covariates with the highest p-values until only significant covariates remain. Initially included covariates were SKY92, r-ISS, gain(1q), del(17p), del(13q), t(4;14), t(11;14), LDH and age. ISS could not be included as it was highly correlated with other covariates (i.e. co-linear); t(14;16) was excluded due to the minimal number of present instances. HR: hazard ratios relative to the lowest risk category with 95% confidence intervals (CI), p: likelihood ratio p-value indicating the association of each covariate with OS.

Table 1. Multivariate survival analysis in the HO87/NM18 trial.

		High-risk	Low-risk	HR [95%CI]	p
SKY92		17	122	2.9 [1.4-5.9]	$8.6x10^{-3}$
rISS	I	20	119	1.0	$7.8x10^{-3}$
	II	107	32	1.7 [0.67-4.4]	
	III	12	127	5.5 [1.8-17.0]	
del(13q)		67	72	1.9 [1.1-3.4]	$2.4x10^{-2}$
n = 139; number of events = 53; 4 degrees of freedom					$2.5x10^{-5}$

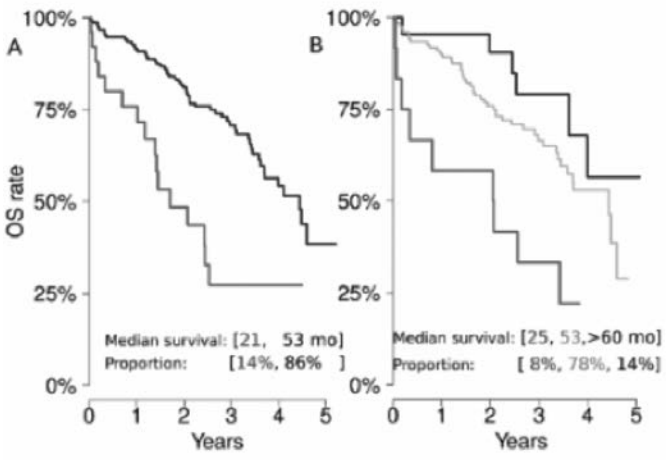

Figure 1. Survival curves for A) the SKY92 classifier and B) the revised ISS. In blue the lowest risk patients, in red the highest risk patients and orange indicating intermediate risk.

Figure 1.

Summary/Conclusions: Here, we compared the SKY92 classifier with revised ISS staging and FISH. These data validate the SKY92 classifier as a robust marker to identify high-risk patients in non-transplant eligible MM patients. In these IMiD treated patients, the SKY92, the revised ISS, and FISH markers such as deletion of 13q retain independent prognostic value.

P678

MULTIPLE MYELOMA AND COMORBIDITY: A POPULATION-BASED STUDY

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Background: The number of multiple myeloma patients has grown with aging populations, and with increasing age the number of comorbidities increases as well. Clinically, it is well known that comorbidity in multiple myeloma patients decreases performance status, increases risk of therapy-related complications and may lead to life-threatening conditions. Currently, the literature on comorbidity in multiple myeloma is very limited and based on small case series. Clinical trials rarely include elderly, frail patients due to eligibility criteria. Population-based studies provide valuable information on survival outcomes in relation to presence/absence of comorbidities in newly diagnosed real-life multiple myeloma patients in the general population.
Aims: To evaluate the prevalence of comorbidities and to study the impact of comorbidities on survival among patients with newly diagnosed multiple myeloma.
Methods: All newly diagnosed patients with multiple myeloma from January 1st 1990 to December 31st 2013 in Sweden were included in the study. Using the Swedish Patient Registry, all discharge diagnosis and discharge listings were gathered from each patient from January 1st 1985. Comorbid conditions were defined as chronic illnesses which demand life-long treatment or follow-up. Only those diagnoses made prior to multiple myeloma were used. Using ICD 8, 9 and 10 codes, comorbid diseases were identified. Kaplan-Meier curves were used to estimate survival. Risk of death was compared among multiple myeloma patients with a comorbid condition to those without a comorbidity, using Cox's proportional hazards regression (adjusting for age, gender, year of diagnosis, and other comorbid conditions).
Results: A total of 13,718 patients with multiple myeloma were included in the study and 21 groups of comorbidities were identified. The most common diseases were cancer, hypertension, heart failure, ischemic heart disease and atrial fibrillation. Among all patients, 55% had no prior history of comorbidity, 23% had one comorbidity, 12% had two comorbidities, and 10% had three or more comorbid conditions. Survival was negatively influenced by the number of comorbidities (Figure 1). The risk of death was significantly increased in patients with atrial fibrillation (HR=1.08; 95% CI 1.00-1.16), heart failure (HR=1.50; 95% CI 1.40-1.61), stroke (HR=1.20; 95% CI 1.11-1.30), psychological disease (HR=1.27; 95% CI 1.16-1.39), chronic lung disease (HR=1.22; 95% CI 1.12-1.32), diabetes (HR=1.14; 95% CI 1.04-1.36), peripheral vascular disease (HR=1.26; 95% CI 1.12-1.42), cancer (HR=1.10; 95% CI 1.04-1.16), dementia (HR=1.65; 95% CI 1.38-1.99), paralysis (HR=1.44; 95% CI 1.15-1.80), inflammatory bowel disease (HR=1.38; 95% CI 1.08-1.74), end stage renal disease (HR=1.57; 95% CI 1.03-2.04), and cirrhosis (HR=1.64; 95% CI 1.10-2.43).

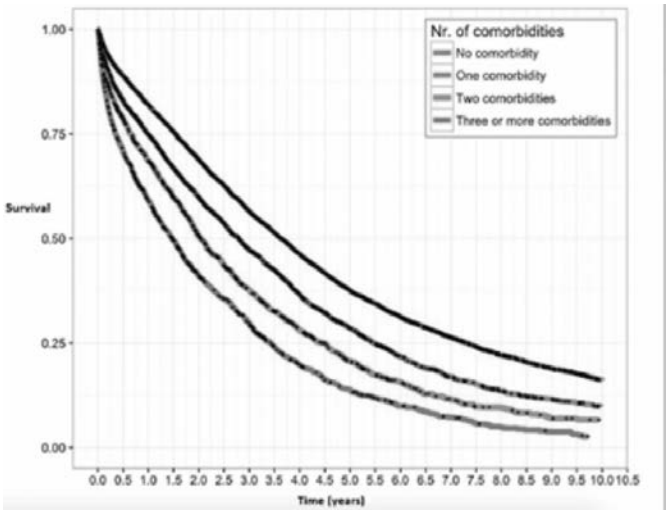

Figure 1.

Summary/Conclusions: In this large, population-based study including almost 14,000 patients, we have shown that comorbidities are common among newly diagnosed multiple myeloma patients and that comorbidities are associated with an inferior survival. Importantly, the number of comorbidities showed a dose-response relationship with inferior overall survival. For example, the median overall survival for patients with 3 or more comorbidities was reduced by more than 50% compared to patients without comorbidities. The importance of comorbidities should be taken into account when evaluating patients and deciding on treatment strategies for individuals with multiple myeloma.

Myeloma and other monoclonal gammopathies - Clinical 4

P679

DETECTION OF NEW EMERGING CLONES DURING TREATMENT BY NGS ALLOWS A BETTER RISK PREDICTION ON MULTIPLE MYELOMA PATIENTS

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Background: Multiple myeloma (MM) is a genetically complex disease, characterized by the presence of multiple clones with differing degrees of drug sensitivity at the time of diagnosis. Consequently, therapeutic response of MM patients is unpredictable and extremely variable, and although the treatments introduced over the last decade have significantly improved the outcome of these patients, most patients eventually relapse. Deep sequencing methods have contributed to increase the knowledge about the clonal heterogeneity of the disease and helped to stablish the three evolution patterns at relapse: linear and branching clonal evolution, and no clonal changes.
Aims: To analyze the diversity and relative dominance of different clones and their evolution throughout the course of disease by NGS of the immunoglobulin repertoire in MM patients. To evaluate if the presence of different clones is associated with increased risk.
Methods: Immunoglobulin repertoire was analyzed by NGS in bone marrow samples from 180 MM patients included in three GEM clinical trials (NCT00461747, NCT00443235 and NCT01237249). The two first clinical trials involve patients younger than 65 years old, and were analyzed with ClonoSeq methodology, the later one involve patients older than 65 years old, and were analyzed with a local NGS method recently validated (Martinez-López et al, Laukemia 2017). A clonotype was identified when at least 400 identical sequencing reads were obtained, or it is present at a frequency of >1%.
Results: Of the 180 MM patients studied, 57 (32%) shows the presence of more than one clone throughout the clinical the course of the disease. The identification of new evolving clones was only possible in the GEM10 clinical trial with the Local NGS method; in this clinical essay, 6% (4/71) of patients shows the development of different clones during treatment. We show that the frequency of the predominant clone at diagnosis of these four patients decreased with treatment, but the frequency of the new ones increased and the patients progressed. When more than one clone is present at diagnosis, the relative dominance of the clones varies throughout the course of disease in an independent manner. There were no differences in median MRD values between patients with one clone or more than one clone (0.0082% and 0.0055% respectively). The presence of more than one clone was not associated with high-risk cytogenetics. The presence of more than one clone at diagnosis does not condition the prognosis in any of the patients and treatments analyzed. Median PFS was 38 and 58 months for patients with one clone or more one clone, respectively (HR=1.136, p= 0.563). Median OS was not reached for patients with one clone, and was 81 months for patients with more than one clone (HR=1.43, p=0.28).
Summary/Conclusions: The analysis of the IG repertoire by the local NGS method during treatment is able to identify and quantify new emerging clones during the treatment that were not detectable at diagnosis. The new clones contributed to increase the MRD levels in the follow-up samples.The presence of of different clones at diagnosis is not associated with higher risk of progression, high risk cytogenetics or higher MRD values.

P680

FINAL RESULTS OF PHASE (PH) 1/2 STUDY OF CARFILZOMIB, POMALIDOMIDE, AND DEXAMETHASONE (KPD) IN PATIENTS (PTS) WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM): A MULTI-CENTER MMRC STUDY

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Background: In the era of increased use of 1st-line and maintenance lenalidomide (LEN), there is growing need for effective ≥2nd-line therapies (tx) for LEN-refractory pts. The combination of carfilzomib (CFZ), pomalidomide (POM), and dexamethasone (DEX) has shown promising activity in advanced RRMM, including for pts refractory to LEN (Shah *et al. Blood*. 2015).
Aims: In this Ph 1/2 study, KPd was evaluated in RRMM, with a focus on pts who are LEN-refractory but proteasome inhibitor (PI)-naïve/sensitive.
Methods: LEN-refractory disease was required for 2nd-line KPd and LEN-refractory/exposure for ≥3rd-line. Ph 1 dose escalation to determine maximum tolerated dose (MTD) followed the TITE-CRM design with 40 pts receiving 3–4mg POM PO (days [d] 1–21), 20–36mg/m^2 CFZ (IV d1, 2, 8, 9, 15, 16 in cycles [C] 1–8, and d1, 2, 15, 16 in C9+), and 40/20mg DEX PO weekly (C1–4/5–8; for all dose levels [DLs]) in 28-day cycles. CFZ was started at 20mg/m^2 for d1, 2 of C1 for all DLs. Per design, 30 PI-naïve/sensitive pts were to be enrolled at MTD across Ph 1/2, with partial response (PR) rate after C4 as 1º efficacy endpoint.
Results: As of 12/1/16, 65 pts have been enrolled, with efficacy and safety data available for 64 pts: 4 at DL1 (20mg/m^2 CFZ, 3mg POM), 29 at DL2 (20mg/m^2 CFZ, 4mg POM), 32 at DL3 (20/27mg/m^2 CFZ, 4mg POM), 0 at DL4 (20/36mg/m^2 CFZ, 4mg POM). Median age was 63 y, median time from diagnosis 5.1 y, median prior tx lines 2, and 94% had refractory disease. Cytogenetic data were available for 59 pts; 33% were high risk per IMWG. There were 9 dose-limiting toxicities, all asymptomatic cytopenias: 8 pts with grade (G) 3 neutropenia and 1 with G4 thrombocytopenia. The MTD was established at DL3. In 64 pts, G3/4 hematologic toxicities included neutropenia (25%) and lymphopenia (14%), and non-hematologic toxicities (all grades) included fatigue (51%), dyspnea (42%), and gastrointestinal (45%). PRs were rapid with a ≥PR rate of 63% after 1 cycle and 77% after 4 cycles. After a median of 20.9 cycles (range, 1–49), ≥minimal response was 95%, ≥PR 84%, ≥very good PR 52%, and ≥near complete response (nCR) 20%. In the 1º population (N=55, 51 LEN-refractory, 28 progressing on LEN maintenance), ≥PR was 84% with 30 treated at MTD. After median follow-up of 21 (1–49) mo, median progression-free survival (PFS) for all 64 pts enrolled was 16.8 mo and 2-y overall survival (OS) was 76.8% with 20 pts remaining on treatment. For standard-risk (n=38) *vs* high-risk pts (n=21), ≥PR was 89% *vs* 81%, ≥nCR was 24% *vs* 10%, median PFS was 22.3 *vs* 10.6 mo, and 2-y OS was 90.8% *vs* 56.0%.
Summary/Conclusions: KPd is well tolerated and highly active (≥PR 84%) with encouraging PFS (median 16.8 mo) in an RRMM pt population that was mostly LEN-refractory and PI-naïve/sensitive. The results support planned evaluation of KPd with daratumumab in RRMM, particularly for high-risk pts.

P681

PANOBINOSTAT INDUCES CD38 UPREGULATION AND AUGMENTS THE ANTI-MYELOMA EFFICACY OF DARATUMUMAB

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Background: Immunotherapy with the anti-CD38 monoclonal antibody (mAb) daratumumab is increasingly being utilized in myeloma patients with relapsed/refractory (R/R) disease after prior treatment with immunomodulatory drugs (IMiDs) and proteasome inhibitors (PIs). However, the efficacy of daratumumab is limited by low CD38 expression on myeloma cells. Here, we investigate the use of the histone deacetylase inhibitor (HDACi) panobinostat to modulate target antigen expression on myeloma in favor of potent mAb-mediated recognition and destruction. We show that panobinostat augments CD38 expression specifically on myeloma cells and demonstrate powerful synergy with anti-CD38 mAb daratumumab.
Aims: Determine the impact of panobinostat on upregulation of CD38 expression on myeloma cells in order to enhance the efficacy of daratumumab.
Methods: Myeloma cells were treated with titrated doses of panobinostat (0, 10, 25 nM) and expression of CD38 and a panel of additional target molecules including SLAMF7, as well as accessory ligands analyzed by flow cytometry at 24, 48 and 72 hours. Antibody-dependent cellular cytotoxicity (ADCC) against panobinostat treated and untreated myeloma cells was analyzed at 4 and 20 hours after addition of PBMC at an effector to target ratio of 25:1 in the presence of daratumumab or an isotype control antibody.
Results: We treated primary myeloma (n=12 patients) with panobinostat (10 *vs* 25 nM) and observed a uniform increase in CD38 expression in each case by flow cytometry. Upregulation of CD38 was already detectable after 24 hours, peaked after 48 hours of exposure to panobinostat and was higher at the 25 nM compared to the 10 nM dose. At 48 hours, the mean fluorescence intensity (MFI) for CD38 expression was 4 (10nM) and 6-fold (25 nM) higher in panobinostat-treated compared to untreated myeloma ($p<0.01$). The increase in CD38 was equal in patients with previously untreated (n=5) and R/R myeloma (n=7); and could be confirmed in a panel of myeloma cell lines, including MM1.S and OPM-2. The panobinostat-induced upregulation of CD38 was rapidly reversible after drug withdrawal. Further, the increase in CD38 expression after panobinostat treatment was specific for myeloma and neither observed this phenomenon in a panel of leukemia and lymphoma cell lines, nor on primary CD8+ and CD4+ T cells that we isolated from peripheral blood of several donors

(n=3). Interestingly, expression of SLAMF7 was not increased after panobinostat treatment at all tested concentrations and time points in both cell lines and primary myeloma. Next, we were interested in determining whether the increase in CD38 expression enabled superior antimyeloma activity of the anti-CD38 mAb daratumumab. Thus, we treated primary myeloma cells from patients (n=4) with panobinostat for 48 hours at 10 nM, as this is the serum level achievable with currently approved dosing regimens. We observed a significant increase in ADCC against panobinostat-treated compared to untreated myeloma in all patients. On average, 78% of panobinostat-treated primary myeloma cells were eliminated by daratumumab within the 4-hour ADCC assay, whereas only 51% myeloma cells were eliminated without panobinostat treatment ($p<0.01$). The synergistic anti-myeloma efficacy of panobinostat and daratumumab was confirmed with a panel of myeloma cell lines.

Summary/Conclusions: Our data demonstrate that the HDACi panobinostat induces upregulation of CD38 on myeloma and a subsequent dramatic increase of daratumumab-mediated ADCC. These data suggest that panobinostat could be used synergistically with daratumumab in a clinical setting to increase response rates and extend duration of responses to daratumumab.

P682

BCL2 EXPRESSION IS A POTENTIAL PREDICTIVE BIOMARKER OF RESPONSE TO VENETOCLAX IN COMBINATION WITH BORTEZOMIB AND DEXAMETHASONE IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background: The anti-apoptotic proteins BCL-2 and MCL-1 have been shown to promote multiple myeloma (MM) cell survival. Venetoclax (VEN) is a potent, selective, and orally bioavailable small-molecule inhibitor of BCL-2. Bortezomib (BTZ) is a proteasome inhibitor that can inhibit MCL-1 activity by increasing the MCL-1 antagonist, NOXA.
Aims: Results presented herein describe correlative biomarker analyses in the ongoing phase 1b study of VEN in combination with BTZ and dexamethasone in patients with relapsed/refractory (R/R) MM (NCT01794507).
Methods: As of 19 Aug 2016, 66 patients were enrolled on study. Baseline bone marrow aspirate samples were available from 52 patients, of which 45 were evaluable for BCL-2 family gene expression by droplet digital PCR in CD138-selected tumor cells. Correlation between *BCL2* (BCL-2), *BCL2L1* (BCL-XL) and *MCL1* (MCL-1) mRNA expression (log2-transformed copies/ul normalized to housekeeping gene) and preliminary efficacy [overall response rate (ORR), time to disease progression (TTP) and duration of response (DoR)] were examined by Log-rank and Wilcoxon tests for binary biomarkers, and by risk ratio from Cox proportional hazard model for continuous biomarkers.

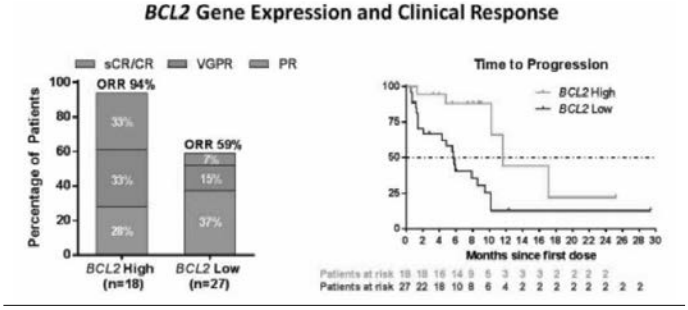

Figure 1.

Results: The ORR was 68% (44/65) for all evaluable patients and 89% (31/35) in patients who had 1-3 prior therapies (31/35). A broad range of *BCL2, BCL2L1* and *MCL1* expression was observed, however higher *BCL2* levels were detected in patients who achieved a partial response (PR) or better (median: 3.01 *vs* 0.87, $p<0.01$). Additionally, higher *BCL2* levels were observed in patients who had 1–3 prior lines of therapy compared to 4 or more lines of therapy (median: 3.03 *vs* 0.94, $p<0.01$). In contrast, no association was observed between *BCL2L1* or *MCL1* gene expression and response or number of prior therapies. Bootstrapping and aggregating thresholds from trees was used to estimate a threshold value for *BCL2* expression that would provide optimum selection of patients likely to have a response. Overall, seventeen of 18 patients with high *BCL2* expression *(≥3.0)* achieved at least a PR (ORR 94%), with 12 patients (66%) achieving VGPR or better (**Figure 1**). Sixteen of 27 patients with low *BCL2* expression achieved at least a PR (ORR 59%), with 6 patients (22%) achieving a VGPR or better. Median TTP (11.6 *vs* 5.7 months) and DoR (10.2 *vs* 7 months) were longer for patients with high *versus* low *BCL2* expression. Responses in high *BCL2* expressors were independent of cytogenetic status as determined by interphase FISH analysis, including t(11;14), t(4;14), del(13q) and del(17p).

Summary/Conclusions: Targeting BCL-2 and MCL-1 with the combination of VEN, BTZ and dexamethasone provides a unique approach for MM treatment. Efficacy results in tumors expressing high *BCL2* levels, including 94% ORR, provide supportive evidence for the evaluation of this combination regimen in the ongoing phase 3 study (NCT02755597) in R/R MM.

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THE IMPACT OF THE INTRODUCTION OF BORTEZOMIB ON DIALYSIS INDEPENDENCE IN MULTIPLE MYELOMA PATIENTS WITH RENAL FAILURE: A NATIONWIDE DUTCH POPULATION-BASED STUDY

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Background: Renal insufficiency is common at presentation in patients with multiple myeloma (MM) and associated with a poor survival. Approximately 10% of the patients require dialysis. Studies have shown that the novel agent bortezomib has a positive effect on recovery of renal function in MM patients with renal insufficiency.
Aims: The aim of this study is to determine the effect of the revised guideline, including the introduction of bortezomib as first line treatment in MM patients with dialysis dependence, on renal function recovery.
Methods: All patients on renal replacement therapy (RRT) in the Netherlands are registered in the Dutch registry Renine. Data on age, gender, start date of RRT, type and switches of RRT or hospitals, primary renal diagnosis, date of death and cause of death are collected. In this nationwide population-based study, we selected all patients with MM registered in Renine between January 2002 and January 2016. No information regarding therapy of MM is provided in Renine. In March 2010, bortezomib was advised as first-line treatment in patients suffering from MM with renal impairment in the Dutch guidelines. Therefore, we divided our cohort in two periods: before the bortezomib guideline (January 1, 2002 till March 29, 2010) and after introduction of the bortezomib guideline (March 29, 2010 till January 1, 2016). Kaplan-Meier and Cox proportional hazards modelling were used to identify significant indicators for dialysis independency.
Results: A total of 700 patients were included in the study (422 patients pre-bortezomib and 278 after bortezomib introduction). In the period after the introduction of bortezomib 15% of patients became dialysis independent compared to 8% in the pre-bortezomib period ($HR_{adj.}$=2.1 (95% CI 1.3–3.3), Fig. 1). In addition, patients who started dialysis in the period after bortezomib was introduced became dialysis independent more rapidly than in the pre-bortezomib period (1.2 compared to 1.7 years; $p < 0.001$). Age < 75 years (vs. ≥ 75 years) and light chain deposition disease (LCDD) as the primary renal disease (vs. amyloidosis) were significantly associated with achieving dialysis independence ($HR_{adj.}$=2.1 (95% CI 1.0–4.2) and $HR_{adj.}$=5.7 (95% CI 2.5–13.2), respectively).

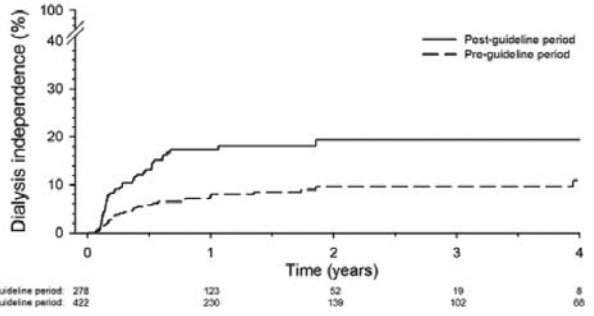

Figure 1.

Summary/Conclusions: In this nationwide population-based study, covering all patients with MM and concomitant renal failure, almost a two-fold increase of patients becoming dialysis independent occurred in the period after the introduction of bortezomib compared to the pre-bortezomib period. This was even more prominent when age was < 75 years and LCDD was the primary renal disease.

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TREATMENT WITH POMALIDOMIDE AND DEXAMETHASONE IN PATIENTS WITH MULTIPLE MYELOMA AND LIGHT CHAIN (AL) AMYLOIDOSIS

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Background: The immunomodulatory agent pomalidomide is active in patients with relapsed/refractory multiple myeloma, including those who failed prior lenalidomide and bortezomib. Phase II clinical trials showed that pomalidomide is also effective in primary AL amyloidosis. After this drug was marketed for multiple myeloma (in Italy since September 2015), it became routinely accessible also to patients with myeloma-associated AL amyloidosis, a particularly fragile population.
Aims: Aim of this study is to report the efficacy of pomalidomide and dexamethasone in patients with multiple myeloma-associated AL amyloidosis.
Methods: The databases of the Pavia Amyloid Research and Treatment Center was searched for patients with a diagnosis of multiple myeloma and AL amyloidosis treated with pomalidomide and dexamethasone (PDex). The patients received 28-day cycles of pomalidomide (4mg from day 1 to 21) and dexamethasone (20/40mg weekly). All patients gave written informed consent for their clinical data to be used for research purposes, in accordance with the Declaration of Helsinki. Thirty patients were treated to date. Hematologic and organ response were assessed according to the International Society of Amyloidosis criteria.
Results: Median age was 65 years (range: 34-85 years) and 22 (73%) patients were man. Heart involvement was present in 13 patients (43%) and kidney involvement in 18 (60%). Four (13%) patients were in Mayo Stage I, 17 (57%) in stage II and 9 (30%) in stage III. Fifteen (50%) subject were in renal stage I, 8 (27%) and 3 (23%) were in renal stage II and III respectively and 5 (16%) patients were on dialysis at the time of PDex initiation. Median bone marrow plasma cell infiltrate was 20% (range: 12-90%). Twenty-three (76%) patients were refractory to all previous lines of therapy. Median time from diagnosis to treatment initiation was 71 months [interquartile range (IQR): 25-120 months]. Adverse events were observed in 5 (17%) of subjects: skin rash and confusion in one patient each and mild increase in serum creatinine in 3 (10%, resolved with the decrease of the dose of pomalidomide). The median number of prior treatments was 3 (range: 2-5 cycles). All patients previously received lenalidomide and an alkylating agent, only 3 patients were not exposed to bortezomib, due to severe peripheral nervous system involvement, 10 (33%) underwent autologous stem cell transplant and 9 (30%) received previous thalidomide-based regimens. The median number of PDex cycles performed was 4 (range: 1-11). Median follow-up of living patients was 8 months (IQR: 3.5-16 months) and 13 (43%) patients died due to progressive disease. Fourteen patients (47%) achieved at least partial response, with 1 complete remission (CR), and very good partial responses (VGPR) in 2 cases (6%). Cardiac responses were observed in 1 of 5 patients with measurable NT-proBNP (20%), but this can be underestimated due to the pomalidomide-related increase of NT-proBNP, and renal response in 3 of the 11 evaluable patients (27%).
Summary/Conclusions: The combination of pomalidomide and dexamethasone is well tolerated and effective in multiple myeloma-associated AL amyloidosis and can be a valuable rescue option in this high-risk population.

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MYOCARDIAL UPTAKE OF 99MTC-DPD IN PATIENTS WITH AL AMYLOIDOSIS

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Background: AL amyloidosis is a free light chain (FLC) deposition disease that can affect the heart. To identify the specific subtype is essential for treatment and prognosis.^{99m}Tc-DPD scintigraphy (SC) has shown high sensitivity and specificity for detecting TTR cardiac amyloidosis, although some cases have been described with AL amyloidosis and DPD uptake. An intense biventricular uptake (Perugini score 2-3 (PS)) is highly suggestive of TTR amyloidosis.
Aims: Our objective is to describe the characteristics of patients with AL amyloidosis and myocardial DPD uptake.
Methods: We reviewed all SC performed at our center in the last 50 months. Indication was ventricular hypertrophy of unknown etiology. We have correlated the image findings with the clinical and/or pathological diagnosis. AL amyloidosis diagnosis was based on histological demonstration of amyloid by Congo Red staining and immunohistochemistry (IHC) in any tissue and confirmation by proteomics (mass spectrometry (MS)) when indicated.
Results: We performed 704 SC for different indications during the study period. 41/61 patients (67.2%) with AL amyloidosisand cardiac involvement underwent a SC: 27 were negative (66%), 7 (17%) showed some degree of uptake (PS score<2 and focal or univentricular uptake) and 7 (17%) had biventricular uptake (PS 2-3). Patients with PS<2 (7): 5 patients underwent an endomyocardial biopsy (EMB). In 4 cases IHC confirmed AL amyloidosis, while in 1 case IHC was not diagnostic and MS confirmed AL amyloidosis. The two remaining patients were diagnosed of AL amyloidosis by IHC unequivocally in other tissues. Patients with PS 2-3 (7): 5 patients underwent an endomyocardial biopsy (EMB). Among them, 3 were diagnosed of AL amyloidosis, both by IHC and MS. Two patients, both of them with a M-spike and abnormal free light chains ratio in serum and increased clonal plasma cells in the bone marrow, showed TTR and faint light chain deposition but MS confirmed the diagnosis of TTR amyloidosis (TTR gene non mutated). The two remaining patients were diagnosed of AL amyloidosis by IHC unequivocally in other tissues. Mean value of NT-proBNP (N-terminal-natriuretic peptide) in patients with AL amyloidosis was 7730 pg/ml for those with a positive uptake in the scintigraphy and 9990 pg/ml for those with a negative result.
Summary/Conclusions: Cardiac 99mTc-DPD SC has been described as a useful technique in the differential diagnosis between AL and TTR amyloidosis. However, up to 30% of cases of AL amyloidosis show some degree of uptake and 10% show a pattern consistent with TTR amyloidosis (biventricular uptake and PS 2-3). Cases have been described with myocardial deposit of both TTR and light chain and up to 10-15% of the population >75 years may show a MC in serum, so it is essential to type accurately amyloid in patients with suspected AL amyloidosis and myocardial uptake in SC. MS is not routinely available in most centers and IHC results may be ambiguous. We consider that MS is mandatory in cases of cardiac amyloidosis with abnormal FLC ratio and positive biventricular ^{99m}Tc-DPD uptake. We did not find any correlation between positive uptake in the SC and NT-proBNP values, as it has been recently suggested.

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WHEN PERFORMANCE OF CYTOGENETICS MATTERS: A POPULATION-BASED STUDY IN THE NETHERLANDS ON NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS

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Background: It was recently shown in both clinical and population-based series that unperformed cytogenetics (UPCs) in intensively treated patients with acute myeloid leukemia was independently associated with poor prognosis, as compared to patients with performed cytogenetics.
Aims: Therefore, we set out to assess whether UPCs is associated with poor outcome in young patients with symptomatic multiple myeloma (MM) who have received induction chemotherapy.
Methods: We identified 358 newly diagnosed patients with MM <66 years in 2014 from the nationwide population-based Netherlands Cancer Registry (NCR). UPCs was used to indicate that no sample was sent in for cytogenetic analysis. Performed cytogenetics were grouped by Revised International Staging System (R-ISS), *i.e.* high-risk (presence of translocations (4;14) or (14;16) or deletion 17p) or standard-risk (presence of other aberrations or no aberrations). Only patients treated with induction chemotherapy, defined as treatment with VCD, PAD, BD or TAD +/- subsequent high dose melfalan and autologous stem cell transplantation (ASCT), were included for analyses. In total, 319 (89%, median age 60 years, 62% male) were treated with induction chemotherapy, 39 patients otherwise or had no therapy. The primary endpoint was progression-free survival (PFS), defined as time from start of first line induction chemotherapy to progression or death, whichever comes first. Patients alive without progression were censored at February 1st, 2016.
Results: In 220/319 (69%) MM patients treated with induction chemotherapy, cytogenetics was performed and 63 of these patients (29%) were cytogenetically high-risk. No statistical significant differences were observed in CRAB criteria or ISS between patients with or without performed cytogenetics. The proportion of patients undergoing ASCT was similar in different cytogenetic groups (standard-risk 85%, high-risk 83% and UPC 77%, p=0.77). Achieving partial response or better was higher, although not statistically significant, in the standard-risk group as compared to the high-risk and UPC groups (93% *vs* 89% *vs* 85%, p=0.92). Response outcome was unknown for 9% of the UPC group, 5% in high-risk group and 4% in the standard-risk group. Median follow-up time was 516 days. PFS for patients in the standard-risk group was highest, as compared to patients in the high-risk or UPCs groups after one year of follow-up (88% *vs* 81% *vs* 74%, p=0.0003).
Summary/Conclusions: Our data show that cytogenetic testing is performed in almost 70% of MM patients <66 years. Although response rates were similar for patients in the UPC, standard- and high-risk groups, PFS was better in the standard-risk group. Patients with unperformed cytogenetics had the poorest outcomes. The reasons are unclear, but a plausible explanation for not performing cytogenetics could be the patients' worse clinical condition at presentation which requires immediate treatment. For the abovementioned outcome measures, data of calendar year 2015 will be added and presented at the European Hematology Association.

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AN UPDATED ADJUSTED COMPARISON SUGGESTS DARATUMUMAB IS ASSOCIATED WITH PROLONGED SURVIVAL COMPARED WITH STANDARD OF CARE THERAPIES IN HEAVILY PRE-TREATED AND HIGH REFRACTORY MULTIPLE MYELOMA PATIENTS

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Background: To contextualize the benefit of novel agents such as daratumumab (DARA) monotherapy for the treatment of patients with heavily pretreated and highly refractory multiple myeloma (MM), it is critical to understand the real-world outcomes of this patient population on current standard of care (SOC) therapies. To determine the comparative effectiveness of DARA *vs* real-world SOC, an adjusted comparison was conducted[1] utilizing data from the DARA monotherapy trials[2] and the International Myeloma Foundation (IMF) chart review.[3]
Aims: The objective of this analysis is to update the adjusted comparison to include additional Swedish patients from the IMF chart review.
Methods: Data for patients treated with DARA 16mg/kg monotherapy were available from clinical trials MMY2002 (n=106) and GEN501 (n=42), while patients treated with SOC therapies were derived from the IMF chart review of patients with MM who had ≥3 prior lines of therapy and were double refractory to a proteasome inhibitor (PI) and an immunomodulatory drug (IMiD) (n=550, original 510, additional Swedish patients 40). Patients from the IMF-cohort who moved into further treatment lines after the line therapy where they fulfilled inclusion criteria, contributed information to the analysis for multiple lines of therapy, with baseline defined as the date of initiation of the actual treatment line, resulting in a sample size of 963 treatment lines from 550 patients. The relative treatment effect of DARA *versus* SOC was estimated using multivariate Cox regression analyses. The methodology utilized individual patient data to compare overall survival (OS). The covariates included were age, gender, prior lines of therapy, albumin, beta-2 microglobulin, prior exposure to pomalidomide and carfilzomib, and PI/IMiD refractory status. Clustering of observations at the treatment-line level within patients was controlled for using the robust sandwich estimate for the covariance matrix. Statistical significance testing was performed using a two-tailed *P*-value of <0.05, and all comparisons between treatment groups were reported with hazard ratios (HRs) and 95% confidence intervals (CIs).
Results: After adjustment for differences in baseline characteristics included in the multivariate model between the DARA and SOC groups, results showed a significant improvement in favor of DARA compared with SOC for OS (HR=0.42 [95% CI 0.31–0.57]). When limiting the comparative analysis to European patients from the IMF cohort (n=341), results for OS are very similar (HR=0.40 [95% CI: 0.28-0.58]).
Summary/Conclusions: Findings from the regression analyses using the updated IMF dataset were consistent with results from the previous analysis3 and suggest that DARA is associated with significant gains in OS compared with SOC therapies for patients with heavily pre-treated and highly refractory MM. Findings for a European subset from the IMF dataset were similar to results from the entire cohort.

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PREDICTORS OF EARLY DEATH RELATED TO ACTIVE MULTIPLE MYELOMA IN ELDERLY PATIENTS RECEIVING OPTIMIZED FRONTLINE TREATMENT COMBINATIONS

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Background: Multiple Myeloma (MM) is predominantly a disease of the elderly and the outcome of these patients is poorer than that of transplant candidates. It is well stablished that those considered frail or unfit have a dismal prognosis, however, even within fit patients, such as those included in clinical trials, there is substantial proportion of early deaths (within the first 2 years after diagnosis). Identification of this "high-risk" fit elderly patients could contribute both to the design of innovative clinical trials, and to avoid the emotional and economical burden of repetitive unsuccessful therapies.
Aims: To analyze the factors associated with early death (within first 2-years) due to active MM in elderly newly diagnosed (NDMM) patients fit enough to be included in clinical trials with optimized therapy with proteasome inhibitors and IMIDs.
Methods: 497 NDMM not transplant candidates treated in two prospective GEM-PETHEMA trials were included in the study: GEM05MAS65 (n=260) used frontline treatment with either bortezomib-melphalan-prednisone (VMP) or bortezomib-thalidomide-prednisone followed by maintenance with bortezomib, thalidomide or bortezomib, prednisone; the GEM2010MAS65 (n=239) compared induction with sequential or alternating cycles of VMP + lenalidomide-dexamethasone. The event was defined as death related to active MM within 2 years from diagnosis, either because of disease progression or early death due to absence of response.

Table 1.

Early death (< 2 years) due to Multiple Myeloma			
Number of patients	Yes (n=77) n (%)	No (n=420) n (%)	P
Age			
< 75 years	38 (49.3)	265 (62.8)	P = 0.026
≥ 75 years	39 (50.7)	157 (37.2)	
Response			
CR (sCR + CR IF-)	13 (20)	164 (41.3)	
VGPR	31 (47.7)	170 (42.8)	P < 0.001
PR	12 (18.5)	47 (11.8)	
< PR (MR + SD)	9 (12.3)	12 (3.08)	
NA/NE	0 (0)	4 (1.0)	
Duration of response			
DOR < 6 months	25 (38.5)	17 (7.2)	P < 0.001
DOR ≥ 6 months	40 (61.5)	220 (92.8)	
ISS			
ISS 1	7 (9.1)	110 (26.4)	
ISS 2	31 (40.3)	185 (44.5)	P < 0.001
ISS 3	39 (50.6)	121 (29.1)	
R-ISS			
R-ISS 1	3 (3.9)	78 (18.7)	
R-ISS 2	56 (72.7)	314 (75.5)	P < 0.001
R-ISS 3	18 (23.4)	24 (5.77)	
β2-microglobulin			
β2-micro < 5.5	41 (54.7)	304 (73.2)	
β2-micro ≥ 5.5	34 (45.3)	111 (26.8)	P= 0.001
LDH			
Normal	57 (75.0)	381 (91.6)	p<0.001
Abnormal	15 (25.0)	35 (8.4)	
Cytogenetics			
High risk	20 (16.9)	61 (16.9)	P= 0.001
Standard risk	41 (67.1)	300 (83.1)	
CD45_Diagnosis			
Negative	2 (66.2)	96 (77.7)	p= 0.040
Positive	20 (33.8)	196 (22.3)	
CD27_Diagnosis			
Negative	42 (64.6)	195 (50.5)	p= 0.035
Positive	23 (35.4)	191 (49.5)	

Results: From the 497 patients included, 77 (15%) patients died within 2 years from diagnosis due to active MM. When we compared this latter cohort with the remaining patients, the profile of the high risk group was characterized (Table 1) by a higher proportion of patients >75 years, advanced ISS and R-ISS stage, higher β2-microglobuline (β2-M) levels (>3.5 and 5.5mg/dl) and abnormal LDH; increased incidence of high-risk cytogenetic features (HR CA), $CD45^-$ clonal plasma cells, and lower incidence of $CD27^+$ MM phenotype. Patients dying in <2 years were also characterized by a higher proportion of non-responding patients as well as unstained responses (unsR). The multivariate analysis showed that the risk of early death due to active MM was related to four independent prognostic factors: age >75y (p=0.008), abnormal LDH (p=0.000), ISS 3 (p=0.008), and presence of HR CA (p=0.019. These four variables enabled the definition of a scoring system composed of 6 categories (0-5) (age>75 y=1 point, ISS 3=1, LDH>normal=2, HR CA= 1). Using a cut-off point of ≥4 the positive predictive value (PPV) was 57.9%, negative predictive value (NPV) was 87.4% and the positive likelihood ratio 7.9. Upon adding the

unsR (duration of response (≥PR) <6 months) to the baseline score we were able to build a new score in which the unsR had a 3 points weight. A score punctuation ≥5 segregates a subgroup of patients with poor outcome (PPV: 83.3%, the NPV: 84.02%).
Summary/Conclusions: The risk of early death due to active disease in elderly patients was related to four independent prognostic factors: age >75y, high LDH levels, advanced ISS, and presence of HR CA. A score ≥ 4 identify a subgroup of patients with high probability of death within 2 years despite optimized treatment.

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MPL ACTIVATION DIRECTLY INDUCES FIBROCYTE DIFFERENTIATION TO CAUSE MYELOFIBROSIS

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Background: Myelofibrosis (MF) may be caused by various pathogenic mechanisms, such as elevated circulating cytokine levels, cellular interactions, and genetic mutations. However, the underlying mechanism of MF remains unknown. A recent study showed that the neoplastic clone of fibrocytes, spindle-shaped fibroblast-like blood cells derived from monocyte lineage, was essential in primary MF pathogenesis; serum amyloid P, which suppressed fibrocyte differentiation, markedly improved survival and MF in a murine xenograft model (J Exp Med 2016; 213: 1723-1740). Regarding cytokines, the thrombopoietin (TPO) signaling pathway was assumed to be closely associated with promoting MF. Mice transplanted with TPO-overexpressing bone marrow cells showed symptoms such as MF and splenomegaly (Blood 1997; 90: 4369-4383). Romiplostim (Rom), a TPO-receptor agonist, induced MF in rats and some immune thrombocytopenic purpura patients (Blood 2009; 114: 3748-3756). Fibrocytes and TPO played certain roles in MF pathogenesis, but the nature of their relationship remains unknown.
Aims: We investigated the relationship between myeloproliferative leukemia protain (MPL; TPO receptor) activation and fibrocyte differentiation in promoting MF. The secondary goal was to discover a unique fibrocyte marker in monocyte or macrophage population.
Methods: Murine fibrocyte cell lines were established from transgenic mice harboring the temperature-sensitive large T-antigen gene of simian virus 40 under IL-13 and M-CSF conditions. Murine fibrocyte cell lines and human peripheral blood mononuclear cells (PBMCs) were cultured with or without Rom to evaluate if MPL activation promoted fibrocyte differentiation, and the ratio of spindle-shaped cells was calculated. Rom was administered on day 1 and 8 to induce an MF-like phenotype in C57BL/6J mice, and clodronate liposomes (CLs; day −4, −1, 4, and 7) were used to eliminate monocytes and macrophages.
Results: Flow cytometric analysis revealed that all murine fibrocyte cell lines stained positive for fibrocyte cell markers, including collagen I, CD45, CD34, CD11b, and CD68. Murine fibrocyte cell lines expressed MPL and responded to Rom or murine TPO to differentiate into mature fibrocytes, specifically with a longer spindle shape and stronger collagen I expression than when cultured with IL-13 and M-CSF alone. Rom also increased the number of mice spleen cell fibrocyte colonies in the presence of IL-13 and M-CSF. The administration of 1mg/kg of Rom once a week induced an MF-like phenotype in all mice within 2–3 weeks and increased the number of fibrocytes in the spleen. Treatment with CLs eliminated fibrocyte precursors and prevented severe MF and splenomegaly. Human cultured fibrocytes also expressed MPL, and Rom increased the number of spindle-shaped fibrocytes induced from human PBMCs. The SLAMF7^{high} MPL^{high} subpopulation was clearly separated from the SLAMF7^{low} MPL^{low} population in human CD14^{+} monocytes. A significantly higher frequency of fibrocyte differentiation was observed in the SLAMF7^{high} MPL^{high} population. The number of SLAMF7^{high} MPL^{high} cells was significantly greater in MF patients than in healthy donors. Conversely, their numbers did not increase in MF patients treated with ruxolitinib.
Summary/Conclusions: MPL activation directly induced fibrocyte differentiation from monocytes and macrophages expressing MPL, and the elimination of these cells reversed the MF phenotype. Our findings confirmed a link between fibrocytes and the TPO/MPL signaling pathway and indicated that the combination of MPL and SLAMF7 could be a useful fibrocyte marker in monocytes or macrophages.

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ENGRAFTMENT OF PRIMARY MYELOFIBROSIS BONE MARROW-DERIVED CD14+ MONOCYTES IN NOD-SCID-g MICE

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Background: Progressive bone marrow (BM) fibrosis in patients with PMF is thought to arise from non-hematopoietic stromal cells stimulated by overpro-

duced growth factors. However, in other tissues and organs, fibrosis is associated with monocyte-derived fibrocytes, which express markers of both hematopoietic and stromal cells. Recently, we have reported that clonal neoplastic fibrocytes play a role in the induction of BM fibrosis in primary myelofibrosis (PMF) (Verstovsek, J Exp Med. 2016). We demonstrated that the BM of PMF patients harbors more neoplastic, functionally distinct fibrocytes and fewer MSCs than hematologically normal bone marrow (BM). In addition, we detected an overabundance of fibrocytes in the BM and spleen of an established PMF mouse model and a xenograft mouse model of PMF created using BM-derived low-density cells from patients with PMF.
Aims: Fibrocytes, which make up <1% of BM cells, differentiate from a subpopulation of CD14+ monocytes and are recruited to sites of organ damage where they regulate tissue repair. We hypothesized that clonal neoplastic CD14+ monocytes may play a role in the induction of BM fibrosis in PMF
Methods: To test this hypothesis, we transplanted NSG mice (NOD/Scid (NoD.Cg-prkdcscid //2rgtm1wjl/SZJ) with sorted CD14+ monocytes from patients with *JAK2*^{V617F}-positive PMF or donors with hematologically normal BM.
Results: Here, we show that BM-derived CD14+ cells from patients with *JAK2*^{V617F}-positive PMF or donors with hematologically normal BM engrafted in NSG mice. Transplanted NSG mice with PMF BM-derived CD14+ monocytes developed a myelofibrosis-like phenotype with reticulin fibrosis and abundant neoplastic (*JAK2*^{V617F}) fibrocytes in the BM and spleen. Two months after transplantation, we detected a subpopulation of hCD45+ and hCD68+ cells within the HLA+ population of BM cells. In addition, we found dysplastic megakaryocytes in the BM and spleen of the PMF CD14+ transplanted mice. Immunohistochemistry staining of paraffin embedded BM sections did not detect hCD3, hCD19 or hCD34 cells. However staining with anti-human CD42b antibodies detected human megakaryocytes, suggesting that the dysplastic megakaryocytes detected in PMF CD14+ transplanted NSG mice are human-derived.
Summary/Conclusions: Taken together, our data suggest that neoplastic CD14+ monocytes contribute to the induction of BM fibrosis in PMF. What role CD14-derived megakaryocytes play in the pathogenesis of PMF remains to be determined.

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ESTABLISHMENT OF AN IN VITRO MODEL FOR THE SKEWED MEGAKARYOPOIESIS BY CALRETICULIN MUTATION IN HUMAN CELLS
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Background: Somatic mutations on *calreticulin* (*CALR*) gene are found in a majority of patients with *JAK2*-unmutated *Philadelphia*-chromosome negative myeloproliferative neoplasms (MPNs). We and other groups have recently shown that mutant CALR activates the downstream pathway of thrombopoietin (TPO) receptor MPL, which induces factor-independent growth in human and murine cells. However, roles of mutant CALR in human hematopoietic cell differentiation remain largely elusive.
Aims: We aimed to recapitulate the MPN phenotypes and examine the impact of *CALR* ins5 on human hematopoietic cell differentiation *in vitro*.
Methods: We employed iPS cells (iPSC) established from an essential thrombocythemia (ET) patient and a healthy individual harboring a 5-base insertion mutant (*CALR* ins5) and wild type (*CALR* wt) *CALR* gene, respectively. Hematopoietic progenitor cells (HPCs) were produced from iPSC by "iPS-Sac" method. HPCs were then cultured to induce megakaryocytic cells (MKs) and erythroid cells defined by CD42b and CD235a, respectively. The mechanism of skewed differentiation was assessed by measuring the mRNA expression of lineage determinant genes such as *FLI1* and *KLF1*. To demonstrate the established assay system for the use of compound screening, CALR ins5-dependent megakaryopoiesis was examined by therapeutic compounds.
Results: The number of CD34+ HPCs produced from iPSC was unchanged between *CALR* ins5 and *CALR* wt genotypes, implying that *CALR* ins5 did not affect the establishment of HPC from iPSC. As expected from constitutive activation of MPL by mutant CALR, MKs were induced from *CALR* ins5-HPC even in the absence of TPO in either semi-solid or liquid culture systems, which was not evident with *CALR* wt-HPC. Unlike megakaryopoiesis, both *CALR* ins5 and *CALR* wt HPCs required EPO for the production erythroid cells. However, the number of erythroid cells induced from *CALR* ins5 HPC was significantly decreased compared to that from *CALR* wt HPC, implying that *CALR* ins5 interfered the erythroid cells differentiation from iPSC-derived HPC. This interference was seemingly caused by a premature expression of *FLI1* that blocked *KLF1* expression required for the erthroid cell differentiation in *CALR* ins5 cells. Finally, we showed that the treatment of ruxolitinib greatly reduced megakaryocytic differentiation in both *CALR* ins5 and wt HPCs, demonstrating that ruxolitinib does not possess preferential targeting of CALR ins5 cells.
Summary/Conclusions: We have established an *in vitro* model system that recapitulates the megakaryocytosis caused by mutant CALR, which should be useful tool for the examination of therapeutic strategies against MPN patients harboring CALR mutation.

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QUANTITATIVE PROTEOME HETEROGENEITY IN MYELOPROLIFERATIVE NEOPLASM SUBTYPES AND ASSOCIATION WITH JAK2 MUTATION STATUS
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Background: Apart from well-known genetic abnormalities, several studies have reported variations in protein expression in Philadelphia negative (Ph-) Myeloproliferative Neoplasm (MPN) patients that could contribute towards their clinical phenotype.
Aims: In this context, a quantitative mass spectrometry proteomics protocol was used to identify differences in the granulocyte proteome with the goal to characterize the pathogenic role of aberrant protein expression in MPNs.
Methods: LC MS/MS (LTQ Orbitrap) coupled to iTRAQ labeling showed significant and quantitative differences in protein content among various MPN subtypes [polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF)], and according to the genetic status of JAK2 (*JAK2V617F* presence and *JAK2V617F* allele burden).
Results: A number of differentially expressed proteins were identified with the most frequent being members of the RAS GTPase family and oxidative stress regulatory proteins. Subsequent analysis found that calreticulin (CALR), known to be involved in calcium homeostatsis and apoptotic signaling, was overexpressed in *JAK2V617F* granulocytes compared with *JAK2 wild-type* and independently of the *JAK2V617F* allele burden. Finally it was demonstrated, in a Ba/F3 cell model, that increased calreticulin expression was directly linked to *JAK2V617F* and could be regulated by JAK2 kinase inhibitors.
Summary/Conclusions: In conclusion, these results reveal proteome alterations in MPN granulocytes depending on the phenotype and genotype of patients, highlighting new oncogenic mechanisms associated with *JAK2* mutations and overexpression of calreticulin.

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THE NOVEL SWITCH CONTROL INHIBITOR DCC-2618 COUNTERACTS GROWTH AND SURVIVAL OF VARIOUS NEOPLASTIC CELLS, INCLUDING MAST CELLS, EOSINOPHILS, AND MONOCYTES, IN PATIENTS WITH SYSTEMIC MASTOCYTOSIS
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Background: Systemic mastocytosis (SM) is a myeloid neoplasm defined by abnormal growth and pathologic accumulation of neoplastic mast cells (MC) in various internal organs. The indolent variant of SM (ISM) is associated with an almost normal life expectancy. By contrast, the prognosis in advanced SM, including SM with an associated hematologic neoplasm (SM-AHN), aggressive SM (ASM), and MC leukemia (MCL) is poor with short survival times. Most patients with SM express the D816V-mutated variant of *KIT*, which confers resistance against several tyrosine kinase inhibitors (TKI), including imatinib. DCC-2618 is a novel switch control inhibitor that has been described to block the kinase activity of KIT D816V.
Aims: The aims of this study were to evaluate the effects of the switch control inhibitor DCC-2618 on proliferation and survival of neoplastic MC and other neoplastic and non-neoplastic cell types that may play a role in advanced SM and often expand in AHN patients.
Methods: For our *in vitro* studies we used different human MC lines (HMC-1.1, HMC-1.2, ROSA^{KITWT}, ROSA^{KITD816V}, ROSA^{KITK509I}, MCPV-1.1, MCPV-1.2, MCPV-1.3 and MCPV-1.4) and primary neoplastic MC obtained from patients with SM. In addition, the acute myeloid leukemia (AML) cell lines

MOLM-13, MV4-11, KG-1 and U-937, the eosinophilic leukemia cell line EOL-1, human cultured umbilical vein endothelial cells (HUVEC), the microvascular human endothelial cell line HMEC-1 and primary neoplastic cells obtained from patients with AML, chronic myelomonocytic leukemia (CMML) and (clonal or reactive) hypereosinophilia were used. Cell proliferation was quantified by ^{3}H-thymidine uptake. Apoptosis was determined by flow cytometry and light microscopy. The phosphorylation-status of KIT and BTK was analyzed by Western blotting. The effects of DCC-2618 on histamine secretion in basophils (BA) were analyzed by histamine release assay.

Results: DCC-2618 was found to block the proliferation of all MC lines tested, with lower IC_{50} values measured in *KIT* D816V-negative HMC-1.1 cells (12.3±3.7 nM) and ROSA^{KITWT}cells (41±5 nM) than in *KIT* D816V-positive HMC-1.2 cells (123±36 nM), ROSA^{KITD816V} cells (168±65 nM), and the multi-resistant MC line MCPV-1. The DCC-2618-metabolite DP-5439 showed comparable growth-inhibitory effects in all cell lines tested. DCC-2618 was also found to inhibit proliferation of primary neoplastic MC obtained from patients with ISM, ASM or ASM-AHN and MCL (IC_{50}: 83-460 nM). DCC-2618 induced apoptosis and blocked tyrosine phosphorylation of KIT in all MC lines tested. We were also able to show that DCC-2618 inhibits proliferation and survival in the eosinophilic leukemia cell line EOL-1 (IC_{50} 1.8±1.3 nM) and the *FLT3* ITD-mutated AML cell lines MV4-11 (IC_{50} 147±88 nM) and MOLM-13 (IC_{50} 132±95 nM). In addition, DCC-2618 was found to block proliferation in primary leukemic cells in patients with monoblastic AML and CMML which are the most prevalent types of AHN in advanced SM. DCC-2618 was also found to inhibit growth of cultured human vascular endothelial cells, suggesting that the drug may also counteract SM-related angiogenesis. Finally, DCC-2618 was found to inhibit anti-IgE-induced histamine release from normal BA in a dose-dependent manner (IC_{50}: 1-10 μM).

Summary/Conclusions: DCC-2618 is a new potent switch control TKI that counteracts growth and survival of neoplastic MC, leukemic monocytes, AML blasts, eosinophils, and endothelial cells *in vitro*. Whether DCC-2618 is able to block growth of neoplastic MC and other involved lineages in patients with advanced SM is currently being ascertained in a clinical trial (NCT02571036).

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DISTRIBUTION OF MUTATIONS IN DRIVER AND NON-DRIVER GENES ACCORDING TO CLONAL HEMATOPOIESIS IN ESSENTIAL THROMBOCYTHEMIA AND POLYCYTHEMIA VERA

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Background: Essential thrombocythemia (ET) and polycythemia vera (PV) are clonal myeloid disorders that originate from a multipotential hematopoietic stem cell. Although most women with PV and ET have mutations in *JAK2V617F, CALR* or *MPL*, the proportion of patients presenting clonal hematopoiesis by X chromosome inactivation patterns (XCIP) is variable and its relationship with the presence of non-driver mutations is not well known.

Aims: To study the distribution and dominance of driver and non-driver mutations in the development of clonal hematopoiesis.

Methods: One hundred and twenty-six women (PV n=33, ET n=93) with an informative result of XCIP based on *HUMARA* assessment were included in the study. *HUMARA* analysis was performed by studying the degree of methylation of exon 1 in granulocytes and lymphocytes. Somatic mutations were studied in DNA extracted from granulocytes by NGS using a panel of 51 myeloid-related genes.

Results: Median age of patients at the time of *HUMARA* analysis was 64 years (range:21-92). Mutations in *JAK2* were present in 62% of them, *CALR* in 11%, *MPL* in 8%, and 14% were triple negative (TN). Non-driver mutations were detected in 45 patients (17 PV and 28 ET). The most frequently mutated genes were *TET2* (16%), *DNMT3A* (8%), *ASXL1* (5%), *SF3B1* (5%), *EZH2* (2%) and *RUNX1* (2%).The mutation with the highest variant allele frequency (VAF) was considered the dominant mutation and it corresponded to a driver mutation in 92 patients (*JAK2* n=70, *CALR* n=13, *MPL* n=8) and a non-driver mutation in 9 patients (3 TN cases with *TET2* mutations, 1 TN patient with *TP53* mutation and 4 *JAK2*-mutated patients with mutations in *TET2, CBL, DNMT3A* or *EZH2*). In 12 cases the VAF of the driver mutation (*JAK2* n=9, *CALR* n=1, *MPL* n=2) was similar to the non-driver mutation, being *TET2* the codominant mutation in 6 of them. *HUMARA* analysis was clonal in 66 patients and polyclonal in 60 patients. Clonality was more frequently observed in PV (76%) than in ET (44%, p=0.002). Clonal *HUMARA* was observed in 90% of *MPL*-mutated patients in comparison with 58% in *JAK2*-mutated, 42% in *CALR*-mutated and 11% in TN (p<0.0001). Two patients with TN ET showing clonal hematopoiesis had *TET2* mutations. In *JAK2*-mutated women, the mutant allele load was significantly higher in clonal than in polyclonal cases (43% *vs* 23%, p=0.02) and in PV than in ET (76% *vs* 47%, p=0.01). Eighty percent of patients with non-driver mutations showed *HUMARA* clonality *vs* 37% of patients without non-driver mutations (p<0.0001). The mutated genes significantly associated with a higher frequency of clonal hematopoiesis were *TET2* (p=0.007) and *SF3B1* (p=0.029). Age was significantly associated with clonal hematopoiesis and with the presence of non-driver mutations (median age of 55 and 68 years for polyclonal and clonal *HUMARA* respectively, p<0.0001 and 57 and 71 years for patients without and with additional mutations respectively, p<0.0001). Patients in which the driver mutation was dominant were younger than those in which the non-driver mutation was dominant or codominant (median age 61 *vs* 71 years, p=0.01). In the multivariate analysis, the variables that were associated with a higher probability of clonal hematopoiesis were the presence of non-driver mutations (OR:4.2, 95%CI: 1.6-10.6, p=0.003), age> 65 years (OR:2.7, 95%CI: 1.2-6.5, p=0.02), *MPL* genotype (OR:10.9, 95%CI: 1.2-98.9, p<0.0001) and PV diagnosis (OR 3.6, 95%CI: 1.3-9.7, p=0.01).

Summary/Conclusions: The presence of non-driver mutations is associated with clonal hematopoiesis regardless of age and type of disease.

Elderly patients with clonal hematopoiesis often show non-driver mutations coexisting in equal or greater proportion than the driver mutation suggesting that MPN has originated on preexisting clonal hematopoiesis.

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RUXOLITINIB/NILOTINIB/PREDNISOLONE COMBINATION: A PROMISING NOVEL TREATMENT FOR MYELOFIBROSIS

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Background: Myelofibrosis (MF) is the myeloproliferative neoplasm chromosome Phi- negative with worst prognosis. MF is characterized by stem cell-derived clonal myeloproliferation and reactive cytokine-driven inflammatory bone marrow fibrosis. Ruxolitinib is the first line treatment for MF. It was associated with significant reduction in symptomatic splenomegaly and improved constitutional symptoms. In a previous work (Arenas *et al.* Blood Volume 122, Issue 21 (ASH Annual Meeting Abstrat)) we identified a set of promising synergistic drugs combinations for a ruxolitinib. Nilotinib and prednisolone were selected from them.

Aims: The aim of this work is the study the effect of the combination of ruxolitinib, nilotinib and prednisolone in hematopoietic progenitor cells from patients with MF.

Methods: A ruxolitinib, nilotinib and prednisolone dose-response curves and synergistic studies were performed in hematopoietic progenitors CD34$^+$ from five MF patients. We studied the molecular effect of single drugs and in combination on SET2 cell line with western blot. To adress the antifibrogenic activity of the drugs and their combinations, we pre-incubated HS27 cultures with 100nM of ruxolitinib, 1 μM of nilotinib, 1 μM of prednisolone or their combination during 1 h. After that, we added 2ng/mL TGF-β during 24h to induce fibrogenesis. Finally, the collagen I expression was evaluated by immunocytochemistry (ICC).

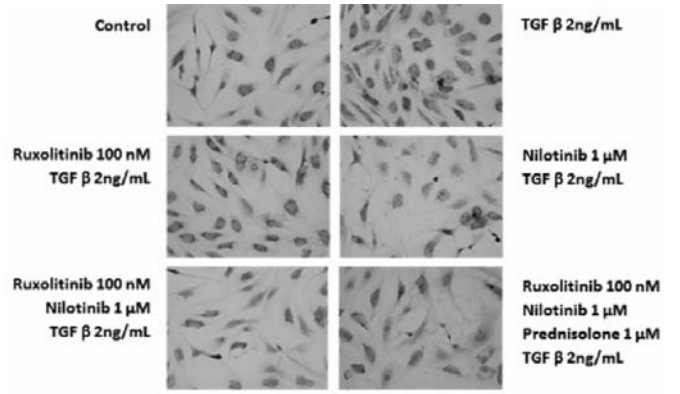

Figure 1: Collagen I expression was evaluated by ICC in HS27 treated with ruxolitinib, nilotinib, prendisolona and their combinations.

Figure 1.

Results: The effects of ruxolitinib, nilotinib and prednisolone resulted in an EC_{50} value of 55nM, 6.6μM and 13.1μM, respectively. A combination index (CI) of less than 1 indicated synergy. All combination had a synergistic behavior (Table 1); moreover, there were two combinations whose CI from all samples indicated synergy: 32nM ruxolitinib, 8 μM Nilotinib plus 0.8 μM prednisolone (CI=0.25±0.11) and 32nM ruxolitinib plus 0.8 μM prednisolone (CI=0.45±0.11). The JAK/STAT signaling pathway was inhibited: the phosphorylation of STAT5 was inhibited by ruxolitinib in 83.2±10.8 % (p-valor<0.05) regarding to control at 30 min and it was maintained at 3 hours (p-valor<0.05). The combinations 32nM ruxolitinib plus 1.6 μM nilotinib (RN) and 32nM ruxolitinib, 1.6μM nilotinib plus 0.8μM prednisolone (RNP) inhibited more than 50% of the phosphorylation of STAT5 at 30 min and maintained at 3 hours. The MAPK signaling pathway was inhibited at 30 min, the phosphorylation of ERK was inhibited in 77.7±16.4

% (p-value<0.05) by ruxolitinib, 42.6±14.4 % by RN and 70.8±11.2 % by RNP (p-valor<0.001). The inhibition was maintained at 3 hours by ruxolitinib (71.3±18.9%) (p-value<0.05). The Akt/PI3K signaling pathway seemed to begin to inhibit at 3 hours by ruxolitinib (57.5+25.2%), nilotinib (38.4±26.8%), RN (30.5±24.03%) and RNP (37.4±16.5%). Then, the antifibrogenic activity of the drugs and their combinations were studied. Nilotinib reduced the mRNA expression of COL1 (39.8±0.9 %) (p-value<0.05). Its combination with ruxolitinib (RN: 48.1±2.9% (p<0.05) and prednisolone (RNP: 37.8±1.9% (p-value<0.05). These results were corroborated by ICC: the inhibition of expression of collagen I was more intense if the HS27 were treated with nilotinib or RN (figure 1).

Summary/Conclusions: In conclusion, ruxolitinib, nilotinib, prednisolone and their combinations had a synergistic behavior to control the proliferation of myeloid cells in MF patients; moreover, they had anti-fibrotic activity in fibroblast cells. For these reasons, the combined ruxolitinib/nilotinib/prednisolone could be a promising therapy to MF and support an ongoing clinical trial in MF patients.

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INTERLABORATORY ASSESSMENT OF MUTATION DETECTION IN MYELOID MALIGNANCIES BY TARGETED NEXT-GENERATION SEQUENCING

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Background: Next-generation sequencing (NGS) technology is being implemented in clinical practice for assessing the mutational status of myeloid neoplasms. The Working Group on Molecular Biology from the Spanish Society of Hematology has performed an interlaboratory assessment of gene mutation analysis by targeted NGS.

Aims: To assess the technical performance of mutation detection by targeted NGS using myeloid panels.

Methods: The technical comparison was established on two rounds with samples previously analysed using NGS panels, Sanger sequencing and/or fragment analysis. First, four DNA samples (S1-S4) from AML patients were shared among 6 laboratories. In a second round, five samples (S5 to S9) were shared among 14 laboratories. The center of origin had previously characterized and confirmed: for the first round, 14 relevant mutations in 10 genes; and for the second round 17 relevant mutations in 7 genes. Each center performed library preparation, sequencing and blind variant analysis following their own routine practice. Detected variants and data regarding main methodological parameters were collected. Detection rate was calculated as the number of laboratories with positive detection out of the number of laboratories that sequenced the particular gene region.

Results: Eight different gene panels were used for library preparation (predesigned in 10 labs and custom in 4). The predominant approach was amplicon enrichment (11/14, 78.6%) and only 3/14 laboratories (21.4%) used capture-based methods. Sequencing was performed with Illumina devices in 9/14 laboratories and Ion Torrent platforms in 5/14. Alignment and variant calling was performed with MiSeq Reporter (n=3), Torrent Suite (n=4) or panel-adjusted analysis pipelines. The median coverage was 2353 reads (range 275-17096). Results are summarized in the table. Overall, most variants were detected by ≥80% of the laboratories. None of the participants was able to detect all the previously characterized mutations using only NGS myeloid gene panels. Variants with lower detection rates can be classified in 3 groups: a) Medium-long insertions/deletions. *FLT3*-ITD was not detected by NGS. *CALR*L367fs and *ASXL1*E635fs were detected in only 67% and 31% of laboratories, respectively. However, a *SRSF2* 24bp deletion with similar characteristics showed a 90% detection rate. b) Mutations with low variant allele frequency (VAF). *CBL*Y371N (VAF 5.24%) and *SF3B1*K700E (VAF 4.09%) were less detected/reported. c) *ASXL1*G646fs. This mutation caused by a guanine duplication in a homopolymeric region is technically challenging and there is controversy as previous reports have considered this alteration as an artifact. The reported VAFs were similar for SNVs whereas for indels higher differences were observed (mean coefficient of variation of 11.91%, and 26.04%, respectively).

Table 1.

Sample	Gene	CDS	AA	% mean VAF (SD)	Nr Labs covering region	Nr Labs detecting variant	% detection (labs covering region)	% detection (total nr labs)
S01	*NPM1*	c.859_860insTCTG	p.Trp288CysfsTer12	41.68 (18.90)	5	5	100	83.33
	IDH2	c.419G>A	p.Arg140Gln	44.73 (8.31)	6	6	100	100
	DNMT3A	c.2645G>A	p.Arg882His	43.77 (1.91)	6	6	100	100
	FLT3	ITD	ITD	NA	6	0	NA	NA
S02	*CEBPA*	c.68_78del11	p.Pro23GlnfsTer81	51.82 (7.17)	4	5	80	66.67
	CEBPA	c.895A>G	p.Ser299Gly	45.32 (3.86)	5	5	100	83.33
	IDH2	c.419G>A	p.Arg140Gln	49.83 (5.23)	6	6	100	100
	NRAS	c.37G>C	p.Gly13Arg	46.32 (2.01)	6	6	100	100
	ASXL1	c.1934dupG	p.Gly646TrpfsTer12	39.82 (4.41)	2	6	33.33	33.33
S03	*DNMT3A*	c.2678G>C	p.Trp893Ser	44.54 (2.32)	6	6	100	100
	TP53	c.652_670del19	p.Val218ArgfsTer28	67.58 (19.42)	5	6	83.33	83.33
	EZH2	c.553G>C	p.Asp185His	80.96 (1.42)	3	3	100	50
	RUNX1	c.167T>C	p.Leu56Ser	33.36 (3.23)	4	6	66.67	66.67
S04	*TP53*	c.392A>T	p.Asn131Ile	47.33 (1.83)	6	6	100	100
S05	*CALR*	c.1092_1143del52	p.Leu367ThrfsTer46	43.72 (13.51)	10	6	66.67	42.86
	ASXL1	c.1900_1922del23	p.Glu633ArgfsTer15	15.80 (4.15)	13	4	30.77	28.57
	ASXL1	c.1934dupG	p.Gly646TrpfsTer12	15.98 (3.41)	13	5	38.46	35.71
	ASXL1	c.2077C>T	p.Arg693Ter	5.57 (1.87)	13	11	84.62	78.57
S06	*CALR*	c.1092_1143del52	p.Leu367ThrfsTer46	44.83 (15.48)	10	6	66.67	42.86
	CBL	c.1100A>C	p.Gln367Pro	39.16 (6.74)	14	13	92.86	92.86
	CBL	c.1111T>A	p.Tyr371Asn	5.24 (0.17)	14	3	21.43	21.43
	SRSF2	c.284C>A	p.Pro95His	44.20 (5.08)	11	9	90	64.29
S07	*JAK2*	c.1849G>T	p.Val617Phe	30.07 (2.92)	14	14	100	100
	CBL	c.1055T>A	p.Leu352Ter	3.78 (1.54)	3	2	66.67	14.29
	SRSF2	c.284_307del24	p.Pro95_Arg102del	37.12 (16.08)	11	9	90	64.29
	ASXL1	c.1934dupG	p.Gly646TrpfsTer12	7.36 (2.25)	13	3	23.08	21.43
S08	*JAK2*	c.1849G>T	p.Val617Phe	9.44 (1.99)	14	13	92.86	92.86
	SF3B1	c.2098A>G	p.Lys700Glu	4.09 (1.05)	11	5	45.45	35.71
S09	*JAK2*	c.1849G>T	p.Val617Phe	35.34 (1.81)	14	14	100	100
	TET2	c.3535delA	p.Arg1179fs	42.04 (1.79)	14	14	100	100
	TET2	c.4447G>T	p.Glu1483Ter	47.37 (1.26)	14	14	100	100

Summary/Conclusions: Gene mutation analysis by targeted NGS in myeloid malignances is highly reproducible between laboratories and allows a comprehensive characterization of the molecular profile. However, medium-long indels, low frequency mutations (<10%), *ASXL1*G646fs detection and variant categorization are critical points that have to be addressed to improve the results. Test system validation is crucial for the implementation of NGS technology.

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METHYLATION AGE IN MPN PATIENTS AS A CORRELATE FOR DISEASE STATUS, ALLELE BURDEN AND THERAPEUTIC RESPONSE

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Background: Myeloproliferative Neoplasms (MPNs) result from genetic and epigenetic dysregulation. Epigenetic therapies, such as Vorinostat (SAHA, MK-0683), a histone deacetylase inhibitor, have been tested as a therapeutic strategy in these patients. Examining the epigenetic landscape in MPN may provide new insights into predicting therapeutic response and therefore enhance the clinical utility of these agents. Probably the best described epigenetic mechanism is DNA methylation (DNAm); in which methyl groups are added to DNA at CpG sites regulating chromatin compaction and gene expression/repression. DNAm is known to be altered by ageing and can reflect the effect of diet, lifestyle or disease on cellular processes. Therefore 'methylation age' (MA) may be a more accurate reflection of disease than 'chronological age' (CA), which is merely a description of how long a person has been alive. Weidner *et al (Genome Biology, 2014)* described how the measurement of DNAm levels at CpGs within 3 genes, *ASPA, ITGA2B, PDE4C* enabled the determination of a reliable MA that reflected CA in normal individuals.

Aims: The aim of our study was correlate MA with disease status, mutational profile and therapeutic response in a cohort of MPN patients treated with Vorinostat.

Methods: MA was calculated following pyrosequencing of bisulfite converted DNA from 40 MPN patients on an investigator initiated non randomised open label phase II multicentre study of Vorinostat (EudraCT #2007-005306-49). Paired samples were analysed at trial entry and after 3 months of therapy to calculate their individual MA scores. Validation of methods used and ageing signature calculation was carried out using cell line and healthy volunteer material.
Results: Samples from 18 Essential Thrombocythaemia (ET) and 22 Polycythaemia Vera (PV) patients (23 F/17 M) with a mean age of 62 years (range 29-81) were assessed. *JAK2V617F* was detected in 77.5% (n=31/40). Complete clinical response (CR) was achieved in 8 patients, partial (PR) in 17, and no response (NR) in 15 patients. MA was on average 8.3 years younger than CA (range -43.4 to +41.6) at time of trial entry and 8.2 years younger (range -36.5 to +33.3) after therapy. This difference between MA and CA was greater in ET patients compared to PV, both at trial entry (-14.0 years *vs* -3.7) and after therapy (-13.0 years *vs* -4.3). A statistically significant link between *JAK2* allele burden and MA was seen: compared to patients with low or no *JAK2* allele burden, patients with high *JAK2* (>60% at baseline) had an older MA at trial entry (64.2 years *vs* 44.5, p=0.0007) and after therapy (64.3 years *vs* 44.6, p=0.0015). This difference was also seen when PV or ET patients were examined separately. Patients with a high *JAK2* allele burden tended to have a MA closer to their CA at trial entry (-0.6 years *vs* -15.3, p=0.0122) and after 3 months therapy (-0.5 years *vs* -15.2, p=0.0072). Although the cohort size was small, within the ET group, NR compared to PR was associated with a younger MA after therapy (41.4 years *vs* 56.3, p=0.0156). Within PV, NR compared to PR was associated with a MA that was older than CA both before (+9.2 years *vs* -14.2 years, p=0.0346) and after therapy (+7.4 years *vs* -13.9, p=0.0347).
Summary/Conclusions: This study suggests a link between MA and *JAK2* mutant allele burden in MPN patients, suggesting that allele burden not only has a role in clinical phenotype and disease evolution but in the overall methylation landscape of the mutated cells. However, the role of MA with respect to therapeutic response needs to be clarified with further studies required to show its full impact.

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ELUCIDATING THE AGE INDUCED HEMATOPOIETIC CELL-INTRINSIC AND EXTRINSIC MECHANISMS IN MYELOPROLIFERATIVE NEOPLASM INITIATION AND PROGRESSION

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Background: The number of detectable somatic mutations increase with age, but this increase is surpassed by the rise in the incidence of cancer in older people. The underlying mechanisms for this disparity remain to be elucidated. Myeloproliferative neoplasm (MPN) is an ideal malignancy model disease to study clonal hematopoiesis, disease initiation and progression during natural aging because the majority of the relevant mutations (such as JAK2 V617F) are catalogued, the disease evolves and progresses slowly allowing the collection of serial samples, and an inducible transgenic mouse models for the disease have been established. Nonetheless, the prevalent occurrence of such clonal events in aged individuals brings up the question, which age-associated factors contribute to initiate hematologic malignancies and what are the rate limiting steps attributable for age-induced myeloid malignancies? We hypothesize that aging induced alterations provides a context that favors acquisition of new mutations, selection for pre-malignant clones, and that activation of mutant JAK2 further augments these changes for increased MPN incidence in aged individuals. Thus, delineation of age associated cellular and molecular mechanisms attributable for increased prevalence of myeloid malignancies will be essential for the development of strategies for early detection and therapeutic targeting of myeloid malignancies.
Aims: The goal of this proposal is to identify age associated hematopoietic cell-intrinsic and cell-extrinsic factors that determine initiation and progression of MPN at young *versus* old age in mouse models carrying a JAK2-V617F or JAK2-exon12 mutation
Methods: To assess the effcet of aging on MPN initiation and progression we studied the young and aged inducible transgenic mouse models of MPN. Integreated omics analysis was peroformed on MPN initiating stem and progenitor cells.
Results: Our results suggest that age related changes in expression patterns resembling MPN can be found in aged wildtype mice. The mutational profiles in patients with pediatric MPN appear to be less complex than in older MPN patients. We are currently investigating the relative contributions and collaborations of age-associated cell intrinsic and extrinsic changes in HSCPs and BM niche in the course and severity of MPN in mouse models carrying a JAK2-V617F mutation, and in naturally aged donors and recipients of bone marrow transplantations.
Summary/Conclusions: Our study provided novel molecular and cellular mechanisms underlying increased incidence of MPN manifestation in old age.The implications of this work goes beyond the MPN malignancy and the comprehensive data sets generated in study will serve as a model to the wider scientific community to study other types of malignancies. This knowledge ultimately will help to define novel strategies to delay or target the onset of MPN in an aging individual.

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PACRITINIB (PAC) *VS* BEST AVAILABLE THERAPY (BAT), INCLUDING RUXOLITINIB, IN PATIENTS (PTS) WITH MYELOFIBROSIS (MF) AND BASELINE THROMBOCYTOPENIA: FOCUS ON ANEMIA IN THE PHASE 3 PERSIST-2 TRIAL

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Background: MF is a life-threatening hematologic malignancy characterized by splenomegaly, debilitating constitutional symptoms, and progressive cytopenias. The approved JAK inhibitor ruxolitinib reduces splenomegaly and symptoms in pts with MF, but is associated with dose-limiting cytopenias and not indicated for pts with platelets <50,000/μL. Red blood cell (RBC) transfusions are the core treatment strategy for anemia in many pts. PAC is an oral kinase inhibitor with specificity for JAK2, FLT3, IRAK1, and CSF1R. In 2 phase 3 studies (PERSIST-1, PERSIST-2), PAC has demonstrated significant and sustained spleen volume reduction (SVR) and symptom control *vs* best available therapy (BAT), regardless of baseline (BL) platelet count.
Aims: This analysis is focused on anemia (at BL and treatment-emergent [TE]) in pts from PERSIST-2, a phase 3 trial of PAC *vs* BAT, including ruxolitinib, in pts with MF and BL thrombocytopenia.
Methods: Pts with MF and BL platelet count ≤100,000/μL were randomized (N=311) 1:1:1 to PAC 400mg once-daily (QD), PAC 200mg twice-daily (BID), or BAT (including ruxolitinib). The co-primary endpoints were the rates of pts achieving ≥35% SVR (by MRI/CT) and ≥50% reduction in total symptom score (TSS; MPN-SAF TSS 2.0) at week 24. Efficacy analyses used the intent-to-treat efficacy population, which included all pts with randomization date allowing them to contribute data for a week 24 end point. The safety population included all pts who received any PAC or BAT (including those who had watchful waiting only). Clinical improvement in hemoglobin (hgb) was defined based on IWG criteria and RBC transfusion end points were defined according to Gale criteria (Table 1).

Table 1.

Table. Efficacy and Safety Outcomes – Spotlight on Anemia

	PAC QD	PAC BID	BAT
Intent-to-treat efficacy population, n	**75**	**74**	**72**
BL hgb <10 g/dL, n (%)	45 (60)	44 (59)	41 (57)
Clinical improvement in hgb[a] at week 24, n (%)	6 (13)	11 (25)	5 (12)
Increase in hgb ≥2 g/dL at week 24, n (%)	3 (7)	5 (11)	2 (5)
Not RBC-TI[b] at BL, n (%)	37 (49)	36 (49)	35 (49)
Reduced transfusion-dependency[c] at week 24, n (%)	7 (19)	8 (22)	3 (9)
RBC-TD[d] at BL, n (%)	17 (23)	14 (19)	14 (19)
RBC-TI[b] at week 24, n (%)	1 (6)	1 (7)	0
SVR ≥35% at week 24, n (%)	11 (15)	16 (22)	2 (3)
Pts with BL hgb <10 g/dL, n/N (%)	7/45 (16)	9/44 (20)	0/41
Pts with BL hgb ≥10 g/dL, n/N (%)	4/30 (13)	7/30 (23)	2/31 (6)
Pts RBC-TD[d] at BL, n/N (%)	1/17 (6)	4/14 (29)	0/14
Pts not RBC-TD[d] at BL, n/N (%)	10/58 (17)	12/59 (20)	2/58 (3)
TSS reduction ≥50% at week 24, n (%)	13 (17)	24 (32)	10 (14)
Pts with BL hgb <10 g/dL, n/N (%)	6/45 (13)	12/44 (27)	5/41 (12)
Pts with BL hgb ≥10 g/dL, n/N (%)	7/30 (23)	12/30 (40)	5/31 (16)
Pts RBC-TD[d] at BL, n/N (%)	2/17 (12)	4/14 (29)	1/14 (7)
Pts not RBC-TD[d] at BL, n/N (%)	11/58 (19)	20/59 (34)	9/58 (16)
Safety population, n	**104**	**106**	**98[e]**
BL grade 3/4 anemia, n (%)	19 (18)	16 (15)	14 (14)
Any grade anemia TEAE, n (%)	29 (28)	25 (24)	15 (15)
Treatment-related anemia, n (%)	15 (14)	14 (13)	7 (7)
Grade 3/4 anemia TEAE, n (%)	28 (27)	24 (23)	14 (14)
Serious TEAE of anemia, n (%)	5 (5)	8 (8)	3 (3)
Dose reduction due to anemia, n (%)	4 (4)	1 (1)	0
Dose interruption due to anemia, n (%)	4 (4)	5 (5)	1 (1)
Discontinuation due to anemia, n (%)	2 (2)	3 (3)	0

TEAE, treatment-emergent adverse event.
[a]A ≥2 g/dL increase at week 24 or becoming RBC-TI for ≥8 weeks prior to week 24.
[b]No RBC transfusions for 3 months. Not RBC-TI at BL indicates pts with transfusion burden who did or did not meet definition of RBC-TD.
[c]A ≥50% decrease in average number of RBC transfusions per month for 3 months.
[d]An average of ≥2 RBC units/month for the preceding 3 months.
[e]Including 19 patients who had watchful waiting only.

Results: At BL, 59% of pts had anemia (hgb <10 g/dL); pts with BL anemia were more likely at BL to have platelet count <50,000/µL (51% *vs* 38%), primary MF (71% *vs* 57%), and high DIPSS score (41% *vs* 14%). For those with BL anemia regardless of whether RBC transfusion-dependent (TD), PAC did not worsen hgb levels and the rate of clinical improvement in hgb was higher for pts in the PAC BID arm (25%) *vs* PAC QD (13%) or BAT (12%) arms (Table). For the 49% of pts not RBC-transfusion independent (TI) at BL, reduction in RBC transfusion-dependency was achieved at higher rates with PAC QD (19%) and PAC BID (22%) *vs* BAT (9%); 2 PAC and 0 BAT pts achieved RBC-TI by week 24. In PAC pts, SVR ≥35% and TSS reduction ≥50% were observed regardless of BL anemia or RBC-TD (Table). At BL, 16% of pts in the safety population had grade 3 anemia. Incidence of TE anemia was highest during the first 16 weeks of PAC (20% and 9% weeks 1-8, 9% and 13% weeks 8-16 for QD and BID, respectively) and first 8 weeks of BAT (10%). For pts with BL hgb <10 *vs* ≥10g/dL, incidence of grade 3/4 TE anemia was similar with PAC QD (26% *vs* 28%, respectively), and lower in pts with BL hgb ≥10 g/dL with PAC BID (26% *vs* 16%) and BAT (20% *vs* 7%). All instances of TE serious anemia with PAC or BAT (Table) were in pts with BL hgb <10g/dL. Dose modifications or discontinuations due to anemia were uncommon (Table). No exposure-response relationship was evident for grade ≥2 TE anemia.

Summary/Conclusions: In pts with MF and BL thrombocytopenia, PAC treatment demonstrated SVR and symptom control, regardless of BL anemia or transfusion burden. PAC treatment led to clinical improvement in hgb and reduction of RBC transfusion needs *vs* BAT. Serious anemia, and dose modifications due to anemia were uncommon. PAC provides a treatment option for pts with MF, including those with BL thrombocytopenia and anemia, for whom available options are limited.

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COMBINATION THERAPY OF POMALIDOMIDE PLUS RUXOLITINIB IN MYELOFIBROSIS: RESULTS FROM COHORT 1 OF THE MPNSG-0212 TRIAL (NCT01644110)

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Background: Therapeutic options to address anemia in patients (pts) with Myelofibrosis (MF) are limited. In our MPNSG-0109 trial investigating pomalidomide (POM) in MF with cytopenia, anemia was improved in 14-29% of pts treated with 0.5-2mg POM once daily (QD) (Schlenk RF, Stegelmann F *et al.*, Leukemia 2016).

Aims: To evaluate synergistic effects of POM plus ruxolitinib (RUX), we are currently investigating the combination therapy within the MPNSG-0212 trial (NCT01644110).

Methods: MPNSG-0212 is designed as multicenter, single-arm phase-Ib/II trial with a target population of 38 pts in the first cohort. Primary endpoints are response rate after 12 cycles (28 days each) according to IWG-MRT (Tefferi *et al.*, Blood 2006) and red blood cell (RBC) transfusion independence criteria (Gale *et al.*, Leuk Res 2011). Secondary endpoints are safety, quality of life, progression-free, and overall survival. Main inclusion criterion is MF with anemia (Hb <10 g/dL and/or RBC transfusion dependency). While POM is given at the fixed dosage of 0.5mg QD, RUX is started at 10mg twice daily (BID) with dose modifications being allowed.

Results: Safety and efficacy data from 38 pts are presented. Median age of the pts is 73 years (range, 49-83); 19 pts (50%) previously received hydroxyurea, RUX, EPO, POM, and/or corticosteroids. Median hemoglobin (Hb) level at study entry was 8.6 g/dL (range, 5.4-11.7); 11 pts (29%) were RBC-transfusion-dependent. Median spleen size by ultrasound was 17.9 cm (range, 12.6 - 28). At baseline, 30 pts (79%) had constitutional symptoms. Mutations of *JAK2*, *MPL,* and *CALR* were present in 28 (74%), 3 (8%), and 7 (18%) pts, respectively; 26 (68%) were intermediate-2 risk and 9 (24%) high-risk according to the DIPSS (Passamonti et al, Blood 2010). Median time on treatment was 12 cycles (range, 2-33). In total, 881 adverse events (AE) CTCAE °1-5 were recorded. Worsening of anemia within the first 6 cycles was the most frequent AE occurring in 13 pts (34%) followed by fatigue in 12 (32%). Treatment interruptions were rare. There were 29 serious AE (SAE) CTCAE °2-5: most frequently, leukemic transformation (n=4), pneumonia (n=3), thoracic pain (n=3), abdominal pain (n=2), cardiac decompensation (n=2) and septic shock (n=2) occurred in 13 pts (34%) of which 5 were fatal (cardiac decompensation, pneumonia, AML and septic shock [n=2]); 5 SAE (Hb, °4; neuropathy, °3, hepatotoxicity, °3; fatigue, °3; and cardiac decompensation, °3) were considered study-related. 16 pts (42%) are currently on study treatment; 22 (58%) discontinued because of AE (n=6), withdrawal of consent (n=5), stable disease (SD) without objective response after 12 cycles (n=4), leukemic transformation (n=4) or death (n=3); 13 pts (34%) responded with spleen reduction (n=9) or ≥2 g/dL Hb increase / RBC transfusion independence (n=4). Of note, mean Hb increased continuously from 8.7 g/dL at baseline to 9.2 g/dL at the end of cycle 12; 12 pts (31%) continued treatment beyond cycle 12 because of response or SD plus clinical benefit (Hb increase <2g/dL, prolongation of transfusion intervals and/or improvement of symptoms); 6 pts (16%) stayed on treatment for >24 cycles.

Summary/Conclusions: In our study in advanced MF, combination of POM plus RUX was feasible with an objective response rate of 34%. Approximately one third of pts was treated beyond cycle 12 due to sustained therapeutic benefit. Based on the favourable safety profile and on results from our MPNSG-0109 trial, a step-wise increase of the POM dosage is intended for the 2nd study cohort to further improve anemia response.

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PACRITINIB (PAC) *VS* BEST AVAILABLE THERAPY (BAT), IN PATIENTS WITH MYELOFIBROSIS (MF) AND BASELINE (BL) THROMBOCYTOPENIA: FOCUS ON RUXOLITINIB (RUX)-TREATED PATIENTS IN THE PHASE 3 PERSIST-2 TRIAL

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Background: MF is a life-threatening hematologic malignancy characterized by splenomegaly, debilitating constitutional symptoms, and progressive cytopenias (anemia and thrombocytopenia). Currently, JAK1/2 inhibitor RUX is the only approved therapy for pts with MF. Although RUX has been shown to reduce splenomegaly and symptoms in pts with MF, it is associated with dose-limiting cytopenias and is not indicated for pts with platelets <50,000/µL. PAC is an oral kinase inhibitor with specificity for JAK2, FLT3, IRAK1, and CSF1R. In the phase 3 PERSIST-2 study of PAC *vs* BAT (including RUX) in pts with MF and BL thrombocytopenia, PAC was significantly more effective in terms of spleen volume reduction (SVR; P=0.001) and appeared to have a better benefit/risk profile *vs* BAT.

Aims: This analysis examines outcomes for pts with MF treated with RUX in the phase 3 PERSIST-2 study.

Methods: Pts with MF and BL platelet count ≤100,000/µL were randomized (N=311) 1:1:1 to PAC 400mg once-daily (QD), PAC 200mg twice-daily (BID), or BAT. BAT included any physician-selected treatments for MF, as well as symptom-directed treatment, or no treatment. The co-primary endpoints were the rates of pts achieving ≥35% SVR (by MRI/CT) and ≥50% reduction in total symptom score (TSS; MPN-SAF TSS 2.0) at week 24. Efficacy analyses used the intent-to-treat efficacy (ITT-E) population, which included all pts with randomization date allowing them to contribute data for a week 24 endpoint. Crossover from BAT to PAC was allowed after week 24 or splenic progression.

Results: RUX was the most commonly received active BAT; 44 (45%) BAT pts received RUX (Figure) and 32 (33%) received only RUX. Of the 44 pts who received RUX on study, 17 (39%) had BL platelet counts <50,000/µL and would not have been candidates for RUX by approved indication (or study protocol). Around half of PAC (46%) and BAT pts (51%) had received prior RUX, including 32/44 (73%) pts treated with RUX on study. One (3%) and 6 (19%) RUX pts (n=32 in ITT-E population) achieved SVR ≥35% and TSS reduction ≥50% at week 24, respectively, *vs* 11 (15%) and 13 (17%) PAC QD and 16 (22%) and 24 (32%) PAC BID pts. For PAC pts with prior RUX, SVR and TSS endpoints were achieved in 6% and 10% with QD, and 13% and 32% with BID, *vs* 20% and 23% with QD, and 28% and 33% with BID for PAC pts without prior RUX. Grade 3/4 adverse events (AEs) were reported in 76%, 70%, and 45% of PAC QD, PAC BID, and RUX pts, most commonly (≥9% in any arm) thrombocytopenia (31%, 32%), anemia (27%, 22%), and neutropenia (9%, 7%) with PAC QD and BID, respectively, and thrombocytopenia (16%) anemia (9%), and neutropenia (2%) with RUX. Dose reductions due to AEs occurred in 20%, 12%, and 11% of pts treated with PAC QD, PAC BID, and RUX, respectively, though the majority of RUX-treated pts began with 5mg dosing (Figure). Discontinuations due to AEs occurred in 19%, 15%, and 16% of pts treated with PAC QD, PAC BID, and RUX, respectively. Half (22/44) of RUX-treated pts crossed over to PAC treatment, at a median of 26.1 (95% CI 25.3-27.3) weeks. Of those 22, 19 pts remained on PAC treatment at the time of data cut-off, 7 for ≥24 weeks of PAC treatment (Figure 1).

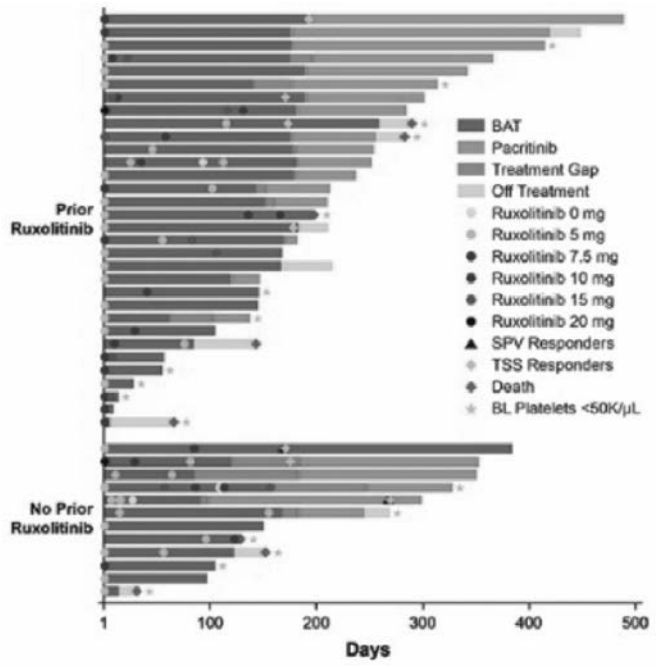

Figure 1.

Summary/Conclusions: In the phase 3 PERSIST-2 study of PAC *vs* BAT in pts with MF and BL thrombocytopenia, although 19% of RUX-treated pts achieved a 50% reduction in TSS, RUX-treated pts rarely achieved SVR ≥35% at week 24. Rates of grade 3/4 AEs were higher with PAC *vs* RUX treatment, though the majority of RUX-treated pts began with 5mg dosing. Rates of dose reductions and discontinuations due to AEs with PAC BID and RUX were similar. Following crossover to PAC in 22 RUX-treated pts, 19 remained on treatment at the time of data cut-off.

P702

SAFETY AND EFFICACY OF RUXOLITINIB (RUX) IN ELDERLY PATIENTS (≥75 YEARS) WITH MYELOFIBROSIS (MF): AN ANALYSIS FROM THE PHASE 3B, EXPANDED-ACCESS JUMP STUDY

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Background: RUX is a potent JAK1/JAK2 inhibitor that has led to reductions in splenomegaly and symptoms in patients (pts) with MF. Although few studies have assessed RUX in elderly pts, a recent analysis including 100 pts >75 y showed that RUX was safe and effective in these pts, with safety and efficacy outcomes similar to those in younger pts (Latagliata *et al. Blood*. 2016;128:4251). JUMP, a large (N=2233), phase 3b, expanded-access trial assessed safety and efficacy of RUX in pts with no access to RUX outside a clinical trial and included a cohort of pts ≥75 y.

Aims: To assess the safety and efficacy of RUX in pts aged ≥75 y.

Methods: Pts with high- or Int-2–MF, or Int-1–risk pts with a palpable (≥5 cm) spleen, were eligible. RUX starting doses were based on baseline platelet (PLT) counts (5mg bid [≥50 to <100×10^9/L], 15mg bid [100 to 200×10^9/L], or 20mg bid [>200×10^9/L]). Pts were ≥18 y; there was no maximum age limit. The primary endpoint was safety and tolerability of RUX. Secondary endpoints included changes in spleen length and symptoms.

Results: This analysis includes 416 pts (primary MF, 66%) who were ≥75 y and started treatment ≥1 y before data cutoff (01 Jan 2016). Baseline characteristics (median) were age, 78 y (range, 75-89 y); male, 57%; spleen length, 10 cm (0-35 cm); blast count ≥1%, 30.3%; hemoglobin, 101 g/L (<100 g/L, 46.9%); PLT count, 249×10^9/L (<100×10^9/L, 6.3%); ECOG PS <2, 84.9%. At data cutoff, more than half of pts remained on treatment or completed treatment per protocol (52.6%). As expected, a greater proportion of elderly pts discontinued RUX due to adverse events (AEs; 23.6%) or death (8.7%) than pts in the overall study (17.7% and 4.1%, respectively). Overall, 72.4% of pts had dose modifications (AEs, 58.4%), and 33.9% had an interruption (AEs, 31.5%). Safety of RUX in elderly pts was consistent with that in the overall population. Median exposure was 11 mo; mean average daily dose was 26.8mg (SD, 10.6). The most common hematologic grade 3/4 AEs were anemia (43.8%; overall, 34.1%) and thrombocytopenia (22.1%; overall, 16.3%). AEs (all grade [grade 3/4]) in >10% of pts included asthenia (18.3% [2.6%]), pyrexia (18.0% [2.6%]), dyspnea (14.4% [4.3%]), diarrhea (13.5% [1.9%]), fatigue (11.8% [2.4%]), peripheral edema (10.8% [0.2%]), and pneumonia (10.1% [7.2%]). Infections in >5% of pts included pneumonia (10.1%), urinary tract infection (7.0%), and bronchitis (5.1%). Herpes zoster occurred in 3.9% of pts. At wk 24, 56.4% (124/220) of pts had a ≥50% reduction from baseline in spleen length (overall, 56.6%), and 19.1% (42/220) had 25%-50% reductions (overall, 23.3%); rates were similar at wk 48 (54.6% [65/119] and 19.3% [23/119]; overall, 61.6% and 18.9%). Most pts (64.2%) achieved a ≥50% reduction at any time (**Figure 1**), similar to the overall population (70.2%). Pts also experienced significant improvements in symptoms. From wk 4 to 48, 42%-48% and 50%-57% of pts achieved a clinically meaningful response on the FACT-Lym TS and FACIT-Fatigue, respectively.

Figure. Best Percent Change From Baseline in Palpable Spleen Length at Any Time by Week 72

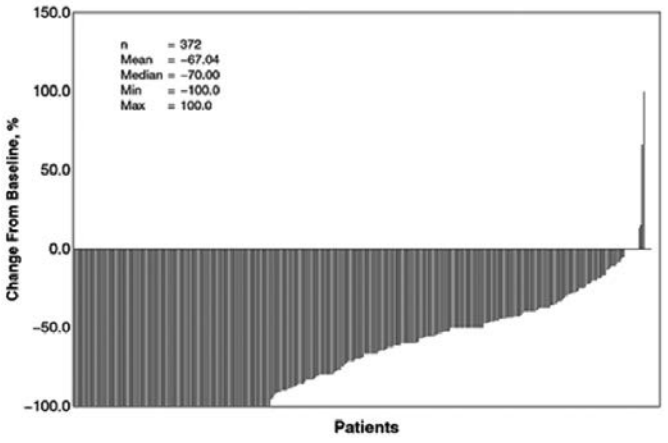

Figure 1.

Summary/Conclusions: This analysis included the largest cohort of elderly pts with MF treated with RUX to date. Consistent with the study by Latagliata et al, RUX was safe and effective in pts ≥75 y, with pts achieving reductions in splenomegaly and symptoms similar to those in the overall population, with comparable rates of AEs. Additionally, findings from our study were consistent with those of the COMFORT studies, which included few pts ≥75 y. Overall, our study provides further evidence that RUX is safe and effective in elderly pts with MF.

P703

PROGNOSTIC RISK MODELS FOR TRANSPLANT DECISION-MAKING IN MYELOFIBROSIS

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Background: Accurate disease risk stratification is crucial for transplant decision-making in myelofibrosis (MF). Although several prognostic models are available, it is unknown if they are equivalent in the way they distribute patients into risk groups and in their discriminatory power to predict survival.
Aims: We have compared the performance of the International Prognostic Scoring System (IPSS), dynamic IPSS (DIPSS), DIPSS-plus, and Rumi's score in a series of 544 MF patients aged 70 years or younger at time of diagnosis.
Methods: The Spanish Registry of Myelofibrosis is a nationwide, longitudinal registry contributed by centers associated to the *Grupo Español de Enfermedades Mieloproliferativas Filadelfia negativas* (GEMFIN). From January 2000 to January 2016, a total of 544 adult patients aged ≤ 70 years with primary MF (n=335) or secondary MF (n=209) had been included in the registry. Cases of the prefibrotic form of MF were not considered. Comparison of the relative power of each prognostic model to discriminate levels of risk was estimated by means of the Harrell's concordance index (C-index) and the R^2explained variation. All the statistical analyses were performed with IBM SPSS 22.0 and Stata 11.
Results: At the study closing date, median follow-up from diagnosis of MF was 3.35 years, 177 patients (33%) had died, and the remaining were censored alive. Sixty-nine patients (13%) had been submitted to allogeneic stem cell transplantation, after a median time of 20 months from MF diagnosis. The median projected survival of the overall series was 9.46 years (95% confidence interval: 7.44-10.59). Median survival was not reached for the low risk category of all classifications (and Rumi's very low risk category). The projected survival for patients in the intermediate-1 group (intermediate in the Rumi's score) and in the high risk group (very high risk in the Rumi's score) was comparable in the four models. By contrast, the Rumi's high risk group had a projected median survival of 9.2 years, whereas that of the intermediate-2 categories by the IPSS, DIPSS, and DIPSS-plus models was 6.6 years, 5.6 years, and 6.5 years, respectively. The number of patients in the intermediate-2 and high risk categories was smaller in the DIPSS than in the IPSS or the DIPSS-plus. Overall, the Rumi's score yielded the highest power to discriminate between risk categories as measured by the concordance index and the R^2 explained variation. However, the IPSS and DIPSS-plus were the best models to discriminate between the intermediate-1 and intermediate-2 risk categories, which is the critical cut-off point for patient selection to transplant.
Summary/Conclusions: In our contemporary series of MF patients only the highest risk categories of the current prognostication systems have a median survival below the 5-year threshold recommended for considering transplantation. Patient selection for transplant is quite dependent on which prognostication model is used for disease risk stratification.

P704

LEUKEMIC TRANSFORMATION AND SECOND CANCERS IN 3649 HIGH RISK ET PATIENTS IN THE EXELS STUDY

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Background: A common therapy for essential thrombocythemia (ET), hydroxycarbamide (HC), has mutagenic properties and there is potential for leukemogenicity and secondary cancers with this agent. In the EXELS study, we reported higher event rates for acute myeloid leukemia (AML) and other malignancies in HC-treated patients compared with those treated with anagrelide (ANA). However, there were large age differences between groups. Here, we account for age difference by estimating standard incidence ratios (SIRs) using country-specific cancer registration data.
Aims: To assess the risk of AML and non-hematological malignancies in patients treated with HC or ANA in the EXELS study.
Methods: Previous exposure to ANA and HC was based on patient history. SIRs were calculated using background rates retrieved from Cancer Incidence in Five Continents (CI5). Risk of AML after study enrolment was estimated by cumulative incidence. Minimum exposure time of 180 days was used to account for disease progression as a confounding factor. Informed consent was obtained before the start of the study.
Results: Overall, 3460 patients were exposed to HC, ANA or both at registration; 481 patients had ANA treatment, 2305 had HC treatment and 674 had been exposed to both drugs. The median age in ANA patients was 51 years, and 71 years in HC patients. One hundred and seventy four cases of non-hematologic cancer, including 35 cases of skin cancer, were recorded. SIRs for all malignancies were close to 1 for all treatment groups, indicating similar risks to the background population. For all skin cancers, including melanoma, the SIR for patients with HC treatment was higher than expected for the normal population and patients on ANA (1.15 *vs* 0.45). When melanoma was excluded, the figures changed only marginally. However, due to the low number of events, the CIs were wide, and no statistically significant difference was found between treatments. Sixty seven AML cases were reported; 39 AML cases were found in the HC group (person-years 8970, SIR 39.7), with another 20 AML cases in the group who switched from HC to ANA (person-years 2934, SIR 91.5). The risk ratio for AML developing in patients who switched from HC more than doubled (RR 2.30-2.52), irrespective of minimum exposure time. In contrast, no AML case was observed in the ANA-only group (person-years 1905, SIR 0) and there were only 3 AML cases in the group who switched from ANA to HC (person-years 802, SIR 68.5). Since the number of AML cases in the ANA group was 0, no statistical comparison could be made. Five AML cases were excluded from analysis since there was uncertainty about which drug was used first.

Table 1.

Table 1. Standardized incidence ratios (SIRs) with 95% confidence intervals (CIs). A minimum time of 180 days on a drug is required to be classified as exposed

	Observed	Expected	Person-years	SIR
Acute myeloid leukemia (C92.0)*				
ET without HC/ANA	0	0.08	331	0.00
ET with HC	39	0.98	8970	39.7
ET with ANA	0	0.08	1905	0.00
ET with HC and then ANA	20	0.22	2934	91.5
ET with ANA and then HC	3	0.04	802	68.5
Skin cancer (C43-C44)				
ET without HC/ANA	0	0.66	331	0.00
ET with HC	28	24.3	8967	1.15
ET with ANA	1	2.21	1905	0.45
ET with HC and then ANA	5	5.38	2941	0.93
ET with ANA and then HC	1	1.05	802	0.95
Skin cancer, other (C44)				
ET without HC/ANA	0	0.56	331	0.00
ET with HC	24	21.2	8968	1.13
ET with ANA	1	1.76	1905	0.57
ET with HC and then ANA	4	4.51	2941	0.89
ET with ANA and then HC	0	0.83	802	0.00
All cancers (C00-C96)				
ET without HC/ANA	0	4.64	331	0.00
ET with HC	159	166	8937	0.96
ET with ANA	8	15.4	1899	0.52
ET with HC and then ANA	53	39.1	2906	1.36
ET with ANA and then HC	7	8.31	795	0.84

ANA: anagrelide; HC: hydroxycarbamide. *Five AML patients with exposure to both HC and ANA not included in the analysis, since there is uncertainty about which drug was given first.

Summary/Conclusions: ET patients have a substantially increased risk for AML development; the SIR for AML was 40-fold higher than expected with HC exposure. In patients who switched from HC to ANA the SIR was approximately 90-fold higher, with 20 AML cases observed *versus* the expected 0.22 cases. The number of AML cases was much lower (n=3) in patients who switched from ANA to HC. It has been proposed that exposure of ET patients failing HC therapy to a second potentially leukemogenic agent should be avoided; yet these data suggest that even after switching to a non-leukemogenic agent, HC-treated patients still have an over 90-fold increased risk of AML. Our data reinforces concerns of a leukemogenic effect of HC, with a markedly higher risk in patients failing HC and switching to another drug, even if that drug is ANA. The caution advocated in the use of HC seems well advised.

P705

EPIDEMIOLOGY, OUTCOME AND RISK FACTORS FOR INFECTIOUS COMPLICATIONS IN MF PATIENTS RECEIVING RUXOLITINIB. A MULTICENTER STUDY ON 373 PATIENTS

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Background: Infectious complications represent one of most frequent cause of morbidity and mortality in Myelofibrosis (MF), the most severe of myeloproliferative neoplasms. Ruxolitinib (RUX), the first approved JAK1/2 inhibitor, significantly ameliorates disease-related splenomegaly and constitutional symptoms. Prospective controlled studies observed a high rate of infectious complications including opportunistic and unusual infections, probably due to its immune-suppressant activity. However, risk factors for infections in MF patients (pts) treated with RUX are still to be investigated.
Aims: To evaluate characteristics, incidence and risk factors for infections in RUX-exposed MF pts.
Methods: Clinical and laboratory data of MF pts treated with RUX were retrospectively collected from the database of 21 Italian Hematology Centers. Infections were defined according to the CTCAE.

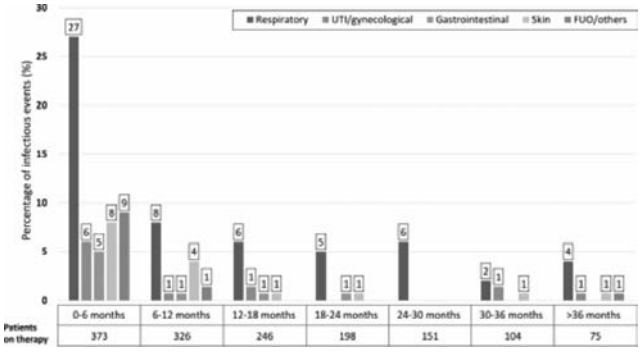

Figure 1.

Results: Overall, 373 pts received RUX between June 2011 and June 2016. At RUX start the clinical features were (median): age 68 years (27-89), ≥65y, 62%; male, 57%; Hb, 10.8g/dL (7-16.7); Hb <10g/dL, 40%; PLT, 246×10^9/L (33-1887); PLT <100×10^9/L, 10%; spleen enlargement, 97%; spleen length ≥10cm, 66%; constitutional symptoms, 52%. International Prognostic Score System (IPSS) was intermediate-1 (15%), intermediate-2 (46%), high (39%). *JAK2*^{V617F} mutation was detected in 255 out of 313 evaluated pts (81%). Karyotype was unfavorable in 15 out of 203 evaluable pts (7%). Previous infectious complications were recorded in 31 pts (8%). After a median RUX exposure of 20 months (range, 1-56), 101 pts (27%) experienced 129 infectious events ([3]grade 3, 33%), for an incidence rate of 14.9 cases for 100 pts/year. The rate of infections tended to decrease over time: 54% occurred within 6 months of therapy, 15% between 6 and 12 months, 9% between 12 and 18 months (p<0.0001). Respiratory tract infections were more frequently observed (73 events, 57%). Cutaneous, urinary tract and gastrointestinal infectious events were diagnosed in 15%, 10% and 7% of cases, respectively. In 14 cases fever of unknown origin was recorded (**Figure 1**). Etiological agents were isolated in 14 cases (11%): bacteria in 9 cases (gram+ 56%, gram- 22%, *C. difficile* diarrhea 22%) and fungi in 2 cases (pulmonary aspergillosis and oesophageal candidiasis); *Mycobacterium tuberculosis* was isolated in 3 cases. Herpes-virus reactivations occurred in 12 cases (9%). No patients reactivated hepatitis B virus. At last follow-up, 88 pts (24%) have died, in 10 cases (11%) due to infectious complication. Among baseline features, age≥65 years at RUX start (p<0.0001), previous infection (p=0.001), primary *vs* secondary MF (p=0.021) and high IPSS (p=0.029) significantly correlated with higher infectious risk. Notably, no differences were observed according presence of large (≥10cm) splenomegaly, higher (>20) total symptoms score, presence of cytopenias, Charlson comorbidity index (>2) and body mass index (<21 and >30). In multivariate analysis, PMF diagnosis (HR 1.6 CI95% 1.07-2.5), age≥65 years (HR 2.1 CI95% 1.3-3.3) and previous infection (HR 3 CI95% 1.7-5.4%) confirmed their negative prognostic association. Interestingly, RUX dosage, spleen response and hematological toxicities during treatment were not associated with infectious risk.
Summary/Conclusions: Infections occurred in around one-third of RUX-treated pts; the rate of infections tended to decrease over time, and were fatal in 11% of the cases. Advanced age, a previous infectious event and diagnosis of PMF seem to be the main contributors to infectious risk.

P706

TREATMENT AND MANAGEMENT OF PATIENTS WITH MPNS-FINDINGS FROM THE INTERNATIONAL MPN LANDMARK SURVEY

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Background: Patient (pts) with myeloproliferative neoplasms (MPNs), including myelofibrosis (MF), polycythemia vera (PV), and essential thrombocythemia (ET), experience a substantial disease burden. Pts are managed with watchful waiting or therapy, including aspirin, hydroxyurea (HU), and other drugs. The international MPN LANDMARK survey evaluated the patient-reported impact of MPNs in pts in 6 countries and identified current management strategies in these pts.
Aims: To analyze treatment strategies used by physicians and pts to manage MPNs.
Methods: This cross-sectional, internet-based survey was conducted in Australia, Canada, Germany, Italy, Japan, and the UK and was administered to pts with MPNs and to physicians treating pts with MPNs. Pts and physicians were recruited independently. We describe disease-management strategies in these pts.

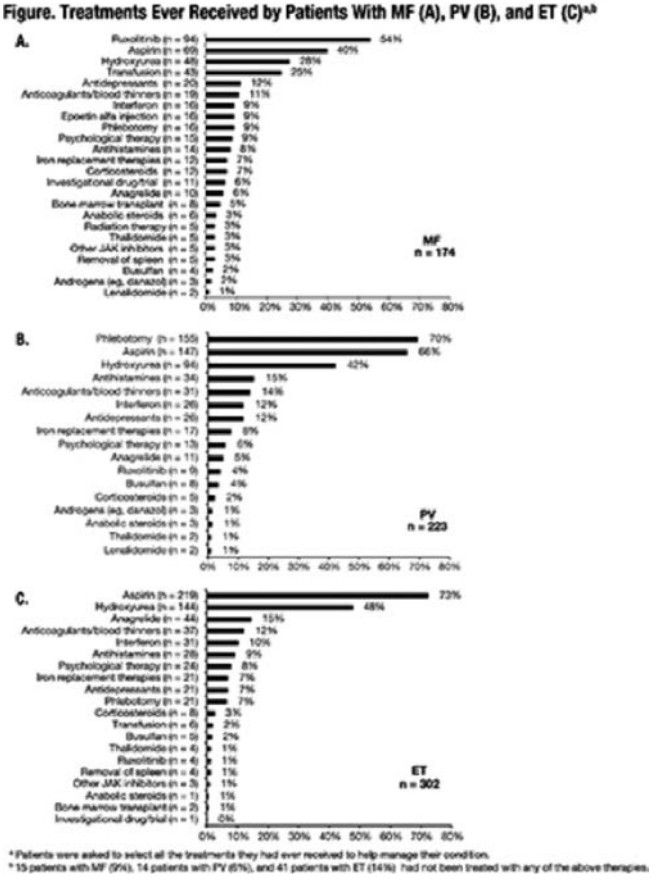

Figure 1.

Results: Overall, 699 pts (MF, n=223; PV, n=174; ET, n=302) and 219 physicians completed the survey. In line with treatment guidelines, main therapies used by pts were ruxolitinib (54%), aspirin (40%), and HU (28%) in MF; phlebotomy (PLB; 70%), aspirin (66%) and HU (42%) in PV; and aspirin (73%), HU (48%), and anagrelide (15%) in ET (**Figure 1.**). Most physicians reported prescribing the following: ruxolitinib (76%), transfusion (54%), and HU (53%) in MF; aspirin (79%), HU (77%) and PLB (67%) in PV; and aspirin (80%), HU (67%), and anagrelide (52%) in ET. Many physicians (51% MF, 47% PV, 49%

ET) chose watchful waiting to manage >25% of their pts at diagnosis; 22% of untreated pts had moderate to high (quartiles 3-4) overall symptom burden. Physicians primarily recommended treatment for pts experiencing severe symptoms (72% MF, 68% PV, 72% ET) or symptomatic splenomegaly (71% MF, 61% PV, 39% ET). PLB was mainly used to treat pts with PV. Of those who received PLB (n=155), 71% were very or somewhat satisfied; 25% were very or somewhat dissatisfied and felt that PLBs had a negative impact on their QOL. Similarly, 37% of physicians felt that PLB had a negative impact on pt QOL; PLB alone was insufficient for disease control in 38% of pts. Pts stopped PLB because physician deemed it no longer necessary (62%), pts felt worse after treatment (10%), and visit frequency was inconvenient (8%). Physician-reported reasons for stopping PLB were that visit frequency was inconvenient (38%), pts felt worse after treatment (35%), and lack of intravenous access (33%). HU use was assessed in pts with PV or ET. Of those who received HU (PV, n=95; ET, n=145), 78% and 74%, respectively, continued to receive HU; 19% and 22% were dissatisfied with HU therapy. Main reasons for stopping HU were lack of efficacy (29% PV, 13% ET) and toxicity (19% PV, 27% ET). Overall, 78% of physicians reported that up to 25% of their pts showed inadequate efficacy or intolerance of HU. Main measures of treatment success among pts were physician feedback (73% MF, 75% PV, 75% ET) and blood counts (72% MF, 67% PV, 74% ET). Lack of efficacy, side effects, and disease progression were key reasons for changing therapies.

Summary/Conclusions: Many pts with MPNs are managed with watchful waiting at diagnosis. Although most of these pts have a low symptom burden, 22% have a moderate to high burden, highlighting the need for proactive and standardized symptom assessments at diagnosis and over the course of treatment. Interestingly, a proportion of physicians and pts felt that phlebotomy had a high negative impact on pt QOL. Overall, pts consider physician feedback and blood counts to be important indicators of treatment success.

P707

SUCCESSFUL LONG-TERM MAINTENANCE OF PV PATIENTS WITH A MONTHLY SCHEDULE OF ROPEGINTERFERON ALFA-2B-AN UPDATE FROM THE PEGINVERA STUDY

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Background: Ropeginterferon alfa-2b is a novel long-acting monopegylated interferon alpha (IFNa) with Orphan designation in Europe and the U.S. Reduced dosing frequencies and favorable tolerability accompanied by robust clinical responses in patients with polycythemia vera (PV) have been reported over the first few years of treatment. Successful long-term, potentially life-long maintenance with high rates of adherence, compliance and treatment outcome remain important goals to be elucidated.

Aims: PEGINVERA phase I/II (NCT: 2010-018768-18), is a prospective, open-label, multicenter study investigating efficacy and safety of ropeginterferon alfa-2b in long-term treatment of patients with confirmed diagnosis of PV, pre-treated or naïve to cytoreductive therapy.

Methods: All patients responding well to the initial bi-weekly administration schedule and participating in the study for longer than one year, had the option to switch to a "once every 4 weeks"-schedule. The 2-week regimen (defined as the time period when all criteria for switching were fulfilled but the patient continued the 2-week regimen) was compared to the 4-week regimen (for a duration of 6 months after switch). The present analysis was focused on maintenance of efficacy.

Results: Data from the last available analysis include 29 patients remaining on study with a median treatment duration of 213 weeks. All 29 patients have completed at least 2 years of treatment (5 patients are in the 3rd year, 7 in the 4th year, 10 in the 5th year and 7 in the 6th year of treatment). Baseline characteristics of the study cohort during short-term treatment and two year safety and efficacy follow-up data were already presented earlier (Gisslinger *et al.* 2015). All of the 29 patients were switched to dosing once every 4 weeks. Median treatment duration at time of switch was 104 weeks (Q1-Q3: 69-124 weeks). All 29 patients remained on the 4-week schedule with a median observation duration of further approx. 2 years for this analysis, reflecting the excellent safety and tolerability profile of ropeginterferon alfa-2b in this setting. The percentage of patients maintaining their best haematological response according to ELN before (i.e. after median 104 weeks of treatment) and 6 months after switching to the 4-week regimen was consistent at 51.7%. Further, need for phlebotomy during the 6 months after switching did not increase (consistent 7/29 patients). Changes in haematological parameters and spleen size were minimal and without clinical relevance. Similarly, the percentages of patients maintaining their best molecular response were 62.1% and 58.6%, respectively (non-significant). Importantly, the majority of patients on ropeginterferon alfa-2b long-term treatment, developed a sustained reduction of mutant JAK2 allele burden to below 10%, a feature that can only be achieved by IFNa based therapies.

Summary/Conclusions: In summary, all patients remaining on ropeginterferon alfa-2b after a median of 2 years of initial treatment were successfully switched to a more convenient monthly long-term maintenance schedule, thereafter no patients discontinued, and all patients could be maintained on this schedule for currently another 2 years (trial still ongoing). These data underscore the expected long-term efficacy with regard to haematological, clinical and molecular parameters and the excellent safety/tolerability of ropeginterferon alfa-2b. Long-term maintenance treatment of PV patients using ropeginterferon-alfa 2b monthly is feasible, efficacious and well tolerable. Continuous patient-individual adaption of dosing regimen, including dose and dosing schedule, is recommended.

P708

NO IMPROVEMENT IN SURVIVAL OVER TIME FOR PHILADELPHIA NEGATIVE MYELOPROLIFERATIVE NEOPLASM PATIENTS WHO TRANSFORM TO ACCELERATED OR BLAST PHASE

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Background: The outcome of patients with Philadelphia negative myeloproliferative neoplasms (MPN) who transform to acute leukemia is abysmal. There have been no advances in targeted therapy for this cohort of patients or individualized treatment based on genomic information. Furthermore, no large studies have investigated the impact of molecular profiling on clinical outcome in patients with accelerated or blast phase of MPN.

Aims: To describe the clinical outcomes of patients with MPN who transform to accelerated or blast phase and evaluate the impact of genomic alterations on outcomes.

Methods: Eligibility criteria included: Prior diagnosis of Philadelphia negative MPN according to WHO 2008 criteria; evidence of transformation to accelerated (10-19% blasts in peripheral blood or bone marrow) or blast phase (≥20% blasts) and seen at Princess Margaret Cancer Center between January 1998 and February 2017. The primary endpoint was overall survival (OS): defined as the time from transformation to death or last follow-up. Secondary endpoints included survival based on curative *versus* non-curative approach and treatment over time. In addition the impact of mutations will be correlated with clinical outcomes and survival.

Figure 1: OS for patients treated from 1998 until June 2011 (arm a) and patients treated from July 2011 until 2017 (arm b)

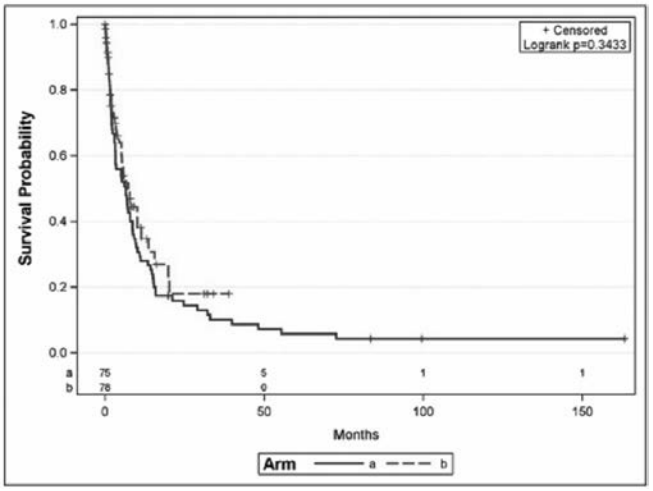

Figure 1.

Results: One hundred and eighty-seven patients who transformed to accelerated or blast phase with a prior diagnosis of MPN were identified at our insti-

tution. Twenty-nine patients were excluded: 17 for myelodysplastic/myeloproliferative overlap (MDS/MPN), six for insufficient information, five for not meeting criteria for accelerated or blast phase and one patient for a diagnosis of systemic mastocytosis. Of the 158 patients included in the study, the median age at the time of MPN diagnosis and leukemic transformation was 59 and 67 years respectively. Prior MPN diagnosis was: polycythemia vera (PV; n=25, 16%), essential thrombocythemia (ET; n=21, 13%), primary myelofibrosis (n=50, 32%), post ET myelofibrosis (PET MF; n=27, 17%), post PV myelofibrosis (PPV MF; n=24, 15%) and MPN-unclassifiable (n=11, 7%). One hundred and forty-two (90%) patients met the criteria for acute myeloid leukemia, thirteen (8%) had accelerated phase and 3 (2%) patients were diagnosed with myeloid sarcoma. Sixty-four (41%) patients were treated with curative intent including 27 (42%) patients who proceeded to hematopoietic cell transplantation, while 94 (59%) received non curative approach including low dose chemotherapy, hypomethylating agent, clinical trial or best supportive care. The median OS for the entire cohort was 6.5 months (95% CI: 5.06-8.01). In patients treated with curative intent median OS was 8.8 *versus* 3.2 months (p=0.003) for patients with non-curative intent. There was no difference in OS between historical controls treated between 1998 and 2011 when compared to a more recent cohort of patients (6.5 *vs* 7.3, p=0.34; see Figure 1). In 105 (67%) patients, NGS molecular profiling of 54 genes (39 hotspot region; 15 complete coding region coverage) was performed on peripheral blood or bone marrow samples using the TruSight Myeloid Sequencing Panel. Mutational data will be correlated with clinical outcomes and will be presented at the EHA annual conference (profiling completed, analysis ongoing).

Summary/Conclusions: Despite advances in systemic therapies and supportive care, there has been no significant improvement in survival for MPN patients who transform to accelerated or blast phase, confirming that current treatment approaches are ineffective. Results of molecular profiling may provide valuable insights and clues as to how to develop an individualized treatment approach for this cohort of patients.

Other Non-malignant hematopoietic disorders

P709

MASITINIB FOR TREATMENT OF SEVERELY SYMPTOMATIC INDOLENT SYSTEMIC MASTOCYTOSIS: ADDITIONAL EFFICACY ANALYSES FROM THE RANDOMIZED, PLACEBO-CONTROLLED, PHASE 3 STUDY

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Background: Masitinib, a selective oral tyrosine kinase inhibitor targeting wild-type KIT, LYN and FYN, was the first drug to demonstrate efficacy in a phase 3 setting (study AB06006) for treatment of patients with severe indolent systemic mastocytosis (ISM) who are unresponsive to existing, optimal symptomatic treatments. In *The Lancet* (Feb 11;389(10069):612-620), Lortholary and colleagues reported a significant and clinically meaningful treatment benefit for masitinib (6mg/kg/day over 24-weeks) *versus* placebo, with primary analysis based on cumulative response (≥75% improvement from baseline, timeframe weeks 8-24, comprising 5 visits at 4-week intervals) in at least one of four severe baseline symptoms (pruritus, flushes, depression, or fatigue) using repeated measures methodology for rare diseases (i.e. a longitudinal analysis with respect to symptoms as opposed to patient response rate at a single point in time). Eligible patients were aged 18–75 years and had ISM according to inclusion criteria that were slightly broader than the WHO classification.
Aims: To aide interpretation of this study's prospectively declared primary endpoint via comparison with additional efficacy analyses based on a cohort restricted to the WHO classification of ISM and more conventional patient-centric response endpoints.
Methods: Randomized, placebo-controlled, phase 3 study that included 135 severely symptomatic ISM patients, including the subvariant smoldering systemic mastocytosis (71 masitinib, 64 placebo), 80% of whom satisfied the WHO classification.
Results: Masitinib showed a significant improvement over placebo according to its pre-specified primary endpoint (mITT population), with a cumulative response of 18.7% *versus* 7.4%, respectively, odds ratio (OR) of 3.6 [95%CI 1.2-10.8], P=0.008 (with re-randomization). This outcome was confirmed in the WHO patient subgroup: 17.8% *versus* 8.0%, respectively, OR=3.25 [0.97-10.88], P=0.0317. Computing the primary analysis (mITT) according to cumulative response per patient (GEE model) was also positive: 26.7% *versus* 12.8%, respectively, OR=2.48 [1.16-5.31], P=0.0212; as was analysis according to individual patient response (Pearson chi-square): 40.3% *versus* 24.2%, respectively, P=0.0062. Response (per patient) on all severe baseline symptoms for at least one visit was: 16.4% *versus* 1.6%, respectively, P=0.0062. Finally, analysis of sustained response in all severe baseline symptoms over multiple visits was highly discriminatory between treatment-arms: for patients with 3 severe baseline symptoms masitinib generated a 12.5% response rate (≥75% improvement in each symptom) for 3 out of 5 visits, *versus* no response for placebo; and for patients with 2 severe baseline symptoms masitinib generated a response rate of 21.1%, 15.8% and 10.5% over at least 1, 2, and 3 visits, respectively, *versus* no response for placebo.
Summary/Conclusions: These post-hoc analyses confirm the clinical relevance, durability, and generalizability of the positive primary endpoint from study AB06006. Findings therefore support the conclusion that masitinib generates a significant therapeutic benefit in patients with severely symptomatic ISM who were unresponsive to optimal symptomatic treatments.

P710

THERAPY RESPONSE AND LONG-TERM OUTCOME OF 71 ADULT PATIENTS WITH HEMATOLOGICAL MALIGNANCY-ASSOCIATED HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS: A SINGLE INSTITUTION EXPERIENCE

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Background: Hemophagocytic lymphohistiocytosis (HLH) is a devastating disorder of uncontrolled immune activation characterized by clinicopathological evidence of extreme inflammation. Hematological malignancy-associated HLH (hM-HLH) has the worst outcome in comparison with any other form of HLH. hM-HLH can occur as the first manifestation of an occult malignancy, before start or during the treatment of known malignancy, or as the sign of a malignancy relapse or transformation to the more aggressive disease form.
Aims: The aim of the present study was to analyze the response to HLH therapy and overall survival of adult patients with hM-HLH.
Methods: From 2008 and onwards, data on adult patients referred to the Hematology Center Karolinska with suspected HLH were prospectively collected. Review concerned records of 142 adults with suspected HLH, hospitalized between Jan 2009 and Dec 2016. Of those, 71 patients with hematological malignancy were diagnosed with HLH and included to the present study. Hematological malignancy was defined as a neoplasm of lymphoid or myeloid origin. In all studied patients, the diagnosis of HLH was based on the HLH-2004 criteria. Infection as a possible additional trigger of HLH was carefully studied in all our hM-HLH patients. EBV and CMV DNA were routinely examined in whole blood, using RT quantitative PCR; other viruses (*e.g.* adenovirus, HSV, VZV, HHV6, influenza) were studied based on indications. Blood and urine cultures were performed in order to reveal any bacterial or fungal infections. Tests for fungal antigens, tuberculosis, and parasites were also performed if indicated. HLH treatment categories have included proapoptotic chemotherapy (etoposide) and immunosuppressive drugs, targeting hyperactivated macrophages (etoposide, corticosteroids, IVIG) and T cells (corticosteroids, cyclosporine A).

Figure Overall survival rate in different subgroups of hM-HLH patients in respect to concomitant infection during chemotherapy (*n*=17) and other patients (*n*=54).

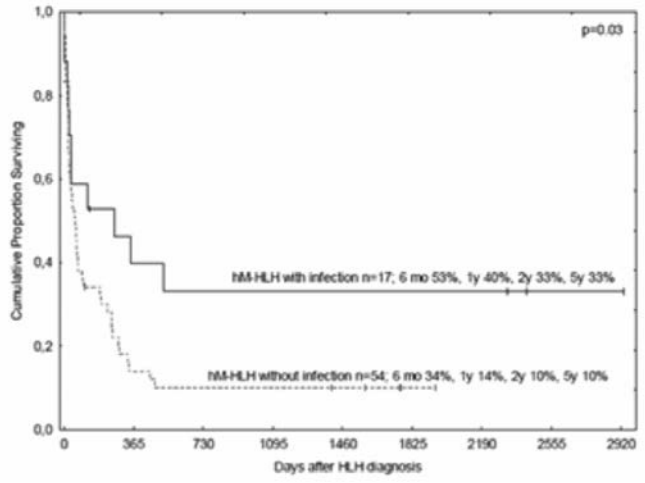

Figure 1.

Results: Seventy-one adults, aged 22–84 years, were diagnosed with aggressive hM-HLH during the 8-year period. Lymphoid malignancy was diagnosed in 42 patients and myeloid malignancy in 29 patients. Fifty-four (76%) patients developed HLH as a first manifestation of an unknown malignancy, during progressive disease, or malignancy relapse. The remaining 24% patients developed HLH during chemotherapy. In 14 patients, HLH therapy started before confirmation of HLH diagnosis, based on suspicion of HLH (mean 6.7±8.4 days; median 2 days; range 1-31 days). Seventeen patients started HLH therapy at the day of HLH diagnosis. In 36 patients HLH therapy started after confirmation of HLH diagnosis (mean 15.9±41.3 days; median 5 days; range 1–242 days). Forty of 71 (56%) patients with active HLH died, of which 20 had signs of progressive malignancy, 16 patients had generalized infection (bacterial - 12 patients, viral - 3 patients, fungal - 4 patients; some patients had more than one type of infection), and 2 patients experienced central nervous system bleeding. Thirty-one (44%) patients responded to HLH therapy and achieved remission of HLH. However, only 13 of 71 (18%) patients with hM-HLH were still alive after a median follow-up time of 50 months, despite the attempted treatment in 67 (94%) cases. The probability of overall survival (OS) from 6, 12, 24 and 60 months after HLH diagnosis were 39, 20, 15 and 15%, respectively. The patients who developed hM-HLH with concomitant infection during chemotherapy had significantly longer OS (p=0.03) compared to patients who had HLH solely attributed to malignancy (Figure 1).

Summary/Conclusions: HLH in the context of malignancy is still considered a challenge of adult hematology. hM-HLH is a highly lethal disorder in adults. The patients who develop hM-HLH with concomitant infection during chemotherapy show better survival than those who had HLH solely attributed to malignancy. Although poor outcome in some patients with M-HLH is related to malignancy progression, in some patients the lack of effective M-HLH therapy may further impede adequate treatment of malignancy.

P711

WHOLE-EXOME SEQUENCING IN CHILDREN WITH IMMUNE CYTOPENIA: THE APPLICABILITY AND CLINICAL IMPACT

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Background: Next generation sequencing is rapidly becoming the main diagnostic tool for precise identification of gene defects in human diseases. Apart from the identification of novel causal genes and pathogenic variants, the main research goal for now is to assess the impact and strategy of whole exome sequencing (WES) use in routine clinical evaluation.
Aims: We aimed to evaluate the benefits and drawbacks of using WES as a diagnostic method in patients with chronic early-onset autoimmune hemolytic anaemia (AIHA), idiopathic thrombocytopenic purpura (ITP) and immune neutropenia, or their combination (Evans syndrome). Most of these patients presented with additional symptoms of immune dysregulation, *e.g.* common variable immunodeficiency (CVID), lymphoproliferation, autoimmune disorders (diabetes mellitus 1, thyreoiditis).
Methods: 30 patients (age 0-39) were evaluated after an examination by a clinical geneticist and signing an informed consent. Sequencing libraries were prepared from peripheral blood DNA using Agilent SureSelectXT Human All Exon V5/6+UTR kit and sequenced with the Illumina NextSeq 500 system with a mean coverage of at least 30x.
Results: In 10 patients (33%) we were able to find likely causative mutations. In 3 patients (siblings) we identified a novel variant leading to CTLA4 deficiency, another novel variant in CTLA4 was identified together with an additional pathogenic variant in TSC1, causing a mixed phenotype. The genetic diagnosis of CTLA4 deficiency allowed for the use of CTLA4 agonist (Abatacept) treatment in 1 patient that led to improvement of his symptoms and disease stabilisation. However, after 6 months, the patient had developed agranulocytosis that led to hematopoietic stem cell transplantation. In 1 patient we were able to identify a gain-of-function variant in STAT3 that was recently described in immune dysregulation. In 3 patients we observed variants in genes typically described in connection with antibody deficiency (TACI, CD40L, and IKBKG). In 1 patient with chronic AIHA and ITP we found a novel heterozygous variant in TERT gene related to dyskeratosis congenita. In 1 patient with multiple congenital abnormalities and Evans syndrome we discovered a heterozygous variant in KMT2D gene causing Kabuki syndrome. The remainder of our patients harboured variants that posed a diagnostic challenge. In 4 of these cases we identified variants in genes involved in the pathogenesis of immune dysregulation, which are observed at lower frequencies also in healthy people (CASP10, PIK3CD). 12 patients (36%) had either only one hit in the genes reported causal in autosomal recessive diseases (e. g. ITK, LRBA) or we have not yet found any relevant aberration. In 4 patients we were able to identify novel variants in genes related to immune dysregulation. However, these variants require extensive validation studies, using patients' primary cells or manipulating established in-vitro or animal models with gene editing techniques, in order to prove the causality.
Summary/Conclusions: WES is a highly useful method that helps to identify the genetic cause of the disease in approximately one third of patients and enables targeted therapy. While targeted sequencing can further reduce costs and make analysis more straightforward, gene panels are quickly becoming obsolete as new causal variants are discovered in the rapidly evolving field of primary immunodeficiencies. Because of the heterogeneity of genetic causes of immune cytopenias, we recommend to use WES over targeted gene panel sequencing.

Support: NV15-30626A, 16-32568A, P302/12/G101.

P712

SEQUENCING OF THE HYPOXIA PATHWAY GENES IN PATIENTS WITH CONGENITAL ERYTHROCYTOSES BY NEXT GENERATION SEQUENCING

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Background: : Erythrocytoses are characterized by an elevated red cell mass. The most widely studied disease is Polycythemia Vera (PV), however, other types of erythrocytoses can be either inherited (Congenital Erythrocytosis-CE) or diagnosed in adult patients with no family history (Idiopathic Erythrocytosis-IE). CE/IE are not associated with myeloproliferation but they can be associated with severe thrombo-embolic or haemorrhagic events, pulmonary arterial hypertension and, rarely, tumours (hemangioblastoma, pheochromocytoma). The 8 genes identified so far as causing CE are involved (i) in the regulation of the hypoxia pathway, PHD2 (also called EGLN1), HIF-2A (EPAS1), VHL, (ii) in proliferation and differentiation of erythroid progenitors (EPOR), or (iii) in mature cell function, haemoglobins (HBB, HBA1, HBA2) or bisphosphoglyceratemutase (BPGM). However, in 80% of cases the cause remains unknown meaning that no proper diagnosis can be made, no prognosis or advice can be provided to CE/IE patients and their families, and no curative treatment exists
Aims: to (i) identify, (ii) collect and (iii) analyze the genomic abnormalities in patients suspected of CE
Methods: We created and developed a national network in France to analyze the genomic abnormalities in patients suspected of CE. The selection of patients was performed in order to exclude patients with Polycythemia Vera or obvious secondary erythrocytosis related to lung, cardiac or renal disorder. Next generation sequencing (NGS) has been used to analyse the presence of mutations in 28 genes (enlarged hypoxia pathway and other candidate genes).
Results: To date, samples from 140 patients have been recorded, among whom 46 have been tested using NGS approach. Variants in 14 (30%) patients [13 males and 1 female; median age 50 y. (12-71)] with unknown significance have been detected, including 4 in *PHD* genes, 5 in *HIF* genes, 4 in *LNK* genes (*SH2B3*) and 1 in *JAK2* gene. In patients with variants, a familial history of erythrocytosis was noted in 3. No independent thrombotic complication was reported in the 15 patients. In 2 patients (one with a *PHD2* and one with a *JAK2* variants), the erythropoietin was low, whereas for the others, the erythropoietin was normal. Of note, the median age of the patients was surprisingly high, suggesting that the diagnostic was not previously performed due to the absence of available tests. Functional studies were performed on *PHD2* variants: a significant decrease in the hydroxylase activity was noted for one variant, but not for the others. On the other hand, a decrease in the stability along time of the PHD2 protein was observed for two variants, underscoring the different mechanisms involved in the impairment of the PHD2 activity
Summary/Conclusions: NGS is a useful tool to explore mutations in CE, but identifies genetic variants in only 30% of patients with such disorder. Further exams including whole exome sequencing are planned to achieve a right diagnosis in the 70% remaining CE patients. *In vitro*, *in cellulo* and *in vivo* (including zebrafish model) functional studies are currently performed to validate the clinical relevance of the variants identified in the hypoxia pathway. They are compared to variants identified in the development of tumors in order to dissect the molecular mechanisms of this finely tuned pathway

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CHARACTERIZATION OF CD34+ HEMATOPOIETIC PRECURSORS IN INDOLENT SYSTEMIC MASTOCYTOSIS AND THEIR POTENTIAL ROLE IN EARLY DISSEMINATION OF THE DISEASE

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Background: Recent studies show that most systemic mastocytosis (SM) patients, including indolent SM (ISM) with (ISMs+) and without skin lesions (ISMs-), carry the *KIT* D816V mutation in PB leukocytes.
Aims: To investigate the potenctial association between the degree of involvement of BM hematopoiesis by the *KIT* D816V mutation and the distribution of different maturation-associated compartments of bone marrow (BM) and peripheral blood (PB) CD34+ hematopoietic precursors (HPC) in ISM, and identify the specific PB cell compartments that carry this mutation.
Methods: The distribution of different maturation-associated of BM and PB CD34+ HPC from 64 newly-diagnosed (*KIT*-mutated) ISM patients and 14 healthy controls was analyzed by flow cytometry. In 18 patients distinct FACS-purified PB cell compartments were also investigated for the *KIT* mutation.
Results: ISM patients showed higher percentages of both BM and PB MC-committed CD34+ HPC *vs* controls, particularly among ISM cases with MC-restricted *KIT* mutation (ISM_{MC}); this was associated with progressive blockade of maturation of CD34+ HPC to neutrophil lineage from ISM_{MC} to multilineage *KIT*-mutated cases (ISM_{ML}). Regarding the frequency of *KIT*-mutated cases and cell populations in PB, variable patterns were observed, the percentage of *KIT*-mutated PB CD34+ HPC, eosinophils, neutrophils, monocytes and T-cells increasing from ISMs-$_{MC}$ and ISMs+$_{MC}$ to ISM_{ML} patients.
Summary/Conclusions: Positivity for the *KIT* D816V mutation in PB of ISM is usually associated with (early) involvement of circulating CD34+ HPC and multiple myeloid cell populations, *KIT*-mutated PB CD34+ HPC potencially contributing to disease dissemination already at very early stages.

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MONOALLELIC VARIANTS IN GENES RELATED TO FAMILIAL HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS: REPORT FROM THE ITALIAN REGISTRY

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Background: Hemophagocytic Lymphohistiocytosis (HLH) is a life-threatening disease of children and adults caused by an impaired cytotoxic function of NK and CTL cells leading to a potentially fatal hyperinflammatory condition. Biallelic mutations in genes involved in the cytotoxic pathway are responsible for the familial form of the disease (FHL). Monoallelic mutations in the FHL-related genes have been reported in association with HLH and other diseases but their role remains to be understood.
Aims: To describe clinical, functional and genetic features of patients referred to the Italian HLH Registry, harboring monoallelic mutations in FHL-related genes.
Methods: Patients with complete or partial HLH diagnostic criteria and monoallelic mutations in at least one of the FHL-related genes were selected from the Italian Registry. Clinical data were collected by specific forms. Perforin expression and NK-cell degranulation measured as CD107a expression were performed by flow-cytometry. Molecular analysis was performed by Sanger or Next Generation sequencing.
Results: Of the 600 patients reported to the Registry, 54 (9%) were found to have monoallelic mutations in FHL-related genes. Their median age was 5 years (quartiles: 1.7, 3, 13, 50 years). Twenty-nine of the 54 patients (54%) fulfilled at least 5 of the 8 diagnostic criteria: fever (n=49/52; 94%), splenomegaly (n=37/50, 74%), cytopenia (n=43/50, 86%), hypertriglyceridemia (n=28/47, 60%), hypofibrinogenemia (n=1/46, 0.02%), hyperferritinemia (n=47/50, 94%; quartiles: 1.454, 7.937, 17.050, 140.000 ng/ml), hemophagocytosis (n=28/45, 62%), central nervous system involvement (n=12/41, 29%). Five patients died; of 8 who reactivated, 4 underwent bone marrow transplantation. An associated/underlying disease was reported in 34/54 (62%): rheumatologic/autoimmune disorder, 22 (11 Juvenile Idiopathic Arthritis, 2 Kawasaki disease, Systemic lupus erythematosus, Cogan Syndrome, colitis ulcerosa; 6 undefined); lymphoproliferative disease, 5 (2 acute lymphoblastic leukemia, 2 non-Hodgkin and 1 Hodgkin lymphoma), infectious diseases, 6 (2 EBV, 1 CMV, 1 parechovirus, 1 osteomyelitis, 1 myocarditis) and 1 pigment deficiency disease. Functional tests were performed in 32/54 (60%), showing impaired degranulation in 42% (n=13/31) and defective perforin expression in 43% (n=16/37). The genetic study revealed 31 monoallelic variants in *PRF1* (n=10)*, UNC13D* (n=9), *STX11* (n=5), *STXBP2* (n=12), *LYST* (n=1) and *Rab27A* (n=1). Four variants were reported as polymorphism. Of the 25 remaining, 23 were missense (9 predicted as benign and 14 as probably damaging), 1 STOP and 1 frameshift both predicted as probably damaging. Two patients had mutations in 2 different genes.
Summary/Conclusions: 9% of patients reported to the Italian HLH Registry carries monoallelic variants in at least one FHL-related genes, including 37% being probably damaging. Altogether these patients are characterized by later onset, partial/milder disease, and partial functional defect. Thus, monoallelic mutation in one FHL-related gene defines a predisposing factor for HLH.

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PRIMARY AND CONGENITAL ERYTHROCYTOSIS IN PEDIATRICS: THE EXPERIENCE OF ITALIAN CENTERS

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Background: Erythrocytosis, (i.e. increased levels of hemoglobin /hematrocit (Hb/Htc) >95percentile for age and sex), is rarely found in pediatric or adolescent age. Presence of familial cases, presentation at birth or presence of known mutations, as well as exclusion of secondary causes identifies primary (PE) or congenital secondary forms (CE). However, many cases still lack evident etiological definition (Idiopatic E.). Moreover, natural course and treatment are still anecdotally reported.
Aims: Here we present our experience in a large and heterogeous series of children with absolute erythrocytosis. The aims is to identify a possible clinical and diagnostic approach to children with erythrocytosis
Methods: All children with E. who lacked evidence of reactive origin were consecutively referred to our laboratory for molecular evaluation. Molecular analysis of the main involved genes (VHL, HIF2A, EPOR, JAK2, PHD2) was performed by allele specific PCR, PCR on direct DNA sequencing. Erythopoietic Colony Essay (EEC) was performed on peripheral blood with and without cytokines. Clinical features and treatment choices were reported by referring clinicians (table 1).

Table 1.

Category	Families (n)	Children (adults) (n)	Gene Defects /transmission	EPO	Splenomegaly	Thrombosis	Vascular symptoms	Notes
Familial	2	2M, 2(1M/2F)	2 VHL (heterozygous)	Elevated	YES	17/02	YES	YES
Familial (defect not known)	3	5 (3M/2F) 2 Ad M	2 families with siblings (AR?) 1 fam AD	Elevated	YES	18.3/62	YES	ASA,Phlebotomy ?
Congenital E	sporadic	1 (M)	[illegible] (Type 4)	Elevated	no	18/61	no	no
Glioma	2	4 2M/2F 1 Ad M	[illegible]	N	NO	18.5/48 15.7/40 F	Y / N	SWANN
		1F	[illegible] Polimac	N	NO	17/48	no	NO
Down, comorbidity		2 M 2F Renal, CNS, [illegible]	1 [illegible] VHL (c.463+a>g) (GANGLIONUROMA)	N	NO	17.3/48.5	Y Cerebral [illegible]	NO phlebotomy
SD		4M 1F	1 [illegible] polymorphic	N	NO	17/48	no	NO phlebotomy
Sporadic, classic PD		18M /3 F	NO			18.8/48.1	8 YES (headach e, abdominal pain, [illegible], & dizziness, pruritus) 13 NO	
PV		1F	JAK2V617F	Suppressed	Y	18.7/52	Y	ASA, phlebotomy

Results: Patients were group according to the definitons of absolute Eryhtrocytosis. A total of 44 pediatric cases were identified (less than 18 years old). There were 7 families, where 5 adults were also found polyglobulic. However, in only 4 families a defect was identified (2 VHL, and 2 Hb variants). One HIF positive case was found sporadic. Most Hb variants were not symptomatic, while all other familiar cases had splenomegaly and vascular symptoms.Among non familial, non genetic cases, 5 children were affected by Down Syndrome; 4 children had severe renal or cerebral disease. In one 4 year old girl, with a polymorphic VHL variant, who presented with arterial hypertension, a small size ganglioneuroma was found after a 5yrs follow-up. In 21 cases non causes could be identified. They were mostly male (n18); presented at adolescent age with advanced puberal status (n17); many were symptomatic (6). Only one 9 year old girl was diagnosed with Polycytemia vera (JAK2V617F positive). Treatment varied according to physician decisions and presence of vascular symptoms, 6 children received ASA and 11 were phlebotomysed. In two older patients severe vascular complications were observed (arterial thrombosis), even with Htc<45%.
Summary/Conclusions: This series shows the heterogeneity of Eryhtrocytosis as found in pediatrics. Extensive clinical and genetic analysis are required but still a large number of cases lack clear definitions. The usefulness of antiaggregation and phlebotomy is not proved.

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NEUROLOGICAL INVOLVEMENT IN EVANS SYNDROME AND CHRONIC HEMOLYTIC AUTOIMMUNE ANEMIA OF CHILDREN: DESCRIPTION, EVOLUTION AND GENETICS

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Background: Neurological involvement is poorly described in autoimmune cytopenias (AIC), containing immune thrombocytopenia (ITP), autoimmune hemolytic anemia (AIHA) and Evans syndrome (ES). This association suggests an underlying primary immunodeficiency (PID).
Aims: To describe neurological involvement observed in autoimmune cytopenias, evolution under treatment and PID profiles in these patients.
Methods: OBS'CEREVANCE is a French nationwide prospective cohort including children with an AIC since 2004. Patients with a neurological involvement were analyzed excluding neurological symptoms related to a traumatism or isolated febrile seizures. Clinical, radiological and biological data were collected from primary centers. Centralized radiological review was performed. Genetic analyses were performed by Sanger gene sequencing or gene-panel based next generation sequencing.
Results: On October 2016, among the 1,167 patients of the cohort (371 AHAI, 615 ITP, 181 ES), a significant neurological involvement was observed in 8 patients from 7 centers. With a median (range) follow-up of 12 years (6-26.5), 7 children had ES (including autoimmune neutropenia in 5) and 1 child had isolated AIHA. Median age at initial cytopenia was 11.5 years (1.6-15.8). At the last follow-up point, AIC were in partial or complete remission for all patients. Neurological symptoms appeared with a mean delay of 6 years (2.5-18) after AIC onset. The symptomatology was: seizures (n=4), cranial nerve palsy (n=2), Brown-Sequard syndrome (n=2) and / or sensory neuronopathy (n=1). No infectious pathogens were identified. MRI showed multiple (n=6) or unique (n=2) inflammatory lesions with hyperintense T2 signal in all patients, gadolinium-enhancing lesions in 7 and perilesional edema in 5. Five patients had a total of 8 biopsies, which confirmed the inflammatory process with macrophagic (n=3) or lymphoplasmocytic (n=5) infiltrates. In 4 cases, a lymphocytic meningitis was associated. Non-neurological organ involvement was present in all patients, mainly pulmonary nodules (n=6) and lymphoproliferation (n=4). All patients had an abnormal immunophenotype, with T-cell (n=7) or B-cell (n=3) deficiency and hypogammaglobulinemia was present in 7 of the 8 cases. Patients have been given steroids (n=6), intravenous immunoglobulins (n=2) or immunosuppressive treatment (n=3, Ciclosporin, Mycophenolate Mofetil and Methotrexate), improving symptomatology and MRI for all. Five patients relapsed and 3 patients had an asymptomatic radiological progression. At the last follow up point, all patients had neurological sequels and 7 persisting radiological abnormalities. Four out of the 6 patients analyzed had a PID: 22q11.2 microdeletion (n=1), heterozygous *CTLA* mutation (n=2) or homozygous *LRBA* mutation (n=1).
Summary/Conclusions: Neurological involvement is a rare and severe late event in the course of childhood ES, or exceptionally AHAI, that may reveal various underlying PID. Complete imaging and pathology examination highlight a causative immune dysregulation and could guide specific therapeutic strategies.

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AUTOIMMUNE NEUTROPENIA OF CHILDHOOD SECONDARY TO OTHER AUTOIMMUNE DISORDERS: DATA FROM THE ITALIAN NEUTROPENIA REGISTRY

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Background: The most frequent Autoimmune Neutropenia (AIN) in childhood is the primary type (p-AIN), whereas in adults AIN is mostly represented by secondary neutropenias, which can be associated to infection, drug administration, immunodeficiency, neoplasms, bone marrow transplantation or other autoimmune disorders.
Aims: To describe clinic and laboratory findings in children affected by AIN secondary to other autoimmune diseases (s-AIN).
Methods: This registry study analyzes 26 patients affected by s-AIN enrolled in the Italian neutropenia registry of A.I.E.O.P. (Associazione Italiana di Onco-Ematologia Pediatrica) over a 15-year time-span: this cohort, the largest ever described, was compared to 263 patients affected by p-AIN enrolled in the Registry in the same period.

Table 1.

Sex	Age at onset (y)	Recovery from AIN	Age at recovery (y)	Associated autoimmunity
M	15,12	yes	15,89	ES (AIN+thrombocytopenia); DAT pos)
F	13,88	yes	14,12	Celiac disease; Autoimmune thyroiditis (anti-TPO pos)
I	2	no		Autoimmune hepatitis; ES (AIN+ thrombocytopenia); DAT pos
F	7,87	no		IDDM; ANA 1:320
M	5,6	no		Celiac disease; ES (AIN+ thrombocytopenia); DAT pos
M	10,82	no		ES (tri-lineage); ANA 1:160; GH deficiency
F	2,76	no		ES (tri-lineage); autoimmune encephalitis
M	13,43	no		ES (AIN+ thrombocytopenia); DAT pos
M	4,02	no		ES (AIN+ thrombocytopenia); ANA 1:320; arthritis
M	0,5	no		ES (AIN + AIHA)
M	17,17	no		ES (tri-lineage); ANA 1:160
I	17,49	no		ES (tri-lineage); ANA 1:640; SLE
F	12,33	no		ES (tri-lineage); ANA 1:160;
F	15,55	no		Autoimmune thyroiditis (anti-TPO pos; anti-TG pos)
F	13,45	no		Autoimmune thyroiditis (anti-TPO pos; anti-TG pos)
I	7,45	no		Autoimmune thyroiditis (anti-TG pos)
M	7,46	no		Autoimmune thyroiditis (anti-TPO pos)
I	16,47	no		Autoimmune thyroiditis (anti-TPO pos; anti-TG pos); ANA 1:1280
M	9,54	no		Autoimmune thyroiditis (anti-TG pos); ANA 1:320
F	11,98	no		GH deficiency; ANA 1:320
I	7,4	no		GH deficiency; ANA 1:320
M	12,49	no		IDDM
F	0,61	no		ANA 1:160; arthralgia; ASMA pos; anti-GAD pos
I	4,2	no		ANA 1:1280; arthralgia
I	2,65	no		ANA 1:320; arthralgia; anti-DNA pos
I	4,1	no		ANA 1:320; arthralgia

ES: ES syndrome; pos: positivity; AIN: autoimmune neutropenia; DAT: direct antiglobulin test; anti-TPO: *anti-thyroid* peroxidase antibodies; IDDM: insulin dependent diabetes mellitus; ANA: *antinuclear antibodies*; *GH*: growth hormone; anti TG: AIHA: autoimmune hemolytic anemia; anti TG: *anti thyroglobulin* antibodies; ASMA: anti smooth muscle antibodies; anti GAD: anti glutamic acid decarboxylase antibodies; anti-DNA: *anti*-double stranded DNA antibodies; SLE: *Systemic lupus* erythematosus.

Results: Specific characteristics of s-AIN patients are presented in Figure 1. The prevalence of former preterm babies among p-AIN (and not s-AIN) patients was significantly higher than in a cohort of 487 consecutively hospitalized children (p=0.008362). The median age of onset of AIN was 0.77 year and 10.07 year in p-AIN and s-AIN respectively (p=1.105e-12). The prevalence of selected IgA deficiency was 3% in p-AIN and 13.6% in s-AIN children: both prevalences were significantly higher than that (0.21%) of a group of 470 controls (p=0.0009 in p-AIN and p=7.239e-12 in s-AIN). Median value of neutrophils was lower in p-AIN (0.45 x 10^9/L) than in s-AIN 0.63 x 10^9/L (p =0.03); median value of lymphocytes was significantly reduced (p=6.29e-11) in s-AIN (1.58 x 10^9/L) *vs* p-AIN (4.36 x 10^9/L) group. Leucopenia (p=1.80e-07) and severe infections (p=0.0001) occurred more frequently in s-AIN; monocytosis (p=0.039) and spontaneous remission (p=3.21e-11) in p-AIN. GCSF was used in 6.9% of the p-AIN and 23.1% of the s-AIN patients (p=0.0045). Neutropenia appeared contemporarily to other autoimmune manifestations in 11/26 s-AIN patients (42.3%), appeared firstly in 8/26 patients (30.7%) (median and mean time of appearance of other autoimmune signs: 440 and 987 days respectively) and later in 7/26 patients (26.9%) (median and mean time of appearance of s-AIN: 558.5 and 865.3 days respectively). Evans Syndrome (ES) and autoimmune thyroiditis (AT) were the most common secondary autoimmune diseases (11 and 7 patients, respectively), whereas 7 s-AIN patients presented not previously reported associations: 3 with GH deficiency, 2 with coeliac disease (CD), 1 with autoimmune hepatitis (AH) and 1 with autoimmune encephalitis. In 6 children s-AIN was associated with more than one defined autoimmune disease and in 4 children with *undefined* autoimmune signs characterized by arthralgia and ANA positivity. Finally, only 2/26 patients presented spontaneous remission: a boy who recovered from ES and one patient, affected by both AT and CD who, after starting gluten-free diet, recovered from s-AIN (and not from AT). A third girl suffering from both AH and bi-lineage ES (thrombocytopenia + AIN) has been maintained, 30 months after the stop therapy, a stable remission from AH and thrombocytopenia (but not from s-AIN).

Summary/Conclusions: p-AIN is in the vast majority of cases a benign and self-limiting disorder typically occurring under 2-3 years old whereas s-AIN is a more severe disease, usually appearing after the first 5 years of life, usually associated to lymphocytopenia and with a highly frequent tendency to become chronic.

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PAROXYSMAL NOCTURNAL HEMOGLOBINURIA TREATMENT DURING PREGNANCY

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a life-threatening disorder with a high risk of thrombosis. Targeted therapy radically changed the prognosis in PNH. Therefore issues of reproductive health in PNH patients are becoming very important. Recently the management of PNH during pregnancy has been challenging because of the high risk of maternal morbidity and frequent pregnancy loss. The combination of targeted therapy with eculizumab and anticoagulants made it possible not only to increase the survival rate, but also to improve the quality of life
Aims: We compared the pregnancy outcomes in PNH patients on eculizumab treatment and retrospective data of pregnancies on symptomatic therapy only.
Methods: Since 1999 we have analyzed 32 pregnancies in PNH patients. 17 patients (group 1) from 2013 exposed to eculizumab during pregnancy with anticoagulants. Other 15 women (group 2) received only symptomatic therapy. The median of PNH granulocyte clone at that time was 74,7% (23-99). PNH diagnosed before the pregnancy in all cases. 64,3% of them had previously received immunosuppressive treatment of aplastic anemia. 18,7% patients registered venous thromboses before conception. 92,9% of patients had been using eculizumab prior to becoming pregnant, mean duration of therapy was 21 months (4-44). Anticoagulation with low molecular weight heparin was used in 85,7% pregnancies.
Results: Clinical manifestations of hemolysis significantly regressed during eculizumab treatment: normalization of LDH was registered in 76,5% patients. Without eculizumab LDH level increased in all pregnant patients. No maternal death and thrombotic events have been observed. 42,9% of patients required a dose adjustment due to breakthrough hemolysis (a dose increase and/or more frequent use of eculizumab). Pregnancy complications were less frequent with eculizumab: abortion threat 35,3% *vs* 85,7%, fetal growth retardation syndrome 7,1% *vs* 21,4%, preeclampsia 5,9% *vs* 14,3%. Transfusion rate was higher without eculizumab (86,7% *vs* 41,2%). Pregnancies resulted in the birth in 100% patients exposed eculizumab and 42,9% on supportive treatment. Mean birth weight 2560 g (450-3550). Most of newborns (87,5%) are healthy, 83,3% of them received breastfeeding without complications both on eculizumab and without it.
Summary/Conclusions: We can conclude that pregnancy outcomes in PNH patients with eculizumab are much better than with symptomatic therapy only. Our data demonstrate the possibility of safe therapy with eculizumab in pregnant women. Pregnancy does not worsen the prognosis of PNH in the case of targeted and adequate supportive therapy. There is no difference in health between infants born by mothers with PNH and the newborns from general population.

Platelet disorders: Clinical

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LONG-TERM RESPONSE TO ORAL ELIGLUSTAT IN TREATMENT-NAÏVE ADULTS WITH GAUCHER DISEASE TYPE 1: FINAL EFFICACY AND SAFETY RESULTS FROM A PHASE 2 CLINICAL TRIAL AFTER 8 YEARS OF TREATMENT

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Background: In Gaucher disease type 1 (GD1), deficient lysosomal acid β-glucosidase activity leads to accumulation of glucosylceramide, primarily in macrophages (Gaucher cells), which deposit in the spleen, liver, and bone marrow, leading to thrombocytopenia, anemia, hepatosplenomegaly, and skeletal disease. Hematologists often identify and manage the disease. Intravenous enzyme replacement therapy (ERT) with recombinant acid β-glucosidase has been the mainstay of therapy for GD1. Eliglustat is an oral substrate reduction therapy approved as first-line treatment for adults with GD1 with poor, intermediate, or extensive CYP2D6-metabolizer phenotypes (>90% of patients). Phase 3 trials demonstrated safety and efficacy of eliglustat in naïve patients (Mistry *et al. JAMA*. 2015) and safety and stability in patients switching from long-term ERT (Cox *et al. Blood.* 2017). We report the final 8-year results of an open-label Phase 2 trial (NCT00358150, Sanofi Genzyme) in previously untreated adults with GD1. These data build on 1-, 2-, and 4-year data showing sustained improvements in hematologic parameters, organ volumes, disease-related biomarkers, and measures of bone health (Lukina *et al. Blood Cells Mol Dis*. 2014).
Aims: Report long-term efficacy and safety after long-term eliglustat treatment.
Methods: Adult GD1 patients who had splenomegaly with thrombocytopenia and/or anemia received 50 or 100mg eliglustat tartrate (equivalent to 42 or 84mg eliglustat) twice daily, dosed by plasma trough levels. Efficacy outcomes included changes in hemoglobin, platelets, spleen and liver volumes, disease-related biomarker levels, skeletal manifestations, and achievement of therapeutic goals for anemia, thrombocytopenia, splenomegaly, and hepatomegaly (Pastores *et al. Semin Hematol.* 2004; Lukina *et al. Blood.* 2010).
Results: Of 26 enrolled patients, 19 completed the trial and 7 withdrew: 2 on the first day of treatment due to asymptomatic nonsustained ventricular tachycardia detected during routine monitoring (plasma levels of eliglustat were undetectable); 1 after 1 year due to progression of a bone lesion (retrospectively identified at baseline); 1 chose to withdraw after 2 years; and 3 due to pregnancy. After 8 years of eliglustat, mean (±SD) hemoglobin level and platelet count increased by 2.1±1.7 g/dL (from 11.3±1.6 to 13.4±1.3 g/dL) and 110% (from 67.5±21.1 to 130.7±59.8 x10^9/L), respectively. Mean spleen and liver volumes (multiples of normal, MN) decreased by 68% (from 17.3±10.4 to 5.1±3.5 MN) and 31% (from 1.6±0.5 to 1.1±0.3 MN), respectively. All patients met ≥3 of 4 long-term therapeutic goals (spleen, 100% of patients; liver, 100%; hemoglobin, 93%; platelets, 53%) by 7-8 years. Median chitotriosidase levels decreased by 84%, CCL-18 by 82%, and glucosylsphingosine (Lyso GL-1) by 88%; plasma GL-1 normalized. Total mean lumbar spine bone mineral density increased by 0.12 g/cm^2; mean Z-score increased by 0.88 (from -1.27±1.02 to -0.39±1.13) and mean T-score by 0.95 (from -1.64±1.07 to -0.69±1.31). Eliglustat was well-tolerated. All quality of life measures (SF-36, fatigue severity score, Gaucher disease severity score) showed improvement over time. Most adverse events in this long-term trial were mild or moderate in severity (98%, 342/348) and considered unrelated (94%, 328/348) to treatment.
Summary/Conclusions: After 8 years of treatment with eliglustat, clinically meaningful improvements in hematologic, visceral, biomarker, and bone parameters continued or were maintained among patients in this Phase 2 trial. No new safety concerns emerged.

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REAL WORLD EVIDENCE ON DRUG UTILIZATION PATTERNS OF ELTROMBOPAG IN ADULT PATIENTS WITH IMMUNE THROMBOCYTOPENIA: REVIEU (REVOLADE™ [ELTROMBOPAG] IN SELECTED COUNTRIES IN THE EUROPEAN UNION) STUDY

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Background: Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by isolated thrombocytopenia, with platelet counts <100x10^9/L. Eltrombopag is an oral small-molecule nonpeptide thrombopoietin-receptor agonist that has shown to increase platelet production. It is approved for the management of patients with chronic ITP (aged ≥1 year) who are refractory to other treatments (eg, corticosteroids, immunoglobulins). The recommended eltrombopag dose in patients with chronic ITP is 25mg once daily, OD (East Asians, 12.5mg OD or 25mg every other day) in pediatrics aged 1-5 years, and 50mg OD (East Asians, 25mg OD) in adults and pediatrics aged 6-17 years at initiation, followed by dose adjustment to a maximum of 75mg OD based on platelet counts. REVIEU study was conducted in accordance with risk management plan in five European Union (EU) countries to document eltrombopag utilization patterns in real-world practice. Here, we report the eltrombopag data on the subset of adult patients (aged ≥18 years) with ITP as primary diagnosis.
Aims: To evaluate the real-world data to determine drug utilization patterns among adult patients with ITP receiving eltrombopag within five EU countries.
Methods: REVIEU study was a multinational, multicenter, retrospective, medical chart review in patients with a documented past treatment with eltrombopag between the period immediately after first approval/launch in May 2010 and September 2014 (ie, dispensed at least once by the pharmacy and patient received at least one dose) for whatever reason. Patients who participated or were participating in a randomized eltrombopag clinical trial were excluded.

Table 1.

Table 1. Proportion of patients with platelet counts by ITP disease phase, and by eltrombopag dose

Platelet count and eltrombopag dose, n (%)	Acute ITP (n=39)		Persistent ITP (n=31)		Chronic ITP (n=216)		Total (N=287)	
	At initiation (n=36)	At dose change (n=25)	At initiation (n=30)	At dose change (n=29)	At initiation (n=211)	At dose change (n=164)	At initiation (n=277)	At dose change (n=218)
25 mg	6 (16.7)	5 (20)	3 (10)	3 (10.3)	31 (14.7)	25 (15.2)	40 (14.4)	33 (15.1)
<30 x 10^9/L	6 (16.7)	4 (16)	1 (3.3)	0 (0)	17 (8.1)	5 (3)	24 (8.7)	9 (4.1)
<30 - <100 x 10^9/L	0 (0)	0 (0)	1 (3.3)	1 (3.4)	11 (5.2)	12 (7.3)	12 (4.3)	13 (6)
100 - <250 x 10^9/L	0 (0)	1 (4)	1 (3.3)	1 (3.4)	3 (1.4)	6 (3.7)	4 (1.4)	8 (3.7)
≥250 x 10^9/L	0 (0)	0 (0)	0 (0)	1 (3.4)	0 (0)	2 (1.2)	0 (0)	3 (1.4)
50 mg	28 (77.8)	19 (76)	26 (86.7)	25 (86.2)	178 (84.4)	139 (84.8)	232 (83.8)	183 (83.9)
<30 x 10^9/L	14 (38.9)	8 (32)	16 (53.3)	7 (24.1)	106 (49.8)	45 (27.4)	136 (48.7)	60 (27.6)
<30 - <100 x 10^9/L	11 (30.6)	4 (16)	10 (33.3)	4 (13.8)	89 (28)	37 (22.6)	80 (28.9)	45 (20.6)
100 - <250 x 10^9/L	1 (2.8)	5 (20)	0 (0)	8 (27.6)	12 (5.7)	30 (18.3)	13 (4.7)	43 (19.7)
≥250 x 10^9/L	2 (5.6)	2 (8)	0 (0)	6 (20.7)	2 (0.9)	27 (16.5)	4 (1.4)	36 (16.1)
75 mg	2 (5.6)	1 (4)	1 (3.3)	1 (3.4)	2 (0.9)	0 (0)	5 (1.8)	2 (0.9)
<30 x 10^9/L	1 (2.8)	0 (0)	1 (3.3)	1 (3.4)	1 (0.5)	0 (0)	3 (1.1)	1 (0.5)
<30 - <100 x 10^9/L	1 (2.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.4)	0 (0)
100 - <250 x 10^9/L	0 (0)	1 (4)	0 (0)	0 (0)	1 (0.5)	0 (0)	1 (0.4)	1 (0.5)
≥250 x 10^9/L	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Results: Overall, 287 adult patients with ITP (chronic [>12 months], 75.3%; persistent [3-12 months], 10.8%; acute [<3 months], 13.6%; unknown [n=1]) were included, majority in Spain (n=128) followed by Italy (n=67), Greece (n=36), France (n=29), and Germany (n=27). Eltrombopag was the first treatment with no prior ITP therapies in 12 (4.2%) [acute, 10.3%; persistent, 6.5%; chronic, 2.8%] patients. A total of 99 (34.6%) patients received one prior therapy (corticosteroids, 79 [27.6%]), 128 (44.8%) patients received two prior therapies (corticosteroids+immunoglobulin, 114 [39.9%]), and 47 (16.4%) patients received three prior therapies (corticosteroids, immunoglobulins, and splenectomy). In total, the majority of patients received at least one prescription of corticosteroids (252, 88.1%) followed by immunoglobulins (180, 62.9%), and splenectomy (64, 22.4%) prior to eltrombopag initiation. Patients received an average daily dose of eltrombopag 45.6mg (chronic ITP, 44.6mg; persistent ITP, 43.1mg; acute ITP, 53.0mg) during the study. Overall, dose changes were reported in 749 adult ITP prescriptions (down-titration, 53.7%; up-titration, 43.7%; no change in dose, 2.7%). 49.1% of dose changes were reported during the first 6 months of treatment (35% in first 3 months). The main reasons for dose change included: disease improvement (30.4%), no treatment response (26.8%) and others (27.1%). Disease improvement accounted for down-titration in 51.2% (206/402) and up-titration in 4.6% (15/327), and no treatment response for up-titration in 54.4% (178/327) and down-titration in 5.0% (20/402) of adult patients with ITP. Proportion of patients with platelet counts by ITP disease phase, and by eltrombopag dose are reported in **Table 1**.
Summary/Conclusions: The majority of adult patients with ITP (75.3%) were diagnosed with chronic ITP, and were treated with eltrombopag as second-line or greater therapy after corticosteroids and immunoglobulins, in line with the approved indication. Eltrombopag was also prescribed in 24.4% of adult patients with acute and persistent ITP. The starting dose followed the summary of product characteristics (SmPC) recommendations in the majority of cases and dose modifications were generally according to platelet counts. Data from REVIEU study have shown that eltrombopag use in the real world setting is largely consistent with the EU label and is considered part of ITP medical therapies.

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BIOLOGICAL CHARACTERIZATION OF ITP PATIENTS THAT ARE NON-RESPONDERS TO TRADITIONAL THERAPIES

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Background: A previous study has suggested a mechanism of Fcγ receptors (FcγR)-independent platelet clearance in immune thrombocytopenia (ITP) (1). Antibody-mediated platelet desialylation may lead to platelet clearance in the liver via hepatic Ashwell–Morell receptors, providing a potential explanation for refractoriness to classical therapies (steroid, IVIG and splenectomy).
Aims: The aim of this study was to analyze the biological features of ITP patients refractory to conventional therapies.
Methods: We performed a prospective study in 8 patients with primary ITP not responding to standard therapies (coticosteroids, IVIG and/or splenectomy) as well as in 8 patients with non-refractory ITP (control group). Mean platelet size, surface expression of platelet glycoprotein (GP) IIb,and the activation marker CD62 were examined by flow cytometry (FC) analysis, as well as desialylation of platelet membrane GPs using fluorescein-conjugated *Ricinus Communis* Agglutinin I (RCA-1), a lectin that binds to galactose residues underlying sialic acids. Patients' sera was also incubated with normal human platelets to analyze their ability to induce desialylation in normal platelets. Analysis of desialylation of plasma proteins was performed by Western blot (FXI, FXII) and HPLC (transferrin). The specificity of platelet autoantibodies was assessed by a solid-phase modified antigen capture ELISA test (MACE). FC results are expressed as ratio of mean fluorescence intensity (MFI) compared to that of a control.
Results: The characteristics of the patients according to the response to conventional treatments (A, no responsers; B, responders) are summarized in Table 1. Non responders exhibited lower platelet counts (p=0.006), higher expression of GPIIb (p=0.049) and loss of platelet sialic acids (p=0.005). Additionally, those who did not respond not only to traditional therapies (corticosteroids, IVIG and splenectomy) but also to thrombopoietin receptor agonists (TPO-RA) (n=5) displayed higher platelet size and alpha-granule secretion. Furthermore, TPO-RA refractory patients' sera desilylated normal platelets, but not plasma proteins. MACE assay revealed that unique positivity for anti-GPIbα antibodies was only detected in those patients classified as non-responders to conventional ITP therapies, including TPO-RA.

Table 1.

Table 1. Patients' characteristics

	Group A: Refractory ITP to conventional treatment (n=8)	Group B: ITP with response to conventional treatment (n=8)	
Age(y) Median (range)	55 (22-79)	30 (20-63)	
Sex (male/female)	4/4	3/5	
Splenectomized	50%	75%	
Platelet count ($x10^9$/L) Mean ± standard error	40 +/- 54 Refractory to TPO-RA(n=5): 4 +/- 6 Response to TPO-RA(n=3): 97 +/- 49	260 +/- 172	*p=0.006
FSC (ratio)	1.81 +/- 1.54 Refractory to TPO-RA(n=5): 1.96 +/- 1.85 Response to TPO-RA(n=3): 0.90 +/- 0.30	1.21 +/- 0.38	p=0.430
GPIIb (ratio)	1.93 +/- 1.02 Refractory to TPO-RA(n=5): 1.91 +/- 1.42 Response to TPO-RA(n=3): 1.33 +/- 0.65	1.06 +/- 0.14	*p=0.049
CD62-P expression in patient platelets (ratio)	4.40 +/- 3.38 Refractory to TPO-RA(n=5): 4.87 +/- 4.16 Response to TPO-RA(n=3): 2.14 +/- 1.06	2.49 +/- 2.78	p=0.064
RCA-1 expression in patient platelets (ratio)	3.01 +/- 2.45 Refractory to TPO-RA(n=5): 3.35 +/- 3.04 Response to TPO-RA(n=3): 1.46 +/- 0.34	1.10 +/- 0.29	*p=0.005
RCA-1 expression in control platelets, patient sera (ratio)	1.57 +/- 0.86 Refractory to TPO-RA(n=5): 1.72 +/- 0.99 Response to TPO-RA(n=3): 1.19 +/- 0.33	1.29 +/- 0.78	p=0.247
Platelet autoantibody specificity	Refractory to TPO-RA(n=5): -GPIbα: 2/5 -GPIIb/IIIa: 2/5 -Both: 1/5 -Negative: 0 Response to TPO-RA(n=3): -GPIbα: 0 -GPIIb/IIIa: 1/3 -Both: 0 -Negative: 2/3	-GPIbα: 0 -GPIIb/IIIa: 1/8 -Both: 1/8 -Negative: 6/8	

Patients who did not achieve complete platelet responses to splenectomy, corticosteroids and / or immunoglobulins were considered refractory (group A). Group A has been subdivided as refractory or not to TPO-RA. Flow cytometry results are expressed as mean fluorescence intensity ratio compared to a parallel healthy control. Abbreviations: TPO-RA, thrombopoietin receptor agonist.

Summary/Conclusions: This study shows a significant higher platelet desialylation in ITP patients who are non-responders to conventional therapies, particularly if they are also refractory to TPO-RA. According to a previous study (1), these results seem to be associated to platelet activation mediated by antiplatelet specific antibodies.

Reference
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SEQUENTIAL USE OF THROMBOPOIETIN RECEPTOR AGONISTS IN ADULT PRIMARY IMMUNE THROMBOCYTOPENIA PATIENTS: A RETROSPECTIVE COLLABORATIVE SURVEY FROM ITALIAN HEMATOLOGY CENTERS

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Background: ITP is a disorder characterized by thrombocytopenia resulting from both increased immune-mediated platelet clearance and inappropriate thrombocytopoieis. TPO-RAs–romiplostim (R) and eltrombopag (E) - offer a new opportunity of treatment with high response rates. However, a small fraction of pts does not respond or loses response - *i.e.* desired platelet (plt) count achieved but not sustained over time - during long-term follow-up, which can not be resumed even if dosage is increased over time, or experience wide fluctuations in plt counts with either agent. Moreover, adverse events (AE) may necessitate treatment discontinuation. Finally, patient's preference may be an important issue considering the different route and timing of administration of the two agents and the alimentary restrictions needed for proper E absorption. Availability of two TPO-RAs for clinical use, with different molecular structure and site of binding within the TPO receptor, has prompted trials of TPO-RA switching with the aim of overcoming treatment limitations of either agent resulting in reported overall response rates of approximately 80% in poor responders to 1st TPO-RA.
Aims: To present the results of a multicenter survey on TPO-RA switch policies and outcome.
Methods: Charts of ITP pts receiving TPO-RAs at 17 collaborating Haematology Centers were reviewed; demographic and clinical data were collected in a dedicated case report form. Pts were grouped and analyzed based on the clinical setting prompting the switch (Table 1). The study was approved by the Hospital Review Board of each participating Center.

Table 1.

Table 1		Reason for switching				
	Patients	1st TPO-RA failure	Loss of response	Fluctuation	Patients' preference	Adverse events
Number	106	51 (48.1%)	20 (18.9%)	11 (10.4%)	8 (7.6%)	16 (15.0%)
eltr-> rom	47 (44.3%)	27 (52.9%)	5 (25%)	2 (18.2%)	0 (0%)	13 (81.3%)
rom -> eltr	59 (55.7%)	24 (47.1%)	15 (75%)	9 (81.8%)	8 (100%)	3 (18.7%)
Max dose used	72 (67.9%)	41 (80.4%)	15 (75%)	5 (45.5%)	5 (62.5%)	6 (37.5%)
No response	37 (34.9%)	26 (51%)	4 (20%)	2 (18.2%)	3 (37.5%)	2 (12.4%)
Response	39 (36.8%)	17 (33.3%)	4 (20%)	4 (36.4%)	3 (37.5%)	11 (68.8%)
Complete Response	30 (28.3%)	8 (15.7%)	12 (60%)	5 (45.4%)	2 (25%)	3 (18.8%)

Results: A total of 546 pts received either R or E between Dec 2009 and Dec 2015. Of these, 106 (19.4%) underwent TPO-RA switch. Table 1 summarizes outcome after switch. Overall 69/106 (65%) of pts achieved, regained or maintained response upon switching. Either one TPO-RA switch sequence was equally effective (p=0.682). Outcome was not associated with gender, age at 1st TPO-RA treatment, splenectomy status. However, number of lines of previous therapies was associated with lower response rates (p=0.020): each additional line of therapy yielded a 30% increase in the odds of being a non responder; a trend toward lower probability of response was observed in pts with longer lasting disease before 1st TPO-RA administration (p=0.066). Adverse events (AE; 16/106 pts) were generally mild and reversible upon discontinuation of either one TPO-RA; 1 arterial (managed with thromboendarterectomy) and 1 venous (standard anticoagulation) thrombotic events were observed which did not recur after switching. AE were characteristic of older pts: each additional year increase in pts age determined a 5% increase in the odds of developing AE.
Summary/Conclusions: Approximately 20% of TPO-RA treated pts were felt by their attending physicians to potentially benefit from a switching policy. Exposure to the 2nd TPO-RA was more effective in pts who had lost response to 1st TPO-RA (80% responders) compared to those who were non responders to 1st TPO-RA (49% responders, p=0.001). It could be speculated that lack of response to either one of the two available TPO-RA identifies a subgroup of pts least likely to respond when switched to the second available TPO-RA. Pts switched for non-efficacy reasons are more likely to maintain a response upon switch (p=0.030). The so far unexplained and unprecedented phenomenon of wide plt fluctuation appears to be linked to the removal of the spleen, the physiological plt reservoir organ

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THROMBOEMBOLIC EVENT MANAGEMENT AND OUTCOMES IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA (CITP) DURING TREATMENT WITH ELTROMBOPAG (EPAG): RESULTS FROM THE EXTEND STUDY

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Background: EPAG is an oral thrombopoietin receptor agonist approved for treatment of previously treated patients (pts; eg corticosteroids, immunoglobulins) with cITP aged ≥1 yr. The EXTEND study, a global, open-label, extension study of pts with cITP who received EPAG or placebo in prior EPAG studies, evaluated long-term safety and tolerability of EPAG. In EXTEND, 19 (6.3%) pts receiving EPAG experienced a total of 24 thromboembolic events (TEEs; Saleh *et al. Blood* 2016;128:1368), which is similar to TEE incidence in cITP pts receiving romiplostim (Kuter *et al. Br J Haematol* 2013;161:411–23) and to one estimate in the general cITP population (Sarpatwari *et al. Haematologica* 2010;95:1167-75).
Aims: To describe management and outcomes of TEEs occurring during EPAG treatment in the EXTEND study.
Methods: Adult pts with cITP received EPAG starting at 50mg/day, with titration to 25–75mg per day or less as required, based on individual platelet count responses (target range ≥50–200x10^9/L). Maintenance dosing continued after minimization of concomitant ITP medication and optimization of EPAG dosing. Pts could remain on EPAG either for 2 yrs in countries where EPAG was commercially available, or for >2 yrs until EPAG became commercially available. The EXTEND primary objective included detection and documentation of AEs, including investigator-reported TEEs.
Results: 302 pts were enrolled and received ≥1 EPAG dose: 67% female; 38% splenectomized; 49% aged 18-49 yrs. Median exposure duration was 2.4 yrs (range, 2 days to 8.8 yrs) and mean daily dose was 50.2 (range, 1-75)mg/day. Overall, 259/302 (86%) pts achieved platelet counts of ≥50×10^9/L at least once and 126/248 (51%) pts maintained continuous platelet counts ≥50×10^9/L for ≥31 weeks. TEEs during EPAG treatment (n=24 events) included deep vein thrombosis (DVT; n=8), myocardial infarction (MI; n=5), cerebral infarction (CI; n=4), transient ischemic attack (TIA; n=3), others (n=4) [Table]. Where available, platelet counts prior to TEE were <150×10^9/L in eight pts and ≥150×10^9/L in nine; 6 pts experienced the TEE at or shortly after achieving their maximum platelet count. Most TEEs resolved with intervention; 15 pts received anticoagulant therapy for 16 TEEs (treatment not recorded, n=3; no anticoagulation, n=5). EPAG was discontinued after a TEE (DVT, n=4; CI, n=3; MI, n=1; other, n=2) in 10 pts (n=9/10 TEE resolved). Seven pts continued to receive EPAG after a TEE (MI, n=3; TIA, n=3; CI, n=1; DVT, n=1; other, n=2; TEE resolved, n=8/10); in two cases, EPAG was continued with no anticoagulant therapy (TIA; both resolved), and in four, anticoagulants were given concomitantly with EPAG (TEE resolved, n=2/4); one of these also underwent percutaneous transluminal coronary angioplasty (MI, resolved). EPAG was interrupted during treatment for TEEs in two pts, one pt had two DVTs and acute MI (n=3/3 TEEs resolved), the other had a DVT (not resolved). TEE risk factors were not recorded for all pts; risk factor effect on the treating physician's management could not be analyzed.

Table 1.

Table. Occurrence of TEEs during eltrombopag treatment, eltrombopag treatment decision and number of resolved events by TEE

		Eltrombopag treatment decision*		
TEE	Total events	Discontinued	Continued	Interrupted
DVT, n (resolved, n)	8 (6)	4 (4)	1 (0)	3 (2)
MI, n (resolved, n)	5 (5)	1 (1)	3 (3)	1 (1)
CI, n (resolved, n)	4 (3)	3 (2)	1 (1)	0
TIA, n (resolved, n)	3 (3)	0	3 (3)	0
Other,† n (resolved, n)	4 (3)	2 (2)	2 (1)	0
Total, n (resolved, n)	24 (20)	10 (9)	10 (8)	4 (3)

*Three patients experienced more than one TEE during eltrombopag treatment
†Cerebral ischemia, pulmonary embolism, pulmonary infarction, thrombophlebitis (n=1 occurrence each)

Summary/Conclusions: This analysis shows that most pts who experienced a TEE had resolution of the event after medical/surgical treatment, most commonly anticoagulant therapy, regardless of whether EPAG was discontinued, interrupted or continued. The decision to restart EPAG following a TEE should be made on a case-by-case basis, with caution (including frequent platelet count monitoring) and only if the benefit is expected to outweigh any risk. If anticoagulation therapy is instituted (as in most cases), it is possible the bleeding risk may shift the risk-benefit to maintenance of EPAG treatment.

P724

SEVERE BLEEDING IN THE ELDERLY WITH PRIMARY IMMUNE THROMBOCYTOPENIA: CHARACTERISTICS, RESPONSE TO THERAPY AND LONG-TERM OUTCOME

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Background: Primary immune thrombocytopenia (ITP) is often diagnosed in the elderly. The elderly patients have been reported to have a higher incidence of severe bleeding manifestations and a higher ITP-related mortality. Nonetheless, few data exist on the characteristics and long-term prognosis of elderly patients with severe bleeding.
Aims: We retrospectively evaluated elderly patients with ITP who had severe bleeding to determine characteristics, response to therapy and long-term outcome.
Methods: We reviewed the medical records of 517 ITP patients over 60 years of age diagnosed at our center (192 men and 325 women) between 1991 and 2012. Therapy was started at diagnosis or during follow up. Bleeding severity was assessed by the Mazzucconi's bleeding assessment. Logistic regression analysis was used to determine which presenting features were associated with the risk of severe bleeding. Cox regression analysis was used to estimate rate ratios(RR) for no remission and mortality.
Results: Among 517 patients with ITP, 10 (1.9%) presented intracerebral hemorrhage(ICH) and 74 (14.3%) presented severe (non-ICH) bleeding during ITP. According to multivariate analysis, risk of severe bleeding in patients was increased with platelet count <10×10^9/L (*P*=0.001, OR=1.682, 95% CI 1.271-2.234), female patients (*P*=0.010, OR=2.148, 95% CI 1.200-3.844), complication of pulmonary disease (*P*=0.001, OR=4.724, 95% CI 1.845-12.092), gum or oral mucosal bleeding (*P*<0.001, OR=2.941, 95% CI 1.658-5.216) and epistaxis (*P*=0.027, OR=1.865, 95% CI 1.074-3.238). Compared to severe (non-ICH) bleeding, ICH was more likely incurred in severe bleeding patients with hypertension (*P*=0.031, OR=2.750, 95% CI 1.266-5.974). Of 103 patients simply observed after diagnosis, 7(6.7%) patients presented severe (non-ICH) bleeding and 3(2.9%) patients presented ICH during ITP. Of 222 patients who had bleeding after treatment, 31 patients (13.9%) presented severe (non-ICH) bleeding and 4(1.8%) patients presented ICH. Compared to observation, treatment did not significantly reduce the risk of severe bleeding (χ^2=1.889, *P*=0.169). The total response rate (CR+R) to initial treatment in patients who presented severe bleeding was 58.1% (43/74), which was lower than that in patients without severe bleeding (70.2%, 238/340, χ^2=4.014, *P*=0.045). The response to steroids, IVIG or combination had no significant difference among

patients with severe bleeding. At the end of follow-up, the estimated 10-year cumulative rate of no remission among patients with severe bleeding was higher than that among patients without severe bleeding (*P*=0.017, RR=1.608, 95%CI, 1.052-2.456). The estimated 10-year cause-specific mortality related to fetal bleeding in patients with severe bleeding was higher than that in patients without severe bleeding (*P*<0.001, RR=9.886, 95% CI, 1.806-54.098). The estimated 10-year mortality among ICH patients was higher than that among severe (non-ICH) patients (*P*<0.009, RR= 4.543, 95% CI, 1.317-15.668).
Summary/Conclusions: Platelet count <10×10^9/L, female patients, complication of pulmonary disease, gum or oral mucosal bleeding and epistaxis are significant predictive factors for severe bleeding in the elderly. Severe bleeding in elderly ITP was associated with more failure of response to treatment, increased long-term risk of no remission and mortality related to fetal bleeding.

P725

ATORVASTATIN IMPROVE THE PROGNOSIS OF ADULT PATIENTS WITH CORTICOSTEROID-RESISTANT IMMUNE THROMBOCYTOPENIA VIA ENHANCING BONE MARROW ENDOTHELIAL CELL FUNCTION

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Background: Immune thrombocytopenia (ITP) is generally considered to be an autoimmune disorder characterized by increased peripheral platelet destruction and reduced platelet production. Corticosteroids represent the standard first-line therapy achieving responses in around 80% of patients. However, for those corticosteroid-resistant ITP patients, who exhibit either no response (NR) to corticosteroids or corticosteroid-dependent, the pathogenesis remains poorly understood and the management is challenging. Emerging evidence from mouse studies has suggested that the cross-talk between megakaryocytes (MKs) and endothelial progenitor cells (EPCs) in the bone marrow (BM) microenvironment regulates MKs maturation and thrombopoiesis. We recently reported that the impaired BM EPCs, which could be quantitatively and functionally improved by atorvastatin in vitro, induced the occurrence of poor graft function following allo-transplant (***Blood****, 2016,128:2988-2999*). However, little is known about the functional role of BM EPCs and how to improve impaired BM EPCs in patients with corticosteroid-resistant ITP.
Aims: To determine whether quantitative and/or functional abnormalities of BM EPCs are involved in the occurrence of corticosteroid-resistant ITP. Moreover, to investigate the effects of atorvastatin and N-Acetyl-L-cysteine (NAC, a ROS scavenger) on the number and function of cultivated BM EPCs derived from patients with corticosteroid-resistant ITP and its underling molecular mechanisms. Finally, to evaluate the efficacy and safety of atorvastatin and NAC to adult patients with corticosteroid-resistant ITP.
Methods: Twenty-three patients with corticosteroid-resistant ITP, 30 patients with newly diagnosed ITP and 17 healthy donors (age 18-55) were enrolled from 2016 to 2017 at Peking University Institute of Hematology. BM EPCs were cultured as previous reported. Atorvastatin and NAC were administrated to the 5-day cultivated BM EPCs in corticosteroid-resistant ITP patients until tested on day 7. The number and function of BM EPCs were evaluated pre- and post-treatment by cell counting, DiI-Ac-LDL and FITC-lectin-UEA-1 double staining, migration, cell proliferation, tube formation, levels of reactive Oxygen Species (ROS) and apoptosis. Proteins expressions for p38, ERK, JNK, Akt were measured by flow cytometry and western blot. Subsequently, a single-center pilot study was performed to evaluate the efficacy and safety of atorvastatin and/or NAC in corticosteroid-resistant ITP patients. The primary end points were complete response (CR), response(R), and overall response (OR). Secondary end points were time to response (TTR) and adverse events.
Results: Human bone marrow EPCs were demonstrated as the spindle shape and the similar expression of CD34, VEGFR2 and CD133 at day 7 of cultivation among the enrolled three cohorts of subjects. Decreased and dysfunctional BM EPCs, which were characterized by impaired proliferation, migration, angiogenesis, and higher levels of ROS and apoptosis, were revealed in corticosteroid-resistant ITP patients compared to those in newly diagnosed ITP. Activation of p-P38 was detected in BM EPCs from corticosteroid-resistant ITP patients. Furthermore, the number and function of BM EPCs derived from corticosteroid-resistant ITP patients were enhanced by atorvastatin or NAC treatment *in vitro* through down-regulation of the p38 mitogen-activated protein kinase (MAPK) pathway. In the single-center pilot study, a total of 12 corticosteroid-resistant ITP patients were recruited to receive either the combination of atorvastatin and NAC or alone. As a result, the CR, R and OR rates were 25% (3/12), 41.7% (5/12) and 66.7% (8/12), respectively. In patients who achieved CR and R, the median (range) TTR was 24 days (7-51 days), with no apparent adverse events.
Summary/Conclusions: The number and the function of BM EPCs were impaired in corticosteroid-resistant ITP patients. Treatment with atorvastatin and NAC *in vitro* and *in vivo* quantitatively and functionally improved BM EPCs derived from corticosteroid-resistant ITP patients through down-regulation of the p38 MAPK pathway. Although the sample size of clinical study is small, with a relatively short follow-up period by now, our data suggest that atorvastatin and NAC are effective and safe in the management of corticosteroid-resistant ITP patients. Therefore, further prospective multicenter randomized clinical trials with larger sample size are needed in the future.

P726

PLATELET DESIALYLATION IS A NOVEL MECHANISM AND A THERAPEUTIC TARGET IN THROMBOCYTOPENIA DURING SEPSIS: AN OPEN-LABEL, MULTICENTER, RANDOMIZED CONTROLLED TRIAL

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Background: Sepsis is a systemic, deleterious host response to infection leading to severe sepsis, and possibly septic shock as defined by the Surviving Sepsis Campaign guidelines. Thrombocytopenia is a common finding in sepsis. Studies in murine models suggested that platelet desialylation was an important mechanism of thrombocytopenia during sepsis. Desialylation-induced platelet removal could possibly be circumvented by adding sialidase inhibitors during sepsis. Oseltamivir, also known as Tamiflu, is a viral sialidase inhibitor that prevents the release of progeny virions. Several studies suggests the feasibility that oseltamivir can be used for the treatment of infection-associated thrombocytopenia.
Aims: To determine whether thrombocytopenia is associated with increased platelet desialylation in septic patients, and whether oseltamivir is an effective treatment to increase platelet counts in severe sepsis.
Methods: We first performed a prospective, multicenter, observational study that enrolled septic patients with or without thrombocytopenia to determine the association between platelet desialylation and thrombocytopenia in patients with sepsis. Next, we conducted an open-label, randomized controlled trial in which the patients who had severe sepsis with thrombocytopenia (platelet counts ≤50×10^9/L) were randomly assigned to receive antimicrobial therapy alone (control group) or antimicrobial therapy plus oseltamivir (oseltamivir group). The study flowchart is shown in Fig. 1. Both groups received appropriate antimicrobial agents and standard medical support based on the guidelines issued by the Surviving Sepsis Campaign. The oseltamivir group additionally received 5 full days of oseltamivir therapy. The oseltamivir was administered orally or through a feeding tube at a dose of 75mg once every 12 hours. Time from randomization to the administration of oseltamivir was less than 24 hours. The antimicrobial agents were continuously administered until 3 days after the resolution of the physiological abnormalities related to the systemic inflammatory response syndrome (SIRS). The primary outcomes were platelet desialylation level at study entry, and overall platelet response rate within 14 days post-randomization. Secondary outcomes included platelet recovery time, the occurrence of bleeding events, and the amount of platelets transfused within 14 days post-randomization**.** The percentages of platelets positive for *Ricinus communis* agglutinin I (RCA-I), *Erythrina cristagalli* lectin (ECL) or Succinyl *Triticum vulgare* lectin (sWGA) analyzed by flow cytometry represented the levels of platelet desialylation. Platelet response was defined as platelet counts returning to or above 100×10^9/L. Platelet recovery time was calculated as the date of randomization to the date when platelet counts were >100×10^9/L. Written informed consents were obtained from the study participants prior to inclusion in the study.

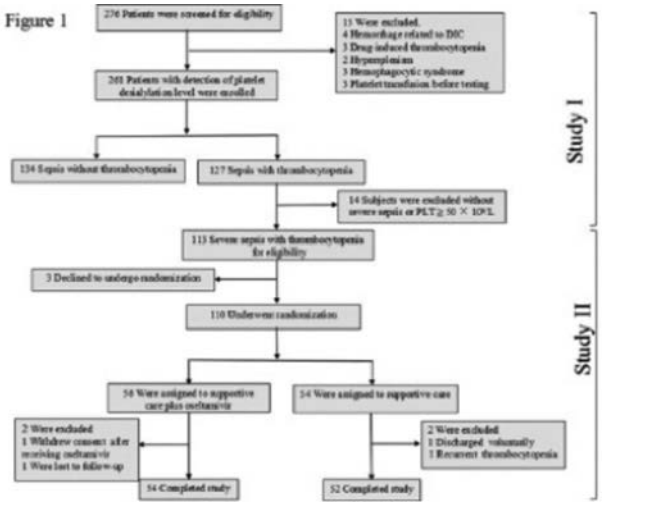

Figure 1.

Results: The platelet desialylation levels increased significantly in the 127 septic patients with thrombocytopenia compared to the 134 patients without thrombocytopenia. A platelet response was achieved in 45 of the 54 patients in the oseltamivir group (83.3%) compared with 34 of the 52 patients in the control group (65.4%; P=0.045). The median platelet recovery time was 5 days (interquartile range 4-6) in the oseltamivir group compared with 7 days (interquartile range 5-10) in the control group (P=0.003). The amount of platelets transfused decreased significantly in the oseltamivir group compared to the control group (P=0.044). The multivariate analysis by Cox proportional hazards models showed that the Sequential Organ Failure Assessment (SOFA) score and platelet recovery time were independent indicators of oseltamivir therapy.
Summary/Conclusions: Thrombocytopenia was associated with increased platelet desialylation in septic patients. The addition of oseltamivir could significantly increase the platelet response rate, shorten platelet recovery time and reduce platelet transfusion. Chinese Clinical Trial Registry, ChiCTR-IPR-16008542.

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SAFETY AND EFFICACY OF LONG-TERM OPEN-LABEL DOSING OF SUBCUTANEOUS (SC) ROMIPLOSTIM IN CHILDREN WITH IMMUNE THROMBOCYTOPENIA (ITP)

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Background: Children with ITP for ≥6 months who completed a romiplostim phase 1/2 or phase 3 parent study could enroll in this open label long term extension study
Aims: To evaluate the safety and efficacy of long-term romiplostim in children with ITP.
Methods: Patients enrolled at 28 sites in the US, Canada, Spain, and Australia. All patients received SC romiplostim once weekly. The initial dose was the final dose from the parent study or 1 µg/kg for patients previously receiving placebo; dose was then adjusted from 1-10 µg/kg to target platelet counts of 50−200×10^9/L. Incidence of adverse events (AEs) was the primary endpoint.

Remission cases	1	2	3	4	5	6	7	8	9
Age, years	18	10	13	8	5	7	7	5	5
Race	W	B	W	W	W	W	B	W	B
ITP, years	9	10	9	2	5	4	3	4	5
ITP Rx, #, prior to studies	2	5	4	2	2	4	2	2	1
Romiplostim, years	5.9	4.3	3.3	0.8	2	1.6	0.8	0.7	1.4
Maximum dose, µg/kg	10	8	9	5	10	1	3	2	1
ITP remission, years	1.1	1.9	0.8	0.8*	0.6*	0.9*	1.3*	0.5*	0.5

B, black; Rx, therapy; W, white. * Still on study.

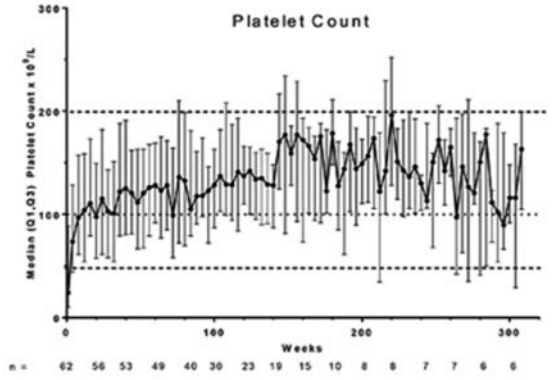

Figure 1.

Results: As of 24 Feb 2016, 66 patients entered this study; 65 received romiplostim for up to 6.2 years. At baseline, median (min–max) age was 11 (3–18) years; 56% were female; 61% were white, 14% African American, 14% Hispanic/Latino, 9% Asian, and 3% other; 9.1% had prior splenectomy. Median (min–max) baseline platelet count was 27.5 (2–458)×10^9/L. Median (min–max) treatment duration was 100 (5–321) weeks. Median (min–max) average weekly romiplostim dose was 4.8 (0.1–10.0) µg/kg, which included escalation to a stable dose. After ~week 200 (n ≤8 patients), the median dose was observed to fluctuate. All 65 patients received their doses per protocol >90% of the time; 18 patients missed ≥1 dose due to noncompliance for a total of 41 times. Reasons for discontinuing treatment (n=22, 33%) included consent withdrawn (n=8), required other therapy (n=4), noncompliance (n=3), administrative decision (n=3), per protocol (n=1), and AE (n=2) (asthenia, headache, dehydration, and vomiting in one patient and anxiety in the other, per investigator, none of the AEs were treatment-related); 43 (65%) patients continued in the study. Fifty-two serious AEs occurred in 17 patients, 3 deemed treatment-related (anemia, epistaxis, and thrombocytopenia). Bleeding AEs occurred in 56 patients; 5 deemed treatment-related (gingival bleeding, petechiae, injection site bruising, injection site hematoma, and epistaxis). No thrombotic events were reported. There were no peripheral blood abnormalities warranting a bone marrow examination. No patients had anti-TPO neutralizing antibodies. From week 2 on, median platelet counts remained >50×10^9/L; platelet counts were >100×10^9/L at most timepoints, despite an observed decrease in the median dose from 4-5 µg/kg to 2-3 µg/kg around week 160 (Figure). Nearly all (94%, 61/65) patients had a platelet response (median platelet counts for a month ≥50×10^9/L). Nine (14%) patients (5 boys and 4 girls, none with prior splenectomy) entered remission (Table), defined here as platelet counts ≥50×10^9/L for 24 weeks with no ITP treatments. Twenty-three (35%) patients received rescue medications.
Summary/Conclusions: Over 6 years of data from this ongoing open-label extension study of romiplostim in children with ITP show that >90% of children achieved a platelet response with romiplostim. The safety profile was overall tolerable, similar to that in past studies. Some children (9/66) with longstanding ITP entered remission after receiving romiplostim.

Quality of life, palliative care, ethics and health economics 2

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IMPACT OF VENETOCLAX ON THE QUALITY OF LIFE OF CLL PATIENTS RELAPSED/REFRACTORY TO B-CELL RECEPTOR (BCR) SIGNALING PATHWAY INHIBITOR TREATMENT

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Background: The prognosis for patients with CLL after B-Cell Receptor inhibitor (BCRi) failure is very poor. Patients with R/R CLL who discontinue and/or progress on BCRi treatment tend to have poor clinical outcomes. Venetoclax (VEN) is an oral BCL-2 inhibitor that has demonstrated deep and durable responses in relapsed/refractory (R/R) CLL patients.
Aims: To assess whether VEN has an impact on health related quality of life (HRQoL) among CLL patients R/R to BCRi treatment and receiving VEN monotherapy.
Methods: The study enrolled patients with CLL who had previously received treatment with ibrutinib and/or idelalisib, have relapsed on treatment, or experienced progression after discontinuation of either agent. Patients are to receive VEN monotherapy for up to two years, or until discontinuation due to disease progression, unacceptable toxicity, or any other reason. Patient-reported HRQoL measures included the EORTC QLQ-C30 and EORTC QLQ-CLL16, which were assessed at Baseline (BL), Week 24, and every 12 weeks thereafter. Mean change from BL to each assessment through Week 48 are reported here. Clinical relevance was based on minimum important difference (MID) of values from BL to each assessment. A change of 5-10 points is considered a "small" change on the EORTC-QLQ-C30. The lower bound of 5 points was used for MID acceptance on both measures.
Results: Clinically meaningful improvements from BL were observed early and were sustained through week 48 in VEN treated patients in the EORTC-QLQ-C30 global health status and the role, social, and emotional functioning scales. Furthermore, early and sustained improvements in fatigue through week 48 were seen in both EORTC-QLQ-C30 and EORTC-QLQ-CLL16 (**Table 1**).

Table 1.

Table 1. Change from Baseline Results in Key Domains on EORTC QLQ-C30 and EORTC QLQ-CLL16

EORTC QLQ-C30 parameter (n)	BL Mean	Visit Mean	Mean Change from BL (95% CI)
Global Health Status[a]			
Week 24 (40)	62.9	75.0	12.1 (3.2, 20.9)
Week 48 (33)	64.1	71.0	6.8 (-1.7, 15.3)
Role Functioning[a]			
Week 24 (40)	74.2	87.9	13.8 (5.1, 22.4)
Week 48 (34)	77.5	91.7	14.2 (6.2, 22.2)
Social Functioning[a]			
Week 24 (40)	75.0	85.8	10.8 (2.7, 19.0)
Week 48 (34)	77.9	86.3	8.3 (0.7, 15.9)
Fatigue[b]			
Week 24 (40)	38.1	23.7	-14.3 (-21.1, -7.5)
Week 48 (34)	35.3	22.5	-12.7 (-21.0, -4.5)
EORTC QLQ-CLL16 parameter (n)	**BL Mean**	**Visit Mean**	**Mean Change from BL (95% CI)**
Fatigue[b]			
Week 24 (40)	35.4	20.0	-15.4 (-23.6, -7.2)
Week 48 (34)	33.3	20.1	-13.2 (-20.8, -5.7)

[a] A positive change in score represents improvement.
[b] A negative change in score represents improvement.

Summary/Conclusions: This interim analysis provides preliminary evidence that demonstrates CLL patients R/R to BCR inhibitors receiving VEN monotherapy experienced improvement in several key aspects of functioning and HRQoL. These results may be important to consider when making therapeutic choices in R/R CLL following relapse or progression on BCRi inhibitors.

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THE ROLE OF PSYCHOLOGICAL VARIABLES FOR TYROSINE KINASE INHIBITORS (TKI) DISCONTINUATION IN CHRONIC MYELOID LEUKEMIA (CML) PATIENTS: IMPLICATION FOR MEDICAL DECISION MAKING PRACTICE

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Background: Treatment-free remission (TFR) is an emerging goal for CML patients (pts) that reach a sustained deep molecular response (DMR), as it can reduce the risk of long-term toxicities that impair quality of life, and mitigate the costs associated with long-term TKI therapy. Therapy discontinuation may represent a great challenge for patients and different factors (not only clinical) may play a role in medical decision, such as psychological and emotional variables. In this respect, it is essential to consider pts' concerns and preferences regarding the discontinuation option.
Aims: This study was aimed at investigating psychological (emotional and cognitive) and clinical factors related with the attitude to opt for discontinuation of therapy in CML pts.
Methods: This is an observational, prospective, no-drug related study conducted in 3 Italian centers with large experience in CML treatment. A detailed battery of questionnaires focusing on health behaviour, risk taking and personality was administered.
Results: One hundred and twenty pts were enrolled (56% males; mean age=50, SD=1.2). Median duration of the disease was 8 years (range 1-39y). 62/120 pts were receiving Imatinib first line. The idea of stopping TKI is appealing for the 81.6% of pts if there is a chance of long-term stable disease and a high probability of response upon restarting a TKI. Pts are more likely to stop their TKI if the risk of relapse is no more than 30% (% Mean=33.62; SD=33.46). Main worries related with the choice to stop TKI are fear of possible disease recurrence, (60.5%), fear of drug resistance if the disease relapses (44.5%) and fear to disappoint family or friends (26.9%). Older pts (>40 years) are more concerned about relapse and subsequent lack of response than younger (x^2=9.65, p=0.02). Finally, pts with higher *passive risk taking* attitude (who are more reluctant and undecided in everyday-life decisions) seemed to be more afraid to lose disease control in CML. ANOVA showed a significant difference between the two groups (F=5.46; p=.021).
Summary/Conclusions: Many studies have confirmed the feasibility and safety of stopping TKI therapy in selected pts, with the potential to drastically modify clinical practice in CML management in the next future. TKI discontinuation appears appealing and challenging at the same time for many CML pts. This study, for the first time, analyses how and when pts would consider this option including implications for health care providers in clinical practice, using both a clinical and psycho-cognitive perspective.

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BUDGET IMPACT ANALYSIS OF BIOSIMILAR RITUXIMAB (CT-P10) FOR THE TREATMENT OF CHRONIC LYMPHOCYTIC LEUKAEMIA IN THE 28 EU MEMBER STATES

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Background: In December 2016, the European Medicines Agency's Committee for Medicinal Products for Human Use has recommended granting marketing authorization to biosimilar rituximab (CT-P10) in all indications of the reference product, including chronic lymphocytic leukaemia (CLL). Compared to the originator rituximab, significant price reductions are expected offering a more affordable treatment option for CLL patients across Europe.
Aims: To assess the budget impact of the introduction of CT-P10 into the treatment of CLL in the 28 EU member states. Moreover, we provide an estimation for the number of additional CLL patients that can be treated with CT-P10 from the cost savings.
Methods: A budget impact analysis was performed to evaluate the one-year cost outcomes under two scenarios with and without the availability of CT-P10. The budget impact was calculated as the difference in costs between the two scenarios. For the major European markets, five-year cost savings were also estimated. Market uptake of CT-P10 was assumed to be 30%. A third party payer's perspective was adopted, and only drug costs were considered. Based on expert opinion, it was assumed that when CT-P10 is entering the market it will be at 50-70% of the official list price of originator rituximab in each country. Costs of administration and monitoring were not incorporated in the calculations, as it can be assumed that these are equal for the reference product and CT-P10. The initial number of patients treated with rituximab was estimated from IMS sales data on total annual consumption of originator rituximab in 2016. Other model parameters such as patients' average body surface area and treatment rate of rituximab among CLL patients, were derived from the published literature. One-way sensitivity analysis was undertaken to test the robustness of model assumptions.
Results: Over a one year time horizon, the cumulative budget impact of adopting CT-P10 is estimated to be €17.80 million in the 28 EU member states (30% discount in drug prices compared to the originator rituximab). Countries responsible for the majority of the cost savings are Germany (€4.06 million), Italy (€3.15 million), France (€2.41 million), Spain (€1.50 million), the UK (€1.34 million), Poland (€0.80 million), Austria (€0.66 million), the Netherlands (€0.59 million), Finland (€0.49 million) and Sweden (€0.43 million). If the cost savings were used to treat additional CLL patients with CT-P10, a total of 1,624 patients could be treated annually throughout Europe. The potential cost savings are in a direct correlation with the price and market uptake of CT-P10. Applying a 40% and 50% discount in drug prices compared to the originator rituximab, cost savings are projected to €23.73 and €29.67 million, from which further

2,526 and 2,706 CLL patients could be treated with CT-P10, respectively. Over five years, the total cost savings are expected to reach €29.81 million in Germany, €23.10 million in Italy, €17.65 million in France, €10.98 million in Spain and €9.83 million in the UK.
Summary/Conclusions: Biosimilar rituximab has the potential to improve the affordability of CLL treatments and easing the burden of healthcare costs in Europe. The cost savings might be even larger in case of a higher price discount for CT-P10. Using the cost savings to treat additional patients would substantially increase the access to better cancer medications, and thus contribute to a longer survival as well as better quality of life outcomes in CLL.

P731

AN INVESTIGATION INTO THE NEEDS AND PRIORITIES OF PATIENTS WITH MULTIPLE MYELOMA DURING REMISSION–IMPLICATIONS FOR RE-DESIGNING PATIENT-CENTRED HEALTHCARE SYSTEMS.
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Background: Therapeutic advances in multiple myeloma (MM) mean that patients have extended periods of remission without need for active anti-myeloma therapy. This provides an opportunity to review how these patients are managed and design patient-centred healthcare systems. Remote monitoring systems have been implemented for other cancer patients in remission.
Aims: We aimed to explore patient needs during stable remission from MM and the influence of these on acceptability of remote monitoring.
Methods: Patients with stable MM in a treatment-free interval selected from outpatient clinics at a tertiary centre completed a survey which explored the acceptability of various methods of remote monitoring. Subsequently semi-structured interviews were conducted by an independent researcher to investigate factors influencing this preference. Interviews were carried out until saturation of themes, transcribed verbatim and thematic analysis was performed using open coding by a doctor, physiotherapist and psychologist.
Results: 78 patients were surveyed; the most acceptable alternative was a telephone clinic (with doctor 77%, nurse 69%). 19 interviews were conducted exploring suitability of a nurse-led telephone clinic (TC) replacing current face to face (FTF) consultations with a doctor. Median age was 61years (range 46-76), and 9 were male. 18 patients were in 1st remission; 16 had most recently received high dose therapy and autograft, 3 had post autograft consolidation. The centre was not the local hospital for 18 patients interviewed. The majority were accepting of TC as an alternative to FTF clinics due to the burden of travel, associated cost and clinic waiting times. These affected patients' physical and psychological well-being, with TC perceived as less burdening. Patients acknowledged reduced needs during remission compared to treatment phase and felt TC would benefit redistribution of consultant time for patients on active therapy. Some patients suggested this service change would be more beneficial for healthcare resourcing rather than them personally. Interpretation of blood results by clinicians was regarded as central to monitoring disease, and for some who were unaware of clinical symptoms, the only way a relapse would be detected. General preference was for bloods to be done locally, leading to concerns about availability of results for TC. Patients were unsure how to monitor their own MM, hence valued the knowledge of their medical team. Doctors were perceived to have more expertise than nurses and this influenced preferences regarding who undertook TC. As a result, patients sought reassurance they could see a doctor if they had any concerns after TC with a nurse. Patients valued continuity under the centre where they were treated due to prior positive experience and the importance of being seen at a tertiary centre renowned for its expertise in MM. This influenced acceptability of TC as long as they remained under the centre's care with preference for continuity of staff involved. Whilst TC was acceptable for patients in remission, some were concerned about how relapse would be managed and expressed preference for FTF when being told they had relapsed.
Summary/Conclusions: Nurse led TCs are an acceptable alternative to FTF consultations for monitoring patients in remission from MM. Design of healthcare systems incorporating TCs need to have robust systems for accessing blood test results, for managing relapse, ready access to doctors and reassurance about the competence and knowledge of practitioners involved.

P732

COST-EFFECTIVENESS OF RITUXIMAB IN ADDITION TO STANDARD OF CARE CHEMOTHERAPY FOR ADULT PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA
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Background: In The Group for Research on Adult Acute Lymphoblastic Leukemia Rituximab (GRAALL-R) study, the addition of the anti-CD20 monoclonal antibody rituximab to standard chemotherapy for Philadelphia chromosome negative, CD20-positive, B-cell precursor Acute Lymphoblastic Leukemia (CD20+ Ph- BCP-ALL) resulted in improved clinical outcomes. However, the cost-effectiveness of rituximab for this indication has not been previously evaluated. We examined this question in the context of the Canadian publicly funded health care system.
Aims: To determine the economic impact in Canada of the addition of rituximab to standard of care (SOC) chemotherapy *vs* SOC alone in newly diagnosed CD20+ Ph- BCP-ALL.
Methods: Standard of care consisted of the two most widely used chemotherapy regimens for adults with ALL in Canada: hyper-CVAD or the Dana Farber Cancer Institute (DFCI) ALL consortium. A decision analytic model included the following health states over a 15-year time-horizon: event-free survival, relapsed/resistant disease, cure (death from causes other than ALL, given ≥5 years EFS) and death. Event-free survival, overall survival and serious adverse event (SAE) rates were taken from the GRAALL-R randomized controlled trial by Maury *et al.* Costs of the model included: first-, second- and third-line treatment and administration; disease management; palliative care; and SAE-related treatments. Model inputs were sourced from public data, literature and provincial cancer agency input. Results are presented using probabilistic sensitivity analysis and Monte Carlo simulation incorporating uncertainty around all model inputs.
Results: Life years increased by 1.33 years (95%CI: 0.10-2.63 years) with rituximab in addition to SOC *vs* SOC alone. Quality-adjusted life-years (QALYs) increased by 1.15 QALYs (95%CI: 0.34-1.93 QALYs) with rituximab in addition to SOC. The incremental cost of rituximab plus SOC was C$46,624 (95%CI: C$28,881-C$64,515), chiefly due to the drug acquisition costs of rituximab. Superior relative EFS associated with rituximab in addition to SOC drove lower second-line treatment and palliative care use, resulting in modest cost savings. The resulting mean Incremental Cost-Effectiveness Ratio (ICER) was C$40,505/QALY. At a willingness-to-pay threshold of C$100,000/QALY, the probability of being cost-effective was 96%. Decision outcomes were robust to the probabilistic and deterministic sensitivity analyses, including the SOC backbone as either hyper-CVAD or DFCI.
Summary/Conclusions: For adults with CD20+ Ph- BCP-ALL, rituximab in addition to SOC is a cost-effective intervention compared to SOC alone, from a Canadian public payer perspective. Rituximab is associated with increased life years and increased QALYs at a reasonable incremental cost.

P733

THE THERAPEUTIC UTILITY OF A SYSTEMATIC PROTOCOL FOR GERIATRIC ASSESMENT IN ONCOHEMATOLOGIC PATIENTS
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Background: The prevalence of hematological malignancies has increased overtime especially in the older population. In the era of immunochemotherapy and targeted therapy, it is important to have a multidimensional and comprehensive assessment that allows the identification of those subsidiaries to intensive therapeutic measures or a more conservative approach. To choose the best treatment for these patients, a geriatric hematology program has been launched.
Aims: Evaluate the utility of the comprehensive geriatric assessment (CGA) in patients with hematologic malignancies on the initial therapeutic decision making. Determine frailty prevalence and short - mid term prognostic impact using a screening tool for its identification.
Methods: Patients diagnosed with hematological malignancies were followed prospectively. Patients age 70 and over were referred to hematological nursing consultation. G8 screening tool was used to identify frailty risk. Patients with G8 score <14 were referred for a complete medical consultation in Geriatric Oncology Clinic for carrying out the CGA. In the comprehensive geriatric assessment, the clinical information obtained included: physical, mental, social and nutritional assessments, as well as an additional screening on geriatric syndromes. The regular medication was reviewed based on the **STOPP/START** criteria. The patients were classified in 3 categories according to the ***Balducci*** classification: 1) **Fit,** 2) **Fragile** and 3) **Poor prognostic**.
Results: We have included 32 patients in the last 9 months, with an average age of 81 (71-89) years. 56% of the sample was female. The main hematological malignancy referred was high grade non-Hodgkin lymphoma (59%). At the time of the evaluation, 87% had ECOG 1 and 56% EuroQoL score of 80. The social, functional and mental profiles are shown in Table 1. According to polipharmacy and comorbidities, data are shown in Table 2. The distribution of patients by frailty scales, are described in Table 3. 56% of the patients were classified as robust, 35% fragile and the rest with poorly prognosis**.** After the evaluation we recommended nutritional measures, control of the polypharmacy and physical exercise. Of the included patients, 22 had been reviewed at 6 months staying alive 95%. 24% required hospitalization after the initial assessment and 13% went to the emergency department.

Tables.

Table 1.

BARTHEL	LAWTON	FAC	GDS	SOCIAL
56% independent. 34% mild dependence.	80% <4 advanced activities.	80% independent walk.	90% do not have cognitive impairment	90% had social support.

Table 2.

CHARLSON	CIRS	>5 DRUGS	>3 diagnostics in addittion to oncological
56% high comorbidity rate.	11.25 on average.	75%	34,3%

Table 3.

FRAIL	SPPB
61% was not fragile	83% had mild or no limitation.

Summary/Conclusions: Our population had a high score of comorbidity and polypharmacy with a high median age. Nevertheless, 9% of the patients benefited from the treatment. In 34% of the cases, the treatment was adjusted because of the frailty criteria. 95% were alive and without clinical impairment. All these findings support the importance of the comprehensive geriatric assessment in the inicial evaluation of elderly patients with hematological malignancies.

P734

RADIATION EXPOSURE FROM CT IMAGING AND CHILDHOOD LEUKEMIA: A NATIONWIDE CASE-CONTROL STUDY

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Background: Pediatric CT imaging offers significant benefits in clinical practice. However, children are more sensitive to carcinogenic effects of ionizing radiation than adults and red bone marrow is especially radiosensitive tissue type. The risk estimates of low doses of ionizing radiation are mainly[1,2] based on extrapolated results of studies done with substantially higher radiation doses and there exists a need to assess the risks of low doses with a more direct approach.
Aims: We assessed the leukemia risk in children after computed tomography imaging studies with high-quality Finnish register data and data from hospital databases.

Table 1.

Table Odds ratios and frequencies of CT scans

	Cases	Controls	TOTAL	
CT scans				**OR *(95% CI)***
0	1043	3268	4311	
1	5	7	12	*2.78 (0.73, 10.5)*
2 or more	5	2	7	*16.9 (1.91, 150)*
1 or more	10	9	19	*4.75 (1.55, 14.5)*
by type				
ALL	8	6	14	*6.50 (1.66, 25.5)*
others	2	3	5	*2.03 (0.25, 16.5)*
by age-group				
2 - <7yo	3	1	4	*9.00 (0.94, 86.5)*
7 - <15yo	8	7	15	*1.69 (0.99, 9.09)*

The reference group for all calculated ORs is zero CT scans.

Methods: We used nationwide, register-based case-control study design to investigate the role of CT imaging in the etiology of childhood leukemia. We identified all childhood (0-15 years) leukemia cases from 1990 to 2011 (N=1093) in Finland and randomly selected thrice as many controls (N=3279) from the Population Registry, individually matched by gender and year of birth. The cases were 81% (N=885) acute lymphoblastic leukemias and 13% (N=142) acute myeloid leukemias. We collected data on all pediatric CT scans from 1975–2011 from the databases of all five university hospitals in Finland and two large central hospitals. In total, we identified 46 CT scans to our subjects. We approximated that this approach covers 81% of all pediatric CT scans performed in Finland from 1975 to 2011. We used a two-year latency period to avoid reverse causation. Conditional logistic regression analyses were adjusted for birth weight (large for gestational age) to control confounding. Cases with Down syndrome were excluded from the analyses.
Results: Overall, 13 cases (1.2%) and nine controls (0.3%) had a record indicating at least one CT examination. Of the relevant CT scans, 50% were performed on the head region and 41.3% the thorax region. The median age at CT scan was 8.12 years (7.46 years for cases and 10.0 years for controls).
In a conditional logistic regression analysis adjusted for birth weight, a significantly increased leukemia risk (OR=4.75, 95% CI 1.55, 14.5) was found for any CT examination (one or more) at least two years prior to leukemia diagnosis. When comparing one CT examination and two or more CT examinations with no examinations the ORs were respectively 2.78 (95% CI 0.73, 10.5) and 16.9 (95% CI 1.91, 150).
Summary/Conclusions: In our preliminary analyses we observed a substantial increase in childhood leukemia risk related to pediatric CT scans. The risk estimates are materially higher than in two earlier studies[1,2] and need to be interpreted with caution. We will seek to estimate radiation doses to the red bone marrow, based on limited data available on CT examinations (body part and examination type).

References

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P735

HEALTHCARE RESOURCE UTILIZATION WITH IXAZOMIB OR PLACEBO PLUS LENALIDOMIDE-DEXAMETHASONE IN THE RANDOMIZED, DOUBLE-BLIND, PHASE 3 TOURMALINE-MM1 STUDY IN RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM)

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Background: Treatment paradigms for RRMM have evolved in recent years with the approvals of multiple novel agents and evidence of benefits for using triplet *vs* doublet therapy and continuous treatment until progression. With more complex regimens and longer treatment duration, costs of treatment and healthcare resource utilization (HRU) are expected to increase, with IV agents having a greater impact on treatment burden than oral agents. The oral proteasome inhibitor ixazomib is approved in the US, EU, and multiple countries worldwide, in combination with lenalidomide-dexamethasone (Rd), for the treatment of RRMM patients (pts) following at least 1 prior therapy. Approval was based on the phase 3 TOURMALINE-MM1 study of ixazomib-Rd *vs* placebo-Rd, which demonstrated significantly improved progression-free survival (PFS; median 20.6 *vs* 14.7 months, HR 0.74) with ixazomib-Rd, with limited additional toxicity and no adverse impact on patient-reported quality of life (QoL; Moreau et al, N Engl J Med 2016).
Aims: HRU was an exploratory endpoint of the TOURMALINE-MM1 trial. The aim of this analysis was to compare HRU with ixazomib-Rd *vs* placebo-Rd, incorporating all non-protocol additional medical care encounters such as inpatient and outpatient admissions and their duration, as well as time lost from work or other activities by pts and their caregivers.
Methods: 722 RRMM pts with 1-3 prior lines of therapy received ixazomib 4mg (n=360) or matching placebo (n=362) on days 1, 8, and 15, plus lenalidomide 25mg on days 1-21 and dexamethasone 40mg on days 1, 8, 15, and 22, in 28-day cycles until disease progression or unacceptable toxicity. The primary endpoint was PFS. HRU was assessed on day 1 of each cycle prior to treatment and every 4/12 weeks during PFS/overall survival follow-up. After a median follow-up of ~23 months, pts had received a median of 17 (range 1-34) and 15 (1-34) cycles of ixazomib-Rd and placebo-Rd, respectively; HRU data are reported from this analysis time point.

Table 1.

Table 1	Ixazomib-Rd (n=360)		Placebo-Rd (n=362)	
Hospitalizations	**Rate ppy (95% CI)**	**Mean length, d**	**Rate ppy (95% CI)**	**Mean length, d**
Any	0.530 (0.471, 0.588)	10	0.564 (0.503, 0.624)	10.8
Acute care unit	0.411 (0.359, 0.462)	6.3	0.463 (0.408, 0.517)	8.2
Palliative care unit	0.069 (0.048, 0.090)	1.3	0.062 (0.042, 0.082)	1.6
Intensive care unit	0.034 (0.019, 0.048)	1.1	0.024 (0.011, 0.036)	0.4
Hospice care	0.022 (0.010, 0.034)	1.2	0.029 (0.015, 0.042)	0.5
Outpatient visits	**Rate ppy (95% CI)**		**Rate ppy (95% CI)**	
Any	3.305 (3.159, 3.451)		3.355 (3.208, 3.502)	
ER	0.143 (0.112, 0.173)		0.109 (0.083, 0.136)	
Study physician	0.889 (0.813, 0.964)		0.939 (0.861, 1.017)	
Other physician/clinic	1.677 (1.573, 1.781)		1.810 (1.702, 1.918)	
Lab	0.037 (0.021, 0.052)		0.032 (0.018, 0.046)	
Radiology/biomedical imaging	0.179 (0.145, 0.213)		0.207 (0.170, 0.244)	
Other	0.589 (0.527, 0.650)		0.436 (0.383, 0.489)	

Ppy, per patient-year

Results: Overall, 152 (42%) pts on the ixazomib-Rd arm had 316 hospitalization events, compared to 156 (43%) pts (335 events) on the placebo-Rd arm. Exposure-adjusted hospitalization rates (0.530 and 0.564 per pt-year [ppy], respectively) and mean length of stay (10 and 10.8 days) were similar between the ixazomib-Rd and placebo-Rd arms (Table 1). Rates of outpatient visits were also similar between arms; 217 (60%) pts on the ixazomib-Rd arm had 1971 visits (median 4) compared to 198 (55%) pts and 1994 visits (median 5) on the placebo-Rd arm. Exposure-adjusted outpatient visit rates were 3.305 and 3.355 ppy, respectively (Table 1). On the ixazomib-Rd arm, 46 (13%) pts missed a total of 527 (median 7) days of work or other activity, compared to 51 (14%) pts and 580 (median 8) days on the placebo-Rd arm. Similarly, 16 (4%) pts' caregivers missed 128 (median 5) days of work or other activity on the ixazomib-Rd arm, compared to 24 (7%) pts' caregivers and 110 (median 4) days on the placebo-Rd arm.
Summary/Conclusions: The ixazomib-Rd triplet regimen did not add to the HRU burden compared to the placebo-Rd doublet, while prolonging PFS. These findings are consistent with the limited additional toxicity burden and the reported lack of an adverse impact on QoL with ixazomib-Rd. In contrast to findings reported for injected agents (Armoiry et al, J Clin Pharm Ther 2011; Gaultney et al, J Clin Pharm Ther 2013; Baz et al, Support Care Cancer 2015), this all-oral triplet regimen did not increase time lost from work, caregiver burden, or the number of inpatient/outpatient visits.

P736

MANAGEMENT, ECONOMIC AND SOCIAL IMPACT OF SUB-CUTANEOUS RITUXIMAB ADMINISTRATION IN LYMPHOPROLIFERATIVE MALIGNANCIES
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Background: Lymphoproliferative disorders (LD) represent a major burden of hematologic malignancies generally treated and followed in Hematological Day Hospital (DH). Diffuse Large B-Cell Lymphoma (DLBCL) and Follicular Lymphoma (FL) are among the most frequent LD treated with chemo-immunotherapies. These therapies are time consuming and costing and may affect the Quality of Life (QoL) of these patients because of their prolonged stay in DH.
Aims: To evaluate, in patients with DLBCL and FL, the economic and social impact of subcutaneous Rituximab administration.
Methods: From March 2016 to December 2016, we investigate in DLBCL and FL, the management advantages of subcutaneous rituximab administration compared to the intravenous formulation. During one week we evaluate in 40 patients the time of intravenous and subcutaneous administration, the type of treatment (rituximab combined or subsequent to chemotherapy, or in monotherapy) and the time required by the pharmacy to prepare each formulation. Moreover, we collected and analyzed data about patients' time expenditure in DH until discharge. Collected data have been categorized as follows: time and human resource employment; drug waste; safety for patient. In order to measure the effect on QoL of subcutaneous formulation, we administered a questionnaire of satisfaction to the patients affected by DLBCL and FL, and their 40 caregivers. Furthermore, we evaluated its role in the optimization of DH management in terms of time, professionals and economic expenditure and in improving patients' safety.
Results: Among the 40 patients, 55% were affected by DLBCL and 45% by FL, 64% were males and 36% females; as for age 68% were over 60 years. The questionnaire examined patient's emotions and perceptions during rituximab administration (anxiety, fear and pain), time required for infusion and interference with daily activities. Overall, 98% of interviewed patients preferred subcutaneous administration because less scared by this formulation and because of the lower waste of time. Among the 40 interviewed caregivers 68% were workers. They considered advantageous the subcutaneous formulation because of the lower waste of time (90%) and the reduced number of workdays lost (80% of workers). With the subcutaneous formulation, we observed a reduction of 38% (equal to 17.5 hours) of time spent at hospital per cycle. However, nurses needed 23% lesser time to handle a patient per cycle (from 144 minutes to 111 minutes), earning 33 minutes/patient per cycle. Furthermore, pharmacy spent 53% lesser time for drug preparation (from 40 minutes to 19 minutes), earning 21 minutes/patient per cycle. Finally, we observed a reduction of clinical risk. As for the cost, using subcutaneous formulation, we saved €254.25 for each dose, with a final saving of €61.021 in a year. Considering that patients who underwent subcutaneous administration of rituximab did not require DH admission, we saved additional for €24.000 for 2016, and caregivers spared 112 work days.
Summary/Conclusions: Our investigation shows that subcutaneous formulation of rituximab requires a lower psychological effort for both patients and caregivers because of a reduced time of administration. In addition, it reduces healthcare professionals and pharmacist's workload, ensures a major safety for patients, and allows an optimized use of DH armchairs. This brings to an increased satisfaction of patients and caregivers. Moreover, the costs analysis demonstrates a significant spending reduction improving planning of therapy sessions and organization.

P737

EFFECT OF IMPROVEMENTS OF SURVIVAL, POPULATION AGING AND IMWG´14 CRITERIA ON INCIDENCE AND PREVALENCE OF MULTIPLE MYELOMA
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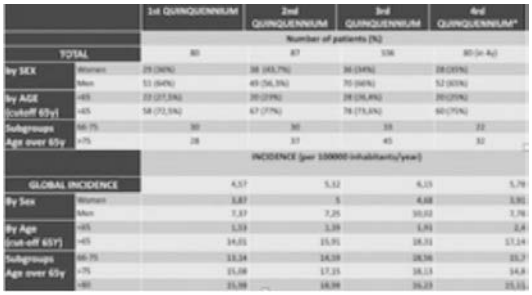
Background: There are some variables that can modify Multiple Myeloma incidence of New Diagnosed (NDMM) and prevalence over the time: Past decade shows a new demographic data in our society: the increment of expectancy of life and an excellent performance status. In the last years we have assisted to an amazing improvement in the management and expectancy of life of Multiple Myeloma (MM) patients. Recent changes in criteria recommendation by IMWG´14 to begin treatment in NDMM patients can increment its incidence. New expensive but very effective and well tolerated antimyeloma (antiMM) agents are in the center of attention of Hematologic and Public Healthcare Systems. There are data of improvement of survival that can increment of prevalence.
Aims: We have analysed our data base and calculate incidence by sex, age and three 5-years periods of time at diagnosis and obtain tendencies to get ready for next decade of ageing people with best antimyeloma agents. We have analysed prevalence of MM patients on last 7 years with cutoff date on 1st of November (2010 to 2016).
Methods: We retrospectively analysed the incidence of patients with new diagnostic of Multiple Myeloma (NDMM) from 1998 to 2012. (Fig.1). Then we divide the cohort in several groups: sex and age at diagnosis (3 groups: <65, 66-75 and 75y) and in four 5-year (quinquennium) period of time (1998-2002, 2003-07, 2008-12, 2013-NOV2016). (Fig. 2). We have calculated the incidence per 100000 inhab/year using census data of our Local Registry of Tumours of our Public Health Area. Characteristics of patients: n= 346. M/F: 206/140. Median age at diagnosis: 74 years (Range: 39-100).
Results: A) INCIDENCE RATES (*see Table*). In the past IMW (Roma-14#PO197) we reported incidence rates form 1998 to 2012. We observed a constant increase of Annual Average of incidence from 4.57 cases/100000 inhabitants/ year from the 1st period to 6.15 in the last. Adjusted by Age Incidence also increase from 14 to 18.5 cases in the O65 group. From 2013 to Nov-2016 global and adjusted by age incidence remains similar to last years data with 80 new cases in the 4 year-period (5,9 cases for global population and 17,2 cases for over65 population). After IMWG´14 criteria to begin treatment in NDMM the incidence was similar to the last 7 years (2008-12 period plus 2013-14) Incidence with 37 NDMM cases (25 O65y group).
B) PREVALENCE RATES (PrevR):
• 2010. 74 patients alive. PrevR: 21.25 /100000 inhabit;
• 2012. 77 pats alive. PrevR: 22.2 /100000 inhabit;
• 2014. 84 pats alive. PrevR: 24.4/100000 inhabit;
• 2016. 103 pats alive. PrevR: 30.3 / 100000 inhabit.

Table 1.



Summary/Conclusions: Although we don´t observe substantial changes on incidence rates of NDMM, we have noted an important rise on prevalence rates of more than 40% from 2010 to 2016 (21.2 to 30.3 pats alive /100000 inhabit.) Several new antiMM drugs are available in the therapeutic arsenal and probably increases the prevalence rates.

Stem cell transplantation - Clinical 2

P738

HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ISOLATED EXTRAMEDULLARY RELAPSE OF ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILDREN

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Background: Although most children affected by Acute Lymphoblastic Leukemia (ALL) are cured with current protocols, relapses still occur in the bone marrow as well in extramedullary sites, mainly the central nervous system (CNS) and the testis.
Aims: Optimal treatment for isolated extramedullary relapse (iEMR) is still controversial. To address this issue, we collected data of patients treated with hematopoietic stem cell transplantation (HSCT) for ALL iEMR from 19 centers, members of the Italian Pediatric Onco-Hematology Association (AIEOP).
Methods: From 1990 to 2015, 281 children (1-18 years) underwent HSCT for ALL iEMR in Italy. Informed consent was obtained from parents or legal guardians. Treatment protocols were based on Berlin-Frankfurt-Münster (BFM) Study Group concept, as well as definitions of iEMR, CNS relapse, and time of relapse (very early/ early/ late). HSCT was performed in second complete remission (CR2) or subsequent remission (CR>2), even patients transplanted with disease were included in the analysis. If a matched familiar (MFD) or a matched unrelated donor (MUD) was available, HSCT was performed from one of these; if not, the single center decided to perform autologous HSCT (Auto HSCT) or haploidentical HSCT (Haplo HSCT).
Results: Of the 281 patients (203 male, 78 female) 167 presented relapse confined to CNS, 73 to testis, 14 to mediastinum,11 to CNS + other sites and 18 to other organs. Thirty one percent of children experienced a late relapse, 34.5% an early relapse, 31% a very early relapse, for 3.5% the time of relapse is not known. Ninety-seven patients underwent auto HSCT, 79 MFD HSCT, 75 MUD HSCT and 30 Haplo HSCT. At transplantation 72.6% of children were in CR2, 21.0% in CR>2 and 6.4% were not in remission Total body irradiation (TBI) was part of 83.6% of conditioning regimens. Overall survival (OS) for the entire cohort was 56% at 10 years and was not influenced by sex, lineage, age, site of relapse, length of first remission, HSCT type (Auto *vs* MFD *vs* MUD *vs* Haplo). Patients transplanted in CR2 had the better OS (64%), those in CR>2 showed an OS of 44%, those transplanted with disease had an OS of 11% (p<0.0001). For HSCT performed before 2000 the OS was 45%, after 2000 was 63% (p 0.0009). TBI containing regimen yielded a better OS (59%) then regimens without TBI (40%) but this difference was not statistically significant (p 0.069). TRM for the entire cohort was 10% at 100 days, 11% at 6 months and 1 year and 16% at 10 years, with no difference between HSCT types. Multivariate analysis was conducted after exclusion of patients with active disease at HSCT: the only factors influencing outcome were number of CR and year of transplantation. Patients in CR>2 have a risk of death 2.3 times greater than those in CR2. Children treated after 2000 have half the risk of death then those treated from 1995 to 2000.
Summary/Conclusions: In this study we present the largest series of patients with ALL iEMR treated with HSCT with a very long follow up. Comparison with published chemo/radiotherapy approaches is favorable, especially for early and very early relapse: in fact the use of HSCT seems to abrogate the impact of some "classical" negative risk factors. Our results suggest that both autologous and allogenic HSCT are efficient treatments for ALL iEMR. Data from contemporary treatment protocols, that include MRD assessment to better stratify the patients, will further clarify the role of HSCT in the treatment of extramedullary relapses.

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PREDICTIVE FACTORS FOR DEVELOPING VENO-OCCLUSIVE DISEASE IN PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA TREATED WITH INOTUZUMAB OZOGAMICIN FOLLOWED BY ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Inotuzumab ozogamicin (IO) is a CD22 monoclonal antibody attached to calicheamycin and targets B lymphocytes in early stages of development. In a randomized study of IO compared with conventional salvage therapy in patients with refractory relapsed B-ALL, patients treated with IO had higher complete response rates (81% *vs* 29%, p<0.001), and a greater proportion of patients proceeded to allogeneic hematopoietic stem cell transplantation (SCT) (41% *vs* 11%, p<0.001). However, patients treated with IO prior to SCT were also noted to have higher rates of veno-occlusive disease (VOD) compared to the SCT group without IO exposure (11% *vs* 1%) (Kantarjian NEJM 2016).
Aims: In efforts to further investigate this finding, we reviewed transplant outcomes for patients with and without IO exposure.
Methods: We performed a nested control comparison of patients transplanted during the years when they were being treated with IO on a number of clinical trials at our institution.
Results: Between 6/2010 and 10/2016, 251 patients with B-ALL with a median age of 35 years (range, 4-70 years) received an allogeneic matched sibling (n=85), matched- or 1-antigen mismatched unrelated (n=90), haplo-identical (n=38), or cord blood donor SCT (n=38) in CR1 (n=103), CR2+ (n=105), or with active disease (n=43). Patients received largely myeloablative regimens (82%) that were busulfan- (64%), fludarabine- (29%) or total body irradiation-based (TBI) (7%). 19% of patients received double alkylator regimens consisting of cyclophosphamide-TBI, fludarabine-melphalan-thiotepa, or busuflan-clofarabine-thiotepa. IO was administered to 69 (27%) patients prior to SCT. A median of 3 cycles of IO were administered (range, 1-5 cycles) at a median of 35 days from SCT (range, 11-254 days). Patients were heavily pre-treated, including 18 who had a prior allogeneic SCT. VOD was noted in 21 patients overall (8%) with median onset 19 days following SCT (range, 7-230 days); fatal VOD was noted in 5 patients (2%). VOD was noted in 11 patients treated with IO (16%), and it was fatal in 2 patients (3%). Factors noted to be significant in contributing to VOD in univariate analysis were prior exposure to IO (HR 3.05, 95% C.I. 1.3-7.2, p=0.01) and receiving a busulfan-based transplant preparative regimen (HR 3.4, 95% C.I. 1.02-12, p=0.05); not receiving a prior SCT was significantly protective (HR 0.3, 95% C.I. 0.1-0.8, p=0.02). Number of IO cycles, time from IO to SCT, age, and donor relation were not found to be significant factors for developing VOD. In efforts to predict the risk for VOD in a patient who has received prior IO, we performed a classification and regression tree analysis (CART) and noted that the combination of IO and a double alkylator preparative regimen was significantly associated with the risk for developing VOD (HR 5.9, 95% C.I. 1.9-18, p=0.002).
Summary/Conclusions: Fatal VOD is a rare occurrence. However, IO exposure prior to SCT increases the risk for any VOD. Furthermore, IO exposure followed by a double alkylator preparative regimen increases this risk nearly 6-fold, and should be avoided in these patients.

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DEFIBROTIDE EFFICACY AND SAFETY IN PATIENTS WITH HEPATIC VENO-OCCLUSIVE DISEASE/SINUSOIDAL OBSTRUCTION SYNDROME (VOD/SOS) DIAGNOSED AFTER DAY 21: ANALYSIS OF FINAL DATA FROM AN EXPANDED-ACCESS PROGRAM

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Background: Hepatic VOD/SOS is a potentially life-threatening complication of conditioning for hematopoietic stem cell transplant (HSCT). VOD/SOS with multi-organ dysfunction (MOD) may be associated with >80% mortality. Although VOD/SOS typically begins <21 days post-HSCT (per Baltimore/modified Seattle criteria), late-onset VOD/SOS has been reported in 15%>20% of cases, sometimes after hospital discharge. The European Society for Blood and Marrow Transplantation (EBMT) proposed new criteria for adults including late-onset VOD/SOS (>21 days post-HSCT) by Baltimore criteria, histological evidence of VOD/SOS, or a version of Baltimore criteria using ≥2 of: bilirubin

≥2mg/dL, painful hepatomegaly, weight gain >5%, or ascites—***plus*** mandatory hemodynamic/ultrasound evidence of VOD/SOS. Defibrotide (DF) is approved to treat severe hepatic VOD/SOS post-HSCT in the EU, and for hepatic VOD/SOS with renal or pulmonary dysfunction post-HSCT in the US.
Aims: This is an analysis of DF efficacy and safety in patients (pts) with late-onset VOD/SOS using final data from an expanded-access study.
Methods: The original expanded-access protocol required VOD/SOS per Baltimore criteria or biopsy by Day+35 post-HSCT, with MOD (renal/pulmonary) by Day+45. The study was amended to include pts with later-onset VOD/SOS, with or without MOD; VOD/SOS per modified Seattle criteria; and VOD/SOS after chemotherapy alone. Pts provided informed consent and received DF 25mg/kg/d (6.25mg/kg q6h) for a recommended ≥21 days. "Late-onset" in this post-hoc analysis was defined as diagnosis >21 days post-HSCT; hemodynamic/ultrasound data (EBMT criteria) were not available.
Results: Of 1000 HSCT pts with a confirmed diagnosis of VOD/SOS and receiving ≥1 dose of DF, 264 (26.4%) had late-onset VOD/SOS, of whom 139 (52.7%) had MOD. By age group, 95/264 (36.0%) were pediatric (aged ≤16 years; 51/95 [53.7%] with MOD) and 169/264 (64.0%) were adults (aged >16 years; 88/169 [52.1%] with MOD). Kaplan-Meier estimated survival at Day +100 (**Figure**) was 52.8% (95% CI, 46.5%–58.7%) across all HSCT pts and 43.9% (95% CI, 35.4%–52.0%) for pts with MOD; for pediatric pts, this was 60.4% (95% CI, 49.5%–69.7%) overall and 45.4% (95% CI, 31.0%–58.6%) for pts with MOD; for adults, Day +100 survival was 48.7% (95% CI, 40.9%–56.0%) overall and 43.0% (95% CI, 32.5%–53.0%) for pts with MOD. Adverse events (AEs) occurred in 75.4% of the total group (80.6% with MOD); 70.5% of pediatric pts (76.5% with MOD); 78.1% of adults (83.0% with MOD). Treatment-related AEs (TRAEs) occurred in 20.8% overall (23.7% in those with MOD); 21.1% of pediatric pts (23.5% with MOD); 20.7% of adults (23.9% with MOD). The most common TRAEs (>3%) were epistaxis, pulmonary hemorrhage, gastrointestinal hemorrhage, and hematuria (each in <5% of pts). For TRAEs leading to study discontinuation (n=25) or death (n=10), the most common was pulmonary hemorrhage.

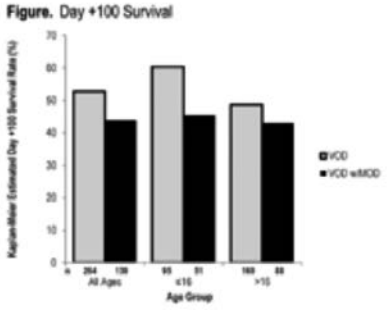

Figure 1.

Summary/Conclusions: In this study, diagnostic criteria requiring onset by day 21 would exclude >26% of pts with VOD/SOS, with more than a third of these being pediatric pts. This highlights the importance of including late-onset VOD/SOS in diagnostic criteria. With DF, 52.8% were estimated to survive to Day +100 (60.4% of pediatric and 48.7% of adult pts). TRAEs for these subgroups were similar to the overall study results. Factors contributing to survival in these pts is a potential area for future exploration.

Support: Jazz Pharmaceuticals.

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ALLO-HCT FOR PAROXYSMAL NOCTURNAL HEMOGLOBINURIA-12 YEARS OF EXPERIENCE

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired clonal abnormality of hematopoietic stem cell leading to lack of phosphatidylinositol glycoproteins, sensitizing cells to complement-mediated lysis. Despite the efficient symptomatic treatment of hemolytic PNH with eculizumab, allo-HCT is the only curative treatment for the disease, although outcomes presented in the past were controversial.
Aims: The aim of this study was to evaluate the safety and effectiveness of allo-HCT in PNH.
Methods: We report 41 allo-HCTs: 37 from MUD and 4 from MRD performed for PNH in 2004-2016. Median age of recipients was 29(20-62) years and donors 30(19-53), median time from diagnosis to allo-HCT was 16(2-307) months. Median size of PNH clone was 80% granulocytes (0.5%>100%). Indication for allo-HCT was PNH with aplastic/hypoplastic bone marrow (19 pts), MDS (2 pts), overlapping MDS/aplasia (3 pts), severe course of PNH with hemolytic crises and transfusion-dependency without access to eculizumab (17 pts). Additional risk factors were Budd-Chiari syndrome and hepatosplenomegaly (1 pt), history of renal insufficiency requiring hemodialyses (2 pts), chronic hepatitis B (1 pt) and C (1 pt). The preparative regimen consisted of treosulfan 3x14 g/m2 plus fludarabine 5x30mg/m2 (31 pts) or treosulfan 2x10 g/m2 plus cyclophosphamide 4x40mg/kg (10 pts). Standard GVHD prophylaxis consisted of cyclosporine-A, methotrexate and pre-transplant ATG in MUD-HCT. 2 pts instead of cyclosporine-A received mycophenolate mofetil and tacrolimus. Source of cells was bone marrow (13 pts) or peripheral blood (28 pts) with median $6.3x10^8$NC/kg, $5.7x10^6$CD34+cells/kg, $24.7x10^7$CD3+cells/kg. Myeloablation was complete in all pts with median 9(1-20) days of absolute agranulocytosis <0.1 G/l. Median number of transfused RBC and platelets units was 9(0-16) and 8(2-18).
Results: All pts engrafted, median counts of granulocytes 0.5 G/l, platelets 50 G/l and Hb 10 g/dl were achieved on days 17.5(10-33), 16(9-39) and 19.5(11-34). Acute GVHD grade I,II and III was present in 16, 7 and 3 pt, limited and extensive chronic GVHD respectively in 11 and 3 pts. LDH decreased by 73%(5%>91%) in first 30 days indicating disappearance of hemolysis. 100% donor chimerism was achieved in all pts. In 1 patient donor chimerism decreased to 81% what was treated with donor lymphocytes infusion (DLI). 3 patients died, 1 previously hemodialysed pt died on day +102 due to nephrotoxicity complicating adenoviral/CMV hemorrhagic cystitis, two other SAA patients with PNH clone<10% died on days +56 due to severe pulmonary infection and +114 due to aGvHD-III and multi organ failure. Complications in survivors were FUO (10 pts), CMV reactivation (13), VOD (1), neurotoxicity (1), venal thrombosis (1), hemorrhagic cystitis (4) and mucositis (8). 38 pts (92.7%) are alive 4.2 (0.4-12) years post-transplant and are doing well without treatment. Complete disappearance of PNH clone was confirmed by flow cytometry in all surviving pts.
Summary/Conclusions: We conclude that allo-HCT with treosulfan-based conditioning is effective and well tolerated curative therapy for PNH.

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A COMPARISON OF CLINICAL OUTCOMES BETWEEN MATCHED SIBLING DONOR (MSD) AND UNRELATED DONOR (URD) STEM CELL TRANSPLANTATION IN ADULT PATIENTS WITH SEVERE APLASTIC ANEMIA

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Background: Allogeneic stem cell transplantation (SCT) using HLA-matched unrelated donor (URD) has been usually regarded as a subsequent option in patients with severe aplastic anemia (SAA), who have failed to immunosuppressive treatment (IST). However, recent improved outcomes of URD SCT lead to its extended role for treating those lacking HLA-matched sibling donor (MSD).
Aims: Through this study, we intended to verify the possibility of URD SCT as a front-line treatment for SAA patients.
Methods: We compared outcomes of consecutive SAA patients who received SCT from 8/8 well-matched URD (WM-URD; n=61) and partially (6/8 or 7/8) matched URD (PM-URD; n=33) with 8/8 matched MSD (n=126) at our institution between Mar 2002 and Dec 2016. Patients receiving MSD and URD SCT were conditioned with fludarabine (180mg/m^2) / cyclophosphamide (100mg/kg IV) plus rabbit ATG (10mg/kg IV), and total body irradiation (fractionated 800cGy)/ cyclophosphamide (100-120m/kg IV) with/without rabbit ATG (2.5mg/kg IV), respectively.
Results: Median age of the WM-URD and the PM-URD groups were significantly lower compared to that of the MSD group (29 yrs, 31 yrs, and 39 yrs; *P* <0.01), with a high proportion of those experiencing IST failures before SCT (80.3%, 90.9%, and 33.3%; *P*<0.01). Median days to neutrophil engraftment of the MSD group was significantly shorter compared to those of the WM-URD and the PM-URD groups (12 days, 16 days, and 16 days; *P*<0.01). The incidences of acute and chronic GVHD of the WM-URD and PM-URD groups were significantly higher compared to those of the MSD group (42.6% and 63.6% *vs* 9.5%; *P*<0.01, and 44.6% and 33.3% *vs* 8.9%; *P*<0.01, respectively). When we compared the incidence of transplant-related mortality (TRM; 10.7% *vs* 7.4% at 6 yrs; *P*=0.53) and overall survival rate (OS; 89.3% *vs* 92.5% at 6 yrs; *P*=0.52) between the WM-URD and the MSD groups, there were no significant difference. However, trends of higher TRM incidence (18.2% *vs* 7.4% at 6 yrs; *P*=0.05) and lower OS rate (81.8% *vs* 92.5% at 6 yrs; *P*=0.05) were observed between the PM-URD and the MSD groups. There was no primary graft failure in either group. Incidence of secondary graft failure of both WM-URD (0% *vs* 18.3%; *P*<0.01) and PM-URD (0% *vs* 18.3%; *P*=0.02) groups were significantly lower compared that of the MSD group. When we adjusted other clinical and transplant-related factors, which include age and IST failure, using multivariate

analysis, the OS rate of the WM-URD group was not significantly different (HR 1.45, 95% CI; 0.52-4.09; *P*=0.48), whereas that of the PM-URD group was significantly lower (HR 2.85, 95% CI; 1.01-8.02; *P*=0.04), compared to that of the MSD group.
Summary/Conclusions: Our study showed that there was no significant difference in OS rate between the WM-URD and the MSD groups. As high incidence of GVHD remains as a problem in the former group, strategies to reduce it are needed in future protocols.

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HAPLOIDENTICAL ALLOGENEIC STEM CELL TRANSPLANT IN SEVERE THALASSEMIA PATIENTS

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Background: Thalassemia free survival after allogeneic stem cell transplantation (SCT) is about 80–90% with either matched related or unrelated donors. However, the probability of finding a HLA-compatible donor is less than 50%. We explored the use of a mismatched related ("Haplo-") donor.
Aims: To evaluate the outcome of SCT with Haplo donors in severe thalassemia patients
Methods: All patients received two courses of pre-transplant immunosuppression therapy (PTIS) with fludarabine (Flu) 40mg/m2/d together with dexamethasone (Dxm) 25mg/m2 for 5 d to facilitate engraftment. After two courses of PTIS, a reduced-toxicity conditioning regimen of rabbit anti-thymocyte globulin (ATG) 1.5mg/kg/d on days SCT -12,-11,-10, Flu 35mg/m2 on days SCT -7,-6,-5,-4,-3,-2 and IV Busulfan (Bu) 130mg/m2 on days SCT
-7,-6,-5,-4 was given followed by T-cell replete peripheral blood progenitor cells (PBPC). GVHD prophylaxis consisted of cyclophosphamide (Cy) 50mg/kg on days SCT +3 and +4 (Post-Cy), and on day SCT +5 tacrolimus or sirolimus was started together with a short course of mycophenolate mofetil.
Results: Fifty-one patients underwent haplo-SCT. Their median age was ten years (range, 2 to 28 years). Forty-nine patients engrafted with 100% donor chimerism. Two of five patients with high titers of donor-specific anti-HLA antibodies suffered primary graft failure. Median time to neutrophil engraftment was 14 days (range, 11 to 18 days). Eight patients developed mild to moderate, reversible veno-occlusive disease, while twelve patients developed acute GVHD grade II, that quickly responded to steroid therapy. Only seven patients developed limited chronic GVHD. Projected overall and event-free survival rates at two years are 95% and 94%, respectively. The median follow up time is 18 months (range;10 to 50 months).
Summary/Conclusions: This haplo-SCT protocol may yield excellent outcomes for thalassemia patients, and provide a treatment option for patients lacking a HLA-matched donor.

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AUGMENTATION OF FLUDARABINE AND BUSULFAN-BASED MYELOABLATIVE REGIMEN WITH THIOTEPA IMPROVES OUTCOMES WITH NO ADDED TOXICITY IN ALLOGENEIC STEM CELL TRANSPLANTATION FOR ACUTE MYELOID LEUKEMIA

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Background: Allogeneic stem-cell transplantation (HSCT, allo-SCT) is the most effective way to control leukemia relapse for patients with acute myeloid leukemia (AML). Busulfan and Cyclophosphamide (Bu/Cy),the current standard of care, in allogeneic transplant for acute myeloid leukemia (AML), is limited by increased treatment related mortality. Myeloablative doses of Busulfan (12·8mg/kg) with Fludarabine (160mg/m^2) (Flu-Bu), has reduced toxicity, however with the limitation of increased relapses. We have tried to improve outcome of Flu-Bu regimen by augmentation with Thiotepa (10mg/kg). Here we compared outcomes of 45 such patients (getting augmented regimen, Flu-Bu with the addition of Thiotepa, (group 2), to 44 patients who received Fludarabine, Busulfan myeloablative reduced toxicity regimen (group 1), during the same period.
Aims: The primary objective of the report was to compare the toxicity and incidence of relapse between the two regimens. Secondary objective was to compare overall survival (OS), and disease-free survival (DFS), the non-relapse mortality (NRM), engraftment kinetics, incidence of acute and chronic graft *versus* host disease (GVHD), and comparison between high and low-risk patients amongst the two groups.
Methods: 89 patients with AML were retrospectively analyzed. 44 patients were conditioned with Flu-Bu (group 1) and 45 patients augmented with Thiotepa (Flu-Bu-TT, group 2). The transplant conditioning regimen, (augmented myeloablative) consisted of 30mg/m^2 intravenous Fludarabine for 5 days (total dose 150mg/m^2), for matched related donors or for 6 days (180mg/m^2), for unrelated or mismatched donors, intravenous Busulfan (3.2mg/kg/day for 4 days, total dose 12.8mg/kg), and intravenous Thiotepa 5mg/kg for 2 days (10mg/kg). The conventional myeloablative regime was identical, however without the addition of Thiotepa.
Results: Toxicities were comparable, with mucositis in 7 patients (15%) in group 1 and 8 patients (17%) in group 2, (p=1.0), severe sepsis in 4 (9%) in group 1 and 3 (6%) in group 2, (p=0.7), severe venocclusive disease in 2% of group 1 and 4% of group 2, (p=1.0) and comparable non- relapse mortality (NRM), (p=0.7). 5-year disease free survival (DFS), (median follow up of 5 years), was significantly better in group 2, 38% for group 1, and 62% in group 2, (p=0.02) and 5-year overall survival showed trend towards benefit in group 2 (62% *vs* 42%, p=0.06). 14/30 (46%) patients in group 1 relapsed, as compared to 4/31 patients, (12%, p=0.005) in group 2, considering NRM as competing risk.

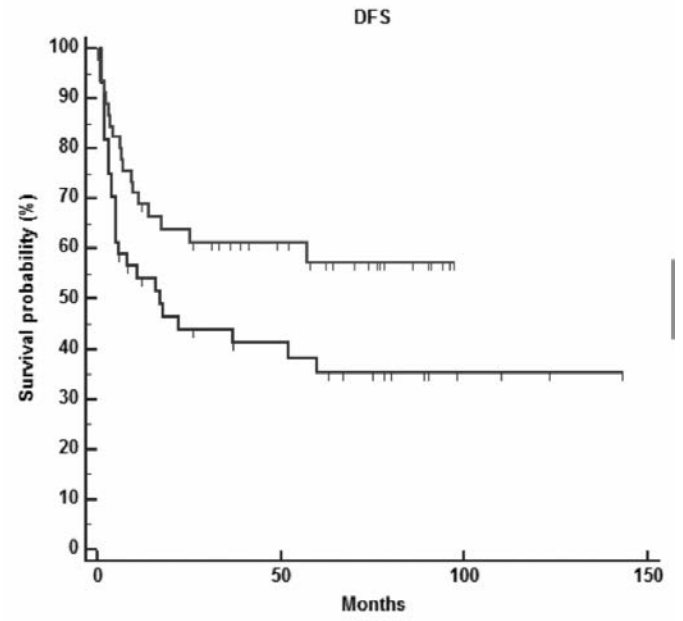

Figure 1.

Summary/Conclusions: In conclusion, the outcome of augmented regimen (DFS and OS) is superior Flu-Bu regime, mainly due to reduction in relapses, with comparable toxicities and could eventually replace Bu/Cy.

P745

PROGNOSTIC TOOLS CAN PROVIDE PERSONALIZED OUTCOMES PREDICTION AFTER ALLOGENEIC HCT IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES

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Background: Current prognostic indices for allogeneic HCT (alloHCT) outcomes often focus on a limited set of factors, be they patient characteristics, disease features, or transplant approaches. We sought to evaluate two comprehensive prognostic models in a large sample of patients undergoing alloHCT with CD34 selection (CD34 alloHCT).
Aims: To evaluate two comprehensive prognostic models: The first combining the HCT-Comorbidity Index (HCT-CI) and Disease Risk Index (DRI); the second applying the Center for International Blood and Marrow Transplant Research (CIBMTR) One Year Survival Outcomes Calculator, which uses large-scale multicenter data reported to the CIBMTR to provide patient-specific predictions on survival 1 year after first alloHCT.
Methods: This retrospective analysis included adult recipients of first alloHCT with CD34+ selected PBSCs from 7/8 or 8/8 donors for AML, ALL, or MDS at a single center between 1/2000 and 12/2015. The Kaplan-Meier (KM) method estimated OS and RFS. The cumulative incidence method for competing risks estimated relapse and nonrelapse mortality. We evaluated univariate association between variables of interest and OS/RFS using the log-rank test. Cox regression models assessed the adjusted effect of covariates on OS/RFS. We then determined predicted 1 year OS for each patient using the CIBMTR Calculator. Patients were divided into groups based on predicted OS probability,

in intervals of 5% +/- 2% (*e.g.* 65 +/-2% probability of survival at 1 year). Corresponding observed 1 year OS was then estimated for each group by the KM method. A kernel smoother was used to visually display the average of observed 1 year survival estimates over the continuous range of predicted OS.
Results: 506 patients with AML (n=290), ALL (n=72), or MDS (n=144) were included. Of these, 470 patients (AML=263, MDS=141, ALL=66) had full data available for the CIBMTR Calculator. On univariate and multivariate analyses, DRI, HCT-CI, and age correlated with significant differences in OS/RFS, while donor HLA match correlated with a significant difference in OS. Stratifying patients based on a composite of DRI (low/intermediate *vs* high/very high) and HCT-CI (0-2 *vs* 3+) revealed significant differences in OS/RFS between the 4 groups (Fig. 1). Compared with a reference group of patients with both low/intermediate DRI and low HCT-CI, those with high DRI and low HCT-CI were at greater risk of death (HR 2.30; 95% CI 1.39-3.81) and relapse or death (HR 2.50; 95% CI 1.55-4.05), more so than patients with a higher HCT-CI but still low/intermediate DRI (HR death 1.80; 95% CI 1.34-2.43; HR relapse/death 1.68; 95% CI 1.26-2.24). When comparing predicted and observed survival, KM estimates of 1 year OS fell within range of that predicted by the CIBMTR Calculator in almost all groups (Fig. 1). In one group, patients had lower observed 1 year OS than predicted (76%, 95% CI 62-93%, *vs* 85 +/- 2%, p=NS). In this group, 29/30 patients (97%) had intermediate or high DRI; 59% had poor prognostic AML/ALL by NCCN criteria (n=12, 44%) or other adverse features such as minimal residual disease pre-HCT (n=4, 15%).

Fig. 1: Survival after CD34 allo HCT by two prognostic models

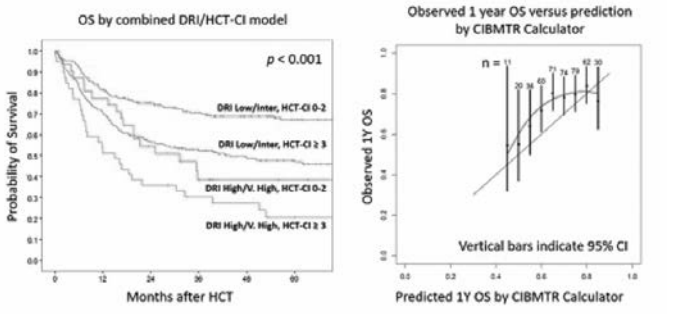

Figure 1.

Summary/Conclusions: Based on a large cohort of patients who underwent CD34 alloHCT for acute leukemia or MDS, we demonstrate that DRI is a major determinant of outcome. The CIBMTR Survival Outcomes Calculator predicts 1 year prognosis with relative precision, though some disease-risk features not reflected in the Calculator may affect outcomes in patients with otherwise good prognosis. Taken together, these prognostic models can assist in predicting outcomes and identifying patients most likely to benefit from CD34 alloHCT. Furthermore, applying the CIBMTR calculator analysis in individual centers may help identify patients with worse outcomes than predicted and guide patient and/or HCT selection.

P746

THROMBOTIC MICROANGIOPATHY AFTER ALLOGENEIC STEM CELL TRANSPLANTATION: IS THERE A PROTECTIVE ROLE FOR URSODEOXYCHOLIC ACID?

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Background: Thrombotic microangiopathy (TMA) after allogeneic stem cell transplantation (alloSCT) may be a severe complication associated with high mortality. Since there is no standard treatment it would be helpful to have efficacious prophylactic measures. Some data support the beneficial effect of ursodeoxycholic acid (UDA) to prevent endothelial-cell damage.
Aims: We retrospectively analysed a total of 671 patients undergoing to reduced intensity conditioning (RIC) alloSCT, comparing the occurrence of overall TMA according with the use or not of UDA.
Methods: Both uni and multivariate analysis were performed including patient and transplant-related variables at the moment of transplant to analyse the risk of developing TMA.
Results: Cumulative incidence for overall TMA was 4.8 (3.4-6.6) at 1 month, 10.1 (7.9-12.5) at 100 days, and 12.7 (10.3-15.4) at 180 days (figure 1). On univariate analysis, TMA was more frequent in lymphoid malignancies, Fludarabine-melphalan based conditioning, unrelated donor, mismatched donor, prophylaxis with sirolimus-tacrolimus (SRL/TKR), prior transplant and non-UDA patients. The probability of overall TMA at 180 days in UDA patients was 9.6% (95% CI: 5.9-14.3), *versus* 14.7% (95% CI: 11.7-18.1) in non-UDA patients. On multivariate analysis the risk factors which remained statistically significant were unrelated donor and the use of SRL/TKR, whereas the use of UDA significantly decreased the risk of TMA (HR:0.4, 95% CI:0.2-0.8, p:0.01). Moreover, in the subgroup of SRL/TKR, 100 days-cumulative incidence of TMA was 11.8% (95% CI: 6.9-18.1) *versus* 25.6% (95% CI: 17.9-33.9) depending on the use or not of UDA, respectively (p: 0.005), whereas in the subgroup of CNI/MTX 100d-Cumulative incidence of TMA was 3.4%(95% CI: 0.6-10.6) *vs* 12.1(95% CI:7.1-18.6) with and without UDA, respectively (p:0.05).
Summary/Conclusions: In conclusion the use of UDA decreases the risk of TMA after alloSCT regardless of type of immunoprophylaxis.

P747

FACTORS PREDICTING GRAFT *VERSUS* HOST DISEASE-FREE, RELAPSE-FREE SURVIVAL AFTER ALLOGENEIC TRANSPLANTATION. COMPARISON ATTENDING TO TWO DIFFERENT DEFINITIONS AND BENEFICT OF HAPLOIDENTICAL DONOR

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Background: Disease free survival is the most common used endpoint for clinical research on allogeneic stem cell transplantation (HSCT), but it doesn't include morbidity endpoints or those which affect their quality of life as graft *versus* host disease (GVHD). Recently, Blood and Marrow Transplant Clinical Trials Network has proposed a composite endpoint: GVHD-free, relapse-free survival (GRFS) for HSCT outcomes. This endpoint includes as event: III-IV acute GVHD (aGVHD), relapse, death or chronic GVHD (cGVHD) requiring systemic treatment. In 2016 EMBT annual meeting a redefinition of this endpoint was proposed changing cGVHD event from those patients with cGVHD requiring systemic treatment (the original one) to those with just severe cGVHD (the redefined one).

Table 1.

	Whole Cohort (n = 616)	Sibling donor (n = 393)	Unrelated donors (n = 186)	Haploidentical donors (n = 36)	p
Median age (yr, range)	49 (16-70)	50 (16-69)	49 (17-68)	60 (16-70)	<0.001
Male gender	363 (59%)	240 (61%)	101 (54%)	22 (60%)	0.3
Sex mismatch	143 (23%)	97 (25%)	35 (19%)	11 (30%)	0.19
Diagnosis					
• AML	201 (33%)	128 (33%)	54 (29%)	18 (51%)	
• ALL	73 (12%)	41 (10%)	32 (17%)	----	
• MDS	87 (14%)	49 (13%)	31 (17%)	7 (19%)	
• CML	39 (6%)	28 (7%)	11 (6%)	----	0.002
• MPS Ph-	12 (2%)	10 (3%)	1 (<1%)	1 (3%)	
• NHL/HL/CLL	152 (25%)	96 (25%)	46 (24%)	10 (17%)	
• MM	53 (9%)	41 (10%)	11 (6%)	----	
Conditioning intensity					
• RIC	390 (64%)	246 (63%)	114 (61%)	30 (83%)	0.06
• MA-chemoT	157 (25%)	103 (26%)	45 (24%)	6 (17%)	
• TBI - based	79 (11%)	42 (11%)	27 (15%)	----	
Cell source					
• PBSCs	532 (86%)	367 (93%)	128 (69%)	35 (97%)	
• BM	64 (10%)	26 (7%)	37 (20%)	1 (3%)	<0.001
• UCB	21 (3%)	----	21 (11%)	----	
ECOG ≥ 2	47 (8%)	34 (9%)	11 (6%)	2 (6%)	0.45
EBMT stage disease					
• Early	277 (45%)	182 (46%)	79 (43%)	15 (41%)	
• Intermediate	235 (38%)	148 (38%)	78 (42%)	8 (22%)	0.008
• Late	105 (17%)	62 (16%)	29 (16%)	13 (39%)	
Year of BMT					
• 1995-2004	194 (31%)	172 (44%)	21 (11%)	----	<0.001
• 2005-2016	423 (69%)	221 (56%)	165 (89%)	37 (100%)	
Prior Autologous SCT	144 (23%)	86 (22%)	47 (25%)	10 (27%)	0.6
Median prior lines (range)	2 (0-9)	1 (0-9)	2 (0-7)	2 (0-6)	0.035
HLA matching					
• Identical	489 (79%)	349 (96%)	109 (59%)	----	
• 1 ó 2 mismatch	91 (15%)	14 (4%)	77 (41%)	----	<0.001
• Haploidentical	37 (6%)	----	----	37 (100%)	
In vivo T-cell depletion (ATG or Alemtuzumab)	54 (9%)	6 (2%)	48 (26%)	----	<0.001
GVHD prophylaxis					
• CSA + MTX	316 (51%)	295 (75%)	20 (11%)	----	
• TCR + MTX	74 (12%)	29 (7%)	45 (24%)	----	
• CI + MMF	60 (7%)	3 (1%)	37 (20%)	----	<0.001
• TCR + SIR	135 (22%)	56 (14%)	79 (43%)	----	
• CI + MMF + CyPT	37 (6%)	----	----	37 (100%)	
• Others	15 (2%)	8 (2%)	5 (3%)	----	
II- IV aGVHD	345 (57%)	194 (50%)	133 (73%)	18 (49%)	<0.001
• Median day (range)	24 (4-362)	30 (4-252)	22 (4-196)	28 (18-362)	0.001
• III-IV aGVHD	95 (16%)	55 (14%)	37 (20%)	3 (9%)	0.09
cGVHD (*)	318 (61%)	222 (66%)	90 (57%)	6 (20%)	<0.001
• Median day (range)	217 (47-1477)	213 (47-1477)	228 (70-1304)	366 (152-604)	0.1
• Moderate or severe cGHVD	225 (43%)	155 (47%)	68 (43%)	2 (6%)	0.001
(*) from evaluated patients alive at day +100					

Aims: We had generated two composite endpoints: in both III-IV aGVHD, relapse or death were considerated events but we defined GRFS1 as the one with cGVHD event including those who required systemic treatment (as the original one) and in GRFS2 just those with severe cGVHD (the EBMT redefined one) and we had compared both.
Methods: We retrospectively analysed 616 patients transplanted (1995-2016) excluding non-malignant diseases, second allo-SCT and those <16 years old age.

Results: Characteristics of patients are shown in **table 1**. With a median follow up for patients alive of 39 months (3-221), the median estimated survival in months and the % at +1 year and +2 years was: 114 months, 70% and 62% overall survival (OS); 23 months, 57% and 49% event free survival (EFS); 6 months, 35% and 26% GRFS1; 11 months, 46% and 38% GRFS2. 147 (24%) and 218 (35%) hadn't any event in GRFS1 and in GRFS2 respectively. In GRFS1, event's incidence was: 90 (15%) for III-IV aGVHD, 170 (27%) for cGVHD, 152 (25%) for relapse and 57 (9%) for death; In GRFS2 was 90 (15%), 65 (11%), 174 (28%) and 65 (11%) respectively. Considering those patients with cGVHD as event in GRFS1, 105 of them hadn't the event as cGVHD at the same time in GRFS2 (since they had cGVHD requiring systemic treatment but not severe cGVHD). For these patients, the alternative event in GRFS2 was: 72 without any event, 22 relapsed and 11 died. In the multivariate analysis, factors associated with better outcomes were:for GRFS1 diagnosis (p=0,04; benefit in NHL/HL/CLL p=0.02, HR 0.71; CI95% 0.53-0.95), >4 prior lines (p=0.03, HR 1.5, CI95% 1.04-2.04,), early EBMT stage (p<0,001 with early as reference; intermediate p=0,002, HR 1.5, CI95% 1.2-1.9; advance p<0,001, 2.0, 1.5-2.6), *in vivo* T-cell depletion (p=0.02, 0.6, 0.39-0.92) and haploidentical donor (p=0.04 with HLA identical as reference, no significance 1 or 2 mismatch [p=0.18], haploidentical p=0.02, 0.43, 0.25-0.74). Only early EBMT disease stage maintained significance in GRFS2 (p<0,001 with early as reference; intermediate p=0,005, 1.5, 1.1-1.9; advance p<0,001, 1.9, 1.4-2.6).

Summary/Conclusions: In our study the percentage of the GRFS endpoint was similar to previously reported. Comparing both proposed definitions, the GRFS2 endpoint define a higher population of patients without any event; so that it is possible that t morbidity is misdiagnosed. The EBMT disease score was the factor with more impact in both; it is interesting to point that although the group is smaller, haploidentical donor is associated with better GRFS1.

P748

EFFICACY AND SAFETY OF DEFIBROTIDE IN THE TREATMENT OF HEPATIC VENO-OCCLUSIVE DISEASE/SINUSOIDAL OBSTRUCTION SYNDROME FOLLOWING HEMATOPOIETIC STEM CELL TRANSPLANTATION: FINAL SUBGROUP RESULTS

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Background: Hepatic veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS) is a potentially life-threatening complication of conditioning regimens for hematopoietic stem cell transplant (HSCT) and may also occur following chemotherapy without HSCT. VOD/SOS with multi-organ dysfunction (MOD) may be associated with >80% mortality. Diagnosis has traditionally been based on the Baltimore criteria or modified Seattle criteria. Defibrotide is approved for treating severe hepatic VOD/SOS post-HSCT in the European Union and for treatment of hepatic VOD/SOS with renal/pulmonary dysfunction post-HSCT in the United States. The defibrotide expanded-access protocol was designed to provide access to defibrotide prior to its approval in the United States and to collect additional data on safety and efficacy in a broader patient population, including those with and without MOD, and following HSCT or chemotherapy without HSCT.

Aims: This is an analysis of defibrotide efficacy and safety in the subgroup of patients developing VOD/SOS following HSCT, using final data from the expanded-access protocol.

Methods: The original expanded-access protocol required VOD/SOS diagnosis by Baltimore criteria or biopsy post-HSCT, with evidence of MOD (renal/pulmonary dysfunction). The study was amended to also include patients without MOD (off-label), with VOD/SOS per modified Seattle criteria, and/or with VOD/SOS following chemotherapy without HSCT (off-label). After patients provided informed consent, defibrotide treatment (25mg/kg/d in 4 divided doses of 6.25mg/kg) was recommended ≥21 days.

Results: This analysis of final data is based on 1000 patients enrolled from 2007–2016 who had confirmed VOD/SOS following HSCT and had received ≥1 dose of defibrotide. Of these patients, 512 (51.2%) had MOD. The median age was 14 years (range 0.10–77.0), with 570 patients (57.0%) aged ≤16 years, (281 [54.9%] of whom had MOD) and 430 patients (43.0%) aged >16 (231 [45.1%] of whom had MOD). Among pediatric patients, 28.2% were aged <1–23 months, 52.5% aged 2–11 years, and 19.3% aged 12–16 years. Primary diseases in ≥10% of the overall HSCT group were acute lymphocytic leukemia (19.8%), acute myelogenous leukemia (26.1%), and neuroblastoma (10.5%). Kaplan-Meier estimated Day+100 survival was 58.9% (95% confidence interval [CI], 55.7%–61.9%) in the overall HSCT group (**Figure**), with rates of 49.5% (95% CI, 45.0%–53.8%) in patients with MOD and 68.9% (95% CI, 64.5%–72.9%) in patients without MOD. In patients aged ≤16 years, Kaplan-Meier estimated Day +100 survival was 67.9% (95% CI, 63.8%–71.6%) and 47.1% (95% CI, 42.3%–51.8%) in patients aged >16 years (**Figure**). In the overall HSCT population, 210 patients (21.0%) had ≥1 treatment-related adverse event (TRAE). TRAEs occurring in ≥2% of patients were pulmonary hemorrhage (4.6%), gastrointestinal hemorrhage (3.0%), epistaxis (2.3%), and hypotension (2.0%).

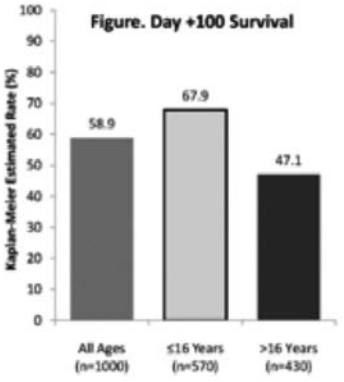

Figure 1.

Summary/Conclusions: This final analysis of the defibrotide expanded-access protocol demonstrates favorable Day +100 survival (58.9%) in patients with confirmed VOD/SOS following HSCT, and 49.5% in those with MOD, a complication typically associated with dismal outcomes. Survival and safety findings, consistent with prior clinical trials, provide supportive evidence for the clinical utility of defibrotide for treatment of VOD/SOS in patients with and without MOD.

Support: Jazz Pharmaceuticals.

Stem cell transplantation - Experimental

P749

GENERATION OF IMMORTAL MURINE HEMATOPOIETIC STEM/PROGENITOR CELL LINES FROM TRANSGENIC MICE

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Background: Research on hematopoietic and leukemic stem cells (LSCs) is currently limited as these cells are infrequent and their immortalization is hardly achieved.
Aims: We aimed to establish a long term *ex-vivo* culture system that allows maintenance and expansion of LSK (lin^-, Sca-1^+, c-Kit^+) cells.
Methods: We adapted a technique described by the L. Carlsson lab and transduced high-purity sorted murine LSKs with *Lhx2*, a LIM-homeobox transcription factor, which has been reported to facilitate *ex vivo* expansion of immature hematopoietic cells.
Results: Lhx2 expressing- hematopoietic progenitor cell (HPC^{LSK}) lines require SCF (stem cell factor) and IL-6 and they can be maintained in a feeder-independent culture for more than 6 months. They preserve LSK markers despite continuous proliferation. HPC^{LSK} cells repopulate lethally irradiated mice and re-feed the entire hematopoietic cell pool. HPC^{LSK} cell lines were established from a range of transgenic mice, underlining the overall applicability of this model. Using this system, we established LSC lines that express BCR/ABL^{p210}, MLL-AF9;$Nras^{G12D}$ or Flt3-ITD; $Nras^{G12D}$. These LSCs home to the bone marrow, differentiate into all lineages and drive myeloid leukemia in mice.
Summary/Conclusions: We created a robust method of expanding hematopoietic stem/progenitor cells. They are immortalized and can be expanded indefinitely. This tool allows analysis of the molecular mechanisms controlling self-renewal in hematopoietic and LSCs as well as drug screening. Our system may represent a breakthrough in (cancer) stem cell biology and assist in the development of new therapeutic avenues to combat LSCs.

P750

INHIBITING BCL2 AND NK CELLS IMPROVES STEM CELL TRANSPLANT OUTCOMES.

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Background: Allogeneic haematopoietic stem cell transplantation (alloHSCT) is the most effective means of preventing relapse of blood cancers, in particular AML. The curative potential of alloHSCT is largely due to the immune mediated graft-versus-leukemia (GVL) effect, which in turn is dependent on the stable engraftment of donor immunity. The dual challenge of alloHSCT is therefore to allow sufficient donor engraftment for haematopoietic and immunological reconstitution that drives the GVL effect while limiting the toxicity of conditioning and the onset of graft-versus host disease (GVHD).
Aims: Optimize the use of BCL2 inhibitors to modify recipient NK cell function in models of alloHSCT in order to minimize GVHD severity and onset.
Hypothesis: *Therapeutic targeting of recipient NK cell frequency or function pre-transplant will allow reduced intensity conditioning (RIC) and promote both donor T cell engraftment and GVL whilst reducing the risks of GVHD.*
Methods: We used a MHC-mismatched mouse model of alloHSCT, where donor BM and T cells from BALB/c ($H2K^d$) mice were injected into irradiated C57BL/6 ($H2K^b$) recipients. On day 0, C57BL/6 WT, or $Bcl2^{fl/fl}$ (*Bcl2* deleted in $NKp46^+NK1.1^+$ cells) recipients were irradiated with either a standard dose of 2 x 600 rad, or 2 x 400 rad, before iv injection of 7.5^{e6} BALB/c BM + 1^{e6} T cells. C57BL/6 WT mice were also treated on day -2 and -1 by oral gavage with 100mg/kg ABT-199 or vehicle, before receiving alloHSCT. Mice were monitored for onset of GVHD and tested for early engraftment (day 7-14 post-transplant), and late engraftment (up to day 50).
Results: We utilized genetic and pharmacological models of BCL2 deficiency to establish the role of recipient NK cells as regulators of donor T cell engraftment and GVHD. Conditional deletion of *Bcl2* in NK cells results in a 90% loss of NK cells *in vivo*. $Bcl2^{fl/fl}$ alloHSCT recipients showed robust donor engraftment, but absence of the pro-inflammatory cytokine storm and substantially less GVHD as determined by clinical scores and gut histology, with RIC compared to WT recipients. Pharmacological inhibition of BCL2 in WT recipients recapitulated the transplant findings in $Bcl2^{fl/fl}$ recipients. We found that BCL2 inhibition by Venetoclax (ABT-199), a BCL2 antagonist approved in the treatment of AML, resulted in NK cell apoptosis in human cells. We extended our observations in $Bcl2^{fl/fl}$ recipients to show that pharmacological inhibition of BCL2 in WT mice with just two doses of ABT-199 resulted in rapid depletion of NK cells. Our preliminary data indicates that alloHSCT WT recipient mice pre-treated with ABT-199 develop full donor engraftment even in the setting of significant RIC, with minimal GVHD.
Summary/Conclusions: Recipient NK cell inhibition may therefore represent a means by which to deliver alloHSCT more safely by reducing conditioning intensity and GVHD.

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MESENCHYMAL STROMAL CELL IRRADIATION INTERFERES WITH THE ADIPOGENIC/OSTEOGENIC DIFFERENTIATION BALANCE IMPROVING THEIR HEMATOPOIETIC-SUPPORTING ABILITY

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Background: Mesenchymal stromal cells (MSC) are precursors of adipocytes and osteoblasts in the bone marrow (BM) niche, and key regulators of the hematopoietic process. After HSC transplantation, MSC remain of host-origin. Total body irradiation has been widely used in conditioning regimen and MSC are shown to be radio-resistant. Nevertheless, the functional effects of irradiation on BM-MSC have not been extensively explored.
Aims: The main objective was to evaluate the effects of irradiation on the MSC in their hematopoietic-supporting capacity.
Methods: Ten BM samples were obtained from healthy donors after informed consent. MSC were obtained and characterized following standard procedures until third passage. Then, one aliquot was gamma-irradiated with a single dose of 2,5Gy whereas non-irradiated MSC from the same sample were used as controls. MSC were characterized following ISCT criteria (flow cytometry and *in vitro* differentiation stainings). Apoptosis was evaluated by flow cytometry using annexinV/7AAD staining. Expression microarrays of irradiated and control-MSC were performed using Human Gene 2.0 ST Array platform (Affymetrix). RT-PCR of key genes involved in the hematopoietic supporting capacity as well as in the differentiation of MSC into osteoblasts and adipocytes was performed in both experimental groups. Finally, long term BM cultures (LT-BMC) were performed as functional assays to test the hematopoietic-supporting ability of irradiated and control MSC. For the latter experiments, $CD34^+$ cells were isolated from leukapheresis and seeded on stromal layers from non-irradiated or irradiated MSC. CFU-GM colonies derived from the LT-BMC were scored weekly.
Results: Flow cytometric characterization of irradiated MSC was comparable to that of control MSC. Similarly, there were no differences in the percentage of viable cells between both experimental groups neither at one hour nor at 72h post irradiation, confirming once more the radio-resistance of MSC. In addition, expression arrays did not show any statistically significant differences in genes involved in hematopoiesis maintenance. However, upon comparing the differentiation ability we interestingly observed that irradiated-MSC differentiation was skewed towards osteogenesis whereas adipogenesis was impaired. In this regard, irradiated-MSC had significantly higher SPP1 expression (involved in late osteogenic differentiation) and lower CBPA and PPAR-gamma (both genes involved in adipogenesis) compared to control MSC. After inducing *in vitro* differentiation, there were no differences in ALP and Alizarin Red staining but the number of adipocytes per field at days 7, 14 and 21 was significantly lower in irradiated MSC (p=0,018 p=0,046 and p=0,018). In addition, angiopoietin and SDF-1, key genes implicated in maintenance of hematopoiesis, were significantly overexpressed in irradiated-MSC (p=0,043 and p=0,028, respectively). Finally, in the functional evaluation of the hematopoietic-supporting ability of MSC by LT-BMC, we observed that the number of CFU-GM colonies generated by the culture was significantly higher in the irradiated group after 4 and 5 weeks (p=0.046 and p=0.018, respectively) compared to the non-irradiated group. Furthermore, the number of adipocytes per field was significantly reduced in the LTBMC.
Summary/Conclusions: Irradiation of MSC with 2,5Gy improves their hematopoietic supporting ability and modifies their differentiation capacity, increasing the osteogenesis and decreasing the adipogenesis.

Funding: PI12/01775 (ISCIII, Spain). Santander-USAL grant to SP.

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DYSFUNCTION OF BONE MARROW MESENCHYMAL STEM CELLS FROM PATIENTS WITH PROLONGED ISOLATED THROMBOCYTOPENIA AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION CAN BE IMPROVED BY N-ACETYL-L-CYSTEINE

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Background: Prolonged isolated thrombocytopenia (PT), is a serious complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT) and defined as the engraftment of all peripheral blood cell lines other than a platelet (PLT) count ≤20×10E+9/L or dependence on PLT transfusions for more than 60 days after allo-HSCT. Several clinical risk factors have been proposed to be associated with PT after allo-HSCT. However, the underlying mechanisms remain to be elucidated. Emerging evidence from mouse studies has suggested that effective hematopoiesis depends on a particular bone marrow (BM) microenvironment in which hematopoietic stem cells reside. MSCs represent a key cellular component of the BM microenvironment, which are potential progenitors for osteoblasts, adipocytes, chondrocytes, and marrow stromal cells. The processes of megakaryocytopoiesis and thrombocytopoiesis result from the interactions between hematopoietic progenitor cells, cytokines, and marrow stromal cells derived from MSCs or MSCs directly. However, the functional role of BM MSCs in the patients with PT has never been reported. Moreover, approaches for improving the dysfunction of BM MSCs in patients with PT are lacking.
Aims: To evaluated the number and function of BM MSCs in patients with PT post-allotransplant. Moreover, to investigate the approach to enhance the number and function of BM MSCs derived from patients with PT and its underling molecular mechanisms in vitro.
Methods: Three cohorts were included: patients with PT (N=25), patients with good graft function (GGF, N=12), defined as persistent successful engraftment after allotransplant, and transplant donors as normal controls (N=10). BM MSCs were cultured as previous reported. All experiments were carried out using BM MSCs derived from passages 2–4. The number and functions of BM MSCs were evaluated by fibroblasts colony-forming unit (CFU-F) assay, cell proliferation assay and senescence-associated β-galactosidase (SAβ-gal) assay. Reactive oxygen species (ROS) levels were evaluated by flow cytometry. Protein expression for p-p38, p38, p-p53, p53 was measured by flow cytometry and western blots. To further investigate the potential effect for repairing the dysfunctional BM MSCs, N-Acetyl-L-cysteine(NAC, a ROS scavenger) and SB203580 (p38 inhibitor) were administrated to the BM MSCs from PT patients. After 2 days *in vitro* culture, the number of SAβ-positive cells was counted, the intracellular levels of ROS and p-p38 were evaluated in BM MSCs by flow cytometry.
Results: Human BM MSCs were demonstrated as spindle shape and typical immunophenotype of MSCs at day 21 of cultivation among subjects with PT, GGF and normal controls. Cultures from all normal BM samples produced confluent layers of adherent cells composed of spindled shaped cells. 2 of the 12 GGF BM and 15 of the 25 PT BM failed to produce any adherent layers within 3 weeks of culture. BM MSCs derived from PT patients expanded more slowly and appeared flattened and larger. Proliferative capacity and CFU-F counts of BM MSCs from PT patients were significantly reduced compared to those of GGF patients and normal controls. Moreover, increased levels of ROS, which was associated with increased number of SAβ-positive cells, were identified in BM MSCs from PT patients. Intracellular p-p38 level was significantly elevated in PT patients compared to those in GGF patients. After NAC treatment in vitro, the proliferative capacity was increased significantly, whereas the number of senescent cells, the intracellular levels of ROS and p-p38 were reduced markedly in BM MSCs from PT patients.
Summary/Conclusions: In summary, the current study demonstrated the number and the function of BM MSCs were abnormal in PT patients following allo-HSCT. Moreover, NAC treatment *in vitro* can partially decreased the ROS level and reversed the senescence phenotype through down-regulation of the p38 MAPK pathway. Our results indicate that the dysfunctional BM MSCs may play an important role in the pathogenesis of PT following allo-HSCT and NAC represents a promising therapeutic approach for repairing the impaired BM MSCs in PT patients post-allotransplant.

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INHIBITORS OF APOPTOSIS PROTEINS (IAPS) MODULATE GASTROINTESTINAL GVHD IN MURINE EXPERIMENTAL BMT MODELS

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Background: The two Inhibitors of apoptosis proteins (IAPs), X-chromosome-liked linked IAP (XIAP) and cellular IAP1 (cIAP1), inhibit apoptosis and play an important role in regulating innate and adoptive immunity. However, the role of IAPs in allogeneic immune responses is not known.
Aims: We determined the role of IAPs in GVHD.
Methods: We utilized clinically relevant, well-characterized murine models of allogeneic BMT. To chemically target IAPs, we utilized AT406 (SM-406), a SMAC mimetic that actively antagonizes all IAPs.
Results: We first utilized AT406 which regulates TNFα in vitro. Given the GVHD potentiating effects of TNFα, we hypothesized that AT406 will mitigate GVHD. After we confirmed that AT406 reduced expression of IAPs in T cells or host target tissues, we utilized B6→BALB/c MHC-mismatched BMT model. BALB/c recipients were lethally irradiated and transplanted withsyngeneic or allogeneic T cells along with bone marrow (BM). Both groups received either AT-406 or its diluent. Contrary to our hypothesis, allo-recipients receiving AT-406 showed worse GVHD. To understand the role of IAPs, we next utilized B6 background XIAP and cIAP1 deficient animals. When donor T cells from B6-cIAP$^{-/-}$ or XIAP$^{-/-}$ animals were compared to B6-WT T cells, the allo-recipients (BALB/c) showed similar GVHD. Same results were also observed in another B6→F1 model. Furthermore, *in vitro* studies showed that XIAP$^{-/-}$and cIAP$^{-/-}$ T cells had comparable proliferation and cytokine secretion as WT-T cells. These data suggested that increase in GVHD mortality following treatment with AT-406 is not due to its effects on donor T cells. To further dissect the increased mortality after AT-406 treatment, we hypothesized that the absence of IAPs in hosts may impact on GVHD. To test this, cIAP$^{-/-}$, XIAP$^{-/-}$ and WT-B6 animals were lethally irradiated and transplanted with syngeneic (B6) or allogeneic (BALB/c) T cells. Compared to WT recipients, both XIAP$^{-/-}$ and cIAP$^{-/-}$recipients showed increased mortality ($p<0.001$) and worse gastrointestinal (GI) GVHD. To explore whether IAPs regulate GVHD through their expression exclusively in host hematopoietic cells, we generated [B6→B6Ly5.2], [cIAP$^{-/-}$→B6Ly5.2] and [XIAP$^{-/-}$→B6Ly5.2] chimeras and utilized them as recipients in 2nd allo-BMT in BALB/c->B6 models. Both [cIAP$^{-/-}$→B6Ly5.2] and [XIAP$^{-/-}$→B6Ly5.2] showed equivalent GVHD mortality to [B6→B6Ly5.2] chimeras. Consistently, dendritic cells (DCs) from XIAP$^{-/-}$ and cIAP$^{-/-}$ animals showed similar functions as WT-B6 *in vitro*, suggesting that IAP expression in host hematopoietic cells is not critical. Next, to test the role of IAPs in non-hematopoietic GVHD target tissues, we made the reverse chimeras, [B6Ly5.2→B6], [B6Ly5.2→XIAP$^{-/-}$] and [B6Ly5.2→cIAP1$^{-/-}$], where IAPs are absent only in the non-hematopoietic host cells. The allogeneic [B6Ly5.2→XIAP$^{-/-}$] and [B6Ly5.2→cIAP1$^{-/-}$] animals demonstrated a significantly worse survival compared to WT [B6Ly5.2→B6] recipient ($p<0.01$). To determine the potential mechanisms for exacerbating GVHD, we tested tunel staining, and the expression of anti- and pro-apoptotic genes (Bcl-2, BIM, BAL) and autophagy (LC3) in the CD326^{+} intestinal epithelial cells from KO and WT animals after allo-BMT. The cIAP$^{-/-}$ and -XIAP$^{-/-}$ animals showed increased number of tunel positive cells and significantly reduced expression of anti-apoptotic protein Bcl-2 and LC-3 but equivalent expression of pro-apoptotic proteins. In addition, the expression ratio of BIM or BAX to Bcl-2 in allo-XIAP$^{-/-}$ animals was significantly increased.
Summary/Conclusions: These data suggest that enhanced apoptosis in the target tissues in the absence of IAPs contributes to greater GVHD severity. Thus expression of functional IAPs in host target tissues is crucial for reducing the damage from GVHD.

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GRAFT-VERSUS HOST DISEASE (GVHD) DEVELOPMENT AFTER BONE MARROW TRANSPLANTATION IS NOT INFLUENCED BY TH9 CELLS

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Background: Th9 cells are a recently defined subset of T helper cells (Th) characterized by the massive production of IL-9. Th9 cells mediate immune responses against helminth infections, exhibit anti-tumor immunity against solid tumors and mediate allogeneic transplant tolerance but they also contribute to immunopathology in allergy and autoimmunity.
Aims: Currently, the role of Th9 cells for GVHD induction and the graft-versus-tumor effect is largely unknown. Therefore, we first explored, whether Th9 cells are induced during GVHD development in two different MHC-mismatched bone marrow transplantation (BMT) models and secondly analyzed, whether transplantation of in vitro-generated Th9 cells mediates GVHD.
Methods: We transplanted allogeneic BM and spleen cells from B6-SJL mice (CD45.1, H-2b) in B6D2F1 mice (CD45.2, H-2bxd) or in B6.bm12 mice (CD45.2, I-Abm12) differing either in 50% of MHC class I and II molecules or only in one MHC class II molecule and analyzed the induction of Th9 cell during GVHD development. To clarify whether in vitro-generated Th9 cells mediate GVHD, we induced Th9 cells *in vitro* from isolated, naïve CD4^{+} T cells on anti-CD3/28 coated plates by TGF-b, IL-4, anti-IFNg and recombinant TL1A and co-injected the Th9 cells together with allogeneic BM in irradiated recipient mice and subsequently monitored GVHD induction.
Results: In both MHC mismatched models used, the transplantation of allogeneic spleen cells and BM leads to GVHD characterized by a time-dependent strong increase of Th1-specific cytokines TNF-a and IFN-g in the serum of the recipient mice. IL-9, however, was undetectable. Additionally, no IL-9 producing allogeneic T cells were identified in the spleen, liver and lung of GVHD-developing animals until 29 days after transplantation, while TNF-a and IFN-g producing cells were strongly increased indicating that Th9 cells are not induced

during GVHD. After *in vitro* differentiation of Th9 cells from naïve T cells we obtained more than 60% of IL-9 producing cells after 5 days of culture. Th9 cells differ in their cytokine profile (IL-9+, IFN-g-, IL-13-) from Th1 and Th2 cells. Transplantation of in vitro-generated Th9 cells together with allogeneic BM cells did not induce GVHD in the MHC-disparate recipient mice, while the transplantation of unselected T cells or in vitro-generated Th1 cells induced GVHD and mediated death in about 60% of the animals. Although no GVHD development was detected, Th9 cells migrated into lymphoid organs and GVHD target organs such as spleen and lung. Surprisingly, when the cytokine phenotype of the transplanted Th9 cells were analyzed after *ex vivo* isolation from spleen and liver at different time points after transplantation, the cells lost their IL-9 production but produced TNF-a and IFN-g pointing to a plasticity of Th9 cells after adoptive transfer. Systemic increase of TNF-a and IFN-g in the serum of mice receiving Th9 cells, however, was not detected.
Summary/Conclusions: Th9 cells are not induced during GVHD development and the adoptive transfer of in vitro-generated Th9 cells does not induce GVHD. However, the transplanted Th9 cells home to spleen and GVHD target organs and start to produce TNF-a and IFN-g without strong systemic increase in these cytokines. Since TNF-a and IFN-g are cytokines associated with an anti-tumor cytotoxicity and Th9 cells are known to eliminate solid tumors, future experiments will define, whether in vitro-generated Th9 cells can be used as a cellular therapy for anti-tumor responses in BM-transplanted hosts.

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IMPROVED HSC ENGRAFTMENT IN A MOUSE MODEL OF HEMATOPOIETIC STEM CELL GENE THERAPY MEDIATED BY MSCS

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Background: Co-transplantation of human mesenchymal stromal cells (hMSC) has been reported to reduce the risk of graft failure and improve hematopoietic stem cells (HSC) engraftment in xenogeneic and determined allogeneic transplants. In addition, we have demonstrated that the co-infusion of MSCs with low numbers of purified HSCs significantly improve the short- and long-term hematopoietic reconstitution in an autologous HSCT experimental model with sublethal conditioning (5Gy).
Aims: The aim of this study is to analyze the effect of MSCs on HSC engraftment in a clinically relevant model of hematopoietic gene therapy.
Methods: We have studied the effect of MSCs co-infusion in a mouse model of HSC gene therapy with risk of engraftment failure in Fanconi anemia mice (*Fanca*$^{-/-}$).
Results: In these experiments, the infusion of low numbers of WT LSK cells (1,500 LSK) in *Fanca*$^{-/-}$ mice resulted in 30% graft failure, which was prevented when 6.10^5 Ad-MSCs were co-infused. Furthermore, when 1,500-3,000 *Fanca*$^{-/-}$ LSK cells transduced with a therapeutic lentiviral vector (PGK-*FANCA*-wPRE*) were transplanted, the infusion of similar cell doses resulted in more than 50% of engraftment failure, which decreased to 30% only when more than 10,000 gene-corrected LSK were infused. Once again, Ad-MSCs co-infusion prevented graft failure in after the infusion with the same number of gene-corrected LSK cells.
Summary/Conclusions: Taken together, our results demonstrate the potential of Ad-MSCs to avoid graft failure in a clinically relevant model of hematopoietic gene therapy with risks of engraftment failure.

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EFFECT OF POMALIDOMIDE ON T CELL POLARIZATION IS MEDIATED THROUGH EPIGENETIC MODIFCATIONS.

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Background: There is conflicting evidence regarding the potential use of IMIDs and particularly pomalidomide after allogeneic stem cell transplantation (allo-HSCT). It has been well described that IMIDs polarize naïve T cells towards a Th1 phenotype increasing IFN-γ cytokine production via the augmentation of T-bet transcription factor. This effect might increase the risk of GvHD after allo-HSCT. Nevertheless, a recent trial has reported a potential benefit on the use of pomalidomide as GVHD treatment.
Aims: In the current study, we have analyzed the effect of pomalidomide in the polarization of CD45RA+ cells and the epigenetic mechanisms that might be involved in this effect.
Methods: Isolated CD45RA+ T cells from healthy donor's Buffy Coats were-stimulated with anti-CD3 plus anti-CD28 in the presence of several cytokines to polarize towards Th1 (IL-12, INF-γand anti-IL4) or Th2 (IL-4, IL-2, anti-IFN-γ and anti-IL-12) for 5 days. Pomalidomide at two different doses (10 and 100 nM) were added into the culture and the effect on T cells polarization was analyzed by flow cytometry after staining with anti-CD25, anti-IFNγ, anti-CD4 and anti-IL2 for Th1 cell polarization and anti-CD25, anti-IL10, anti-CD3 and anti-IL4 for Th2 cell polarization. In addition, the release of cytokines (IL-2, IL-4, IL-6, IL-10, TNF-α and IFN-γ) in cell culture supernants were measured with BD Human Th1/Th2 Cytokine CBA kit (BDBiosciences) and T-bet and GATA-3 expression were analyzed by Western Blot. Chromatin immunoprecipitation (ChIP) assays were performed to assess the trimethylation of H3K4 (associated with gene activation) and the trimethylation of H3K27 (associated with gene repression) in the *TBET* and *GATA-3* gene promoters.
Results: Pomalidomide increased the expression of INF-γ and IL-2 as determined by flow cytometry in Th1 cell culture conditions. By contrast, in the presence of Th2 promoting cytokines, we observed an increase for both IL-10 and IL-4 upon adding pomalidomide to the culture. In addition, the exposure to pomalidomide increased the levels of TNF-α, INF-γ and IL-2 in the Th1 polarizing culture while, under Th2 promoting conditions, an increased concentration of IL-4 and IL-2 in supernatant was observed after exposure to pomalidomide. Furthermore, exposure to pomalidomide led to an increased expression of T-bet as assessed by western-blot in naïve CD45RA+ cells activated with anti-CD3 plus anti-CD28 and supplemented with IL-12, INF-γ and anti-IL4. By contrast, in Th2 polarization conditions, pomalidomide increased GATA-3 expression.We next studied whether or not the effect of pomalidomide in T cell polarization might be mediated by epigenetic mechanisms: in the presence of Th1 promoting conditions there was a significant increase of the activation marker H3K4me3 at the *TBET* promoter and a significant decrease in H3K27me3 upon exposure to the drug while, under Th2 promoting conditions, a significant increase in H3K4me3 at the promoter of *GATA-3* gene was observed among T cells exposed to pomalidomide.
Summary/Conclusions: Pomalidomide favours both Th1 and Th2 cell differentiation of CD45RA+ cells depending on the cytokines present in the medium. Treatment of naïve T cells with pomalidomide induces epigenetic modifications during T cell polarization which might favour the process of differentiation of the naïve T cells.

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MESENCHYMAL STEM CELLS (MSCS) ATTENUATE CUTANEOUS SCLERODERMATOUS GRAFT-VERSUS-HOST DISEASE (SCL-GVHD) THROUGH INHIBITION OF IMMUNE CELL INFILTRATION IN A MOUSE MODEL

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Background: Human chronic graft-versus-host disease (CGVHD) shares clinical characteristics with a murine sclerodermatous GVHD (Scl-GVHD) model that is characterized by skin thickening and lung fibrosis.
Aims: This study investigated the therapeutic effect of mesenchymal stem cells on the development of Scl-GVHD according to each target organ.
Methods: A B10.D2 → BALB/c transplant model of Scl-GVHD was used to address the therapeutic effect of mesenchymal stem cells (MSCs) on the development of CGVHD. M210B4 cells were administered after allo-HSCT at a dose of 3 x 10^5 cells/mouse on days 3, 5 and 7.
Results: The clinical and pathological severity of cutaneous Scl-GVHD was significantly attenuated in MSC-treated recipients relative to Scl-GVHD controls. After MSC treatment, skin collagen production was significantly reduced with consistent downregulation of *TGF-β* expression. Effects of MSCs on molecular markers implicated in persistent TGF-β signaling and fibrosis, such as phosphatase and tensin homolog (PTEN), phosphorylated Smad-2/3 and matrix metalloproteinase-1 (MMP-1), were observed in skin tissue. MSCs neither migrate to the skin nor affect the *in vivo* expansion of immune effector cells, but inhibited their infiltration into skin via downregulation of CCR4 and CCR8 expression on $CD4^+$ T cells and CCR1 on $CD11b^+$ monocyte/macrophages. MSCs diminished expression of chemokines such as CCL1, CCL3, CCL8, CCL17, and CCL22 in skin. MSCs were also dependent on stimulated splenocytes to suppress fibroblast proliferation.
Summary/Conclusions: Our findings indicate that MSCs attenuate the cutaneous Scl-GVHD by selectively blocking immune cell migration and downregulating chemokines and chemokine receptors.

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C57BL/6 SUBTRAINS SHOW DIFFERENCES IN HEMATOPOIETIC REPOPULATION

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Background: C57BL/6 mice are one of the most studied in-bred mouse strains.

Although C57BL/6N (N) and C57BL/6J (J) mice are derived from the same parental C57BL/6 strain, there are key genotypic and phenotypic differences between these sub-strains. However, more than 58% of studies published involving C57BL/6 mice do not indicate the specific sub-strain employed. J mice have a five-exon deletion in the *Nicotinamide nucleotide transhydrogenase* (*Nnt*) gene that results in a non-functional protein. NNT is involved in the resolution of oxidative stress in the mitochondria. Hematopoietic stem cells (HSCs) can reconstitute the entire hematopoietic system after transplantation into hosts whose hematopoietic compartment has been ablated. This is clinically exploited as HSCs transplantation (HSCT) to treat hematologic diseases and represents the only curative therapy for many disorders. During HSCT, HSC are subject to dramatic increases in both intra and extracellular reactive oxygen species (ROS), which compromises their self-renewal, differentiation, and survival. The absence of a functional *Nnt* gene in J-HSC may curtail their ability to resolve elevated ROS post-transplant.

Aims: As elevated oxidative stress compromises hematopoietic stem and progenitor cell (HSPC) function, here we thoroughly interrogated the frequency and function of HSPCs in J and N bone marrow (BM).

Methods: N and J peripheral blood (PB) and BM (n=9) was interrogated by flow cytometry for the absolute frequencies of all major hematopoietic lineages and HSPC compartments, respectively. 5000 J or N CD45.2 HSPCs (Lin-Sca-1+c-Kit+ cells) were transplanted along with 5000 competitor CD45.1 HSPCs into lethally irradiated mice to test for competitive *in vivo* hematopoietic repopulating activity and ROS levels post-transplant. The lineage potential and repopulating activity of multi-potent progenitors (MPP2: Lin-Sca1+cKit+Flt3-CD48+CD150+, MPP3: Lin-Sca1+cKit+Flt3-CD48+CD150-, MPP4: Lin-Sca1+cKit+Flt3+CD48+CD150-) was also tested by transplanting 2000 MPPs from J or N mice into sub-lethally irradiated mice and examining the PB of recipients every 3-4 days for 34 days post-transplant. Sensitivity of HSPCs to oxidative stress was tested by examining ROS levels and the *in vitro* colony forming unit (CFU) potential of HSPCs isolated from N and J mice treated with pI:pC.

Results: The frequency of the major PB lineages and bone marrow HSPC compartments was identical in J and N mice. However, J-HSPCs displayed compromised short-term (4-12 weeks post-transplant) hematopoietic repopulating activity relative to N-HSPCs that was driven by a delay in lymphoid reconstitution. No differences were found in donor contribution to bone marrow HSPC compartments at 20 weeks post-transplant. However, donor-derived MPPs and CLPs displayed a two-fold increase in ROS levels in recipients of J-HSPCs *versus* N-HSPCs at 20 weeks post-transplant. MPPs are responsible for repopulation of the hematopoietic system during this early window post-transplant. Different MPP subpopulations can be defined (MPP2, MPP3 and MPP4) according to their self-renewal potential and specific lineage potential. MPP3s and MPP4s are the first MPP subpopulations to reconstitute the lymphoid lineage after transplant. J-MPP3s and J-MPP4s displayed less *in vivo* repopulation activity than N-MPP3s and N-MPP4s. It is known that pI:pC treatment increases ROS levels in HSPCs. We found about two-fold higher ROS levels in HSPCs isolated from pI:pC treated J mice than N mice with the exception of the myeloid progenitor compartments (CMP, GMP and MEO). J-HSPCs also generated fewer and smaller CFU than N-HSPCs when isolated from pI:pC treated mice. These data indicate that J-HSPCs cannot resolve oxidative stress as efficiently as N-HSPCs, which may be due to lower self-renewal potential after exposure to oxidative stress. Short-term J-lymphoid-biased progenitors (*e.g.* MPPs and CLPs) were especially sensitive to increasing ROS, which very likely drives the short-term loss of *in vivo* repopulating activity.

Summary/Conclusions: Based on these data, we hypothesize that loss of the *Nnt* gene in C57Bl/6J mice sensitizes HSPCs to oxidative stress, which compromises their short-term *in vivo* hematopoietic repopulating activity.

Thrombotic disorders

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GWAS RESULTS IN RED BLOOD CELL PHENOTYPES AND THEIR RELATIONSHIP WITH THROMBOSIS

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Background: Venous thromboembolism (VTE) is a complex and multifactorial disease with a estimated heritability of 60%. Intermediate phenotypes of VTE have been used to identify genetic risk factors. We previously reported a genetic correlation of 5 erythrocyte phenotypes with VTE[1].

Aims: To identify single nucleotide polymorphisms (SNPs) influencing the phenotypic variance of erythrocyte parameters, especially those related to VTE, in Spanish families from the Genetic Analysis of Idiopathic Thrombophilia (GAIT2) Project.

Methods: Genome-wide association analyses (GWAS) with ~10M SNPs were performed for eighteen erythrocyte phenotypes in 935 subjects belonging to 35 extended families with thrombosis of GAIT2. The erythrocyte phenotypes evaluated were: Hemoglobin (Hb), red blood cell count (RBC), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), reticulocyte (RET), low fluorescence reticulocyte (LFR), middle fluorescence reticulocyte (MFR), high fluorescence reticulocyte (HFR), reticulocyte fluorescence index (IRF), haptoglobin (HP), serum iron (Fe), total iron binding capacity (TIBC), saturation index (SI), serum ferritin (FT) and serum transferrin receptor (TFR).

Results: We identified 12 SNPs showing association with the 5 erythrocyte phenotypes previously related to VTE (Table 1). Interestingly, the rs56036145 that showed association with TFR is an intronic variant located in the gene *tissue factor pathway inhibitor 2* (TFPI2), which encodes a protein that inhibits a variety of serine proteases of blood coagulation, such as activated factor VII (FVIIa/TF), FXa, plasmin and plasma kallikrein. These data reinforce our previous report of genetic correlation of TFR with VTE. The most significant SNP-associations were reported.

Table 1. Top SNP-associations with erythrocyte phenotypes related to VTE from GWAS in GAIT2.

Phenotype	ρ_G	SNP	Chr.	Type	Closest gene	P value
Ht	0.52	rs4460	22	Intergenic	PRR5-ARHGAP8	$1{\cdot}10^{-6}$
		rs113104814	11	Intronic	ARHGEF17	$2{\cdot}10^{-6}$
		rs200232896	5	Intronic	PDE4D	$2{\cdot}10^{-6}$
RDW	0.28	rs11394605	2	Intronic	SCRT	$1.6{\cdot}10^{-7}$
IRF	0.45	rs3956837	12	Intronic	PXN-AS1	$2.6{\cdot}10^{-7}$
		rs3774270	3	Intronic	MASP1	$6.6{\cdot}10^{-7}$
SAT	0.7	rs1799945	6	Intronic	HFE	$2.4{\cdot}10^{-7}$
		rs6000553	22	Intronic	TMPRSS6	$2{\cdot}10^{-6}$
		rs792839	3	Intronic	COL8A1/MIR548G	$4.8{\cdot}10^{-7}$ / $6.6{\cdot}10^{-7}$
TFR	0.4	rs9297983	8	Intergenic	FAM110B	$1.1{\cdot}10^{-7}$
		rs2519651	7	Intronic	GNGT1	$3.3{\cdot}10^{-7}$
		rs56036145	7	Intronic	TFPI2	$3.4{\cdot}10^{-7}$

G: genetic correlation with VTE; Chr: Chromosome.

Summary/Conclusions: Several genetic variants involved in the variance of erythrocyte phenotype levels were identified by GWAS. Of note, TFR was associated with a SNP in *TFPI2* that might influence the variance of both TFR levels and VTE risk. These data could be useful to investigate genes related to red blood cell parameters and VTE.

Reference

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This work was supported by RIC RD12/0042/0032, FIS PI12/00612 and FIS PI 15/0269 grants

P760

ESSENTIAL THROMBOCYTHEMIA (ET) AND POLYCYTHEMIA VERA (PV) PATIENTS SHOW AN INCREASED THROMBUS FORMATION IN A DYNAMIC MODEL OF PLATELET ADHESION

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Background: ET and PV are characterized by a high incidence of arterial and venous thrombosis. Platelet (PLT) count is not an independent risk factor for thrombosis in these conditions. However, no information is available on patient PLT qualitative properties, *i.e.* the PLT thrombus formation capacity in a dynamic condition.
Aims: We wanted to evaluate, in a group of ET and PV patients, the PLT thrombus formation capacity by an *ex-vivo* dynamic model of PLT adhesion under flow conditions, and to establish the influence of JAK2-V617F/Calreticulin (CalR)/MPL mutations, hematological parameters, and ongoing therapies.
Methods: One hundred-thirty patients, *i.e.* 78 ET (32 M/46 F; median age=61 years, range 28-86) and 52 PV (26 M/26 F; median age=65 years, range 38-87), and 32 healthy controls (16 M/16 F; median age=44 years, range 27-61) were enrolled after informed consent. For the adhesion assay, peripheral venous whole blood was drawn in sodium citrate, recalcified in the presence of heparin, and perfused over a collagen-coated surface for 4 min. at a shear rate of 1,000 s^{-1}. PLTs were then stained with an anti-CD62P (P-selectin)-FITC antibody to evaluate PLT activation, and annexinV-AlexaFluor647 to detect pro-coagulant phosphatidylserine expression. After staining, phase contrast and fluorescence images of adherent PLTs were taken in random fields using an EVOS® microscope. Results are expressed as the mean±SEM of the % of area covered by all PLTs (% coverage), or as the % of adherent PLTs positive for either P-selectin or phosphatidylserine. Main hematological parameters, therapies, and mutational status were recorded.
Results: PLT adhesion was significantly ($p<0.01$) greater in either ET (45.3±1.7%) and PV patients (48.9±1.6%) compared to healthy controls (37.5±1.7%), while no difference was found between ET and PV patients. The analysis according to the mutational status shows that ET PLT adhesion was highest in JAK2-V617F mutation carriers (n=41; coverage: 47.7±2.4%, $p<0.001$ *vs* controls), followed by CalR-positive patients (n=21; coverage: 45.5± 3.2%, $p<0.05$ *vs* controls, p=n.s. *vs* JAK2-V617F), while, PLT adhesion of MPL-positive (n=3; coverage: 32.1±2.1%) or triple negative (n=13; coverage: 42.6±2.5%) ET patients was not statistically different from controls. In PV, no statistically significant difference was observed between subjects with >50% *versus* those with <50% JAK2-V617F allele burden. According to treatment, we observed that ET patients treated with the combination of aspirin+hydroxyurea presented the lowest PLT adhesion, while in PV no significant difference was observed between different therapeutic regimens. PLT count correlated ($p<0.01$) with PLT adhesion only in CalR-positive ET patients. The analysis of adherent PLT surface markers shows no difference in P-selectin expression between whole patients and controls. Differently, phosphatidylserine expression was significantly reduced ($p<0.01$) in both ET and PV compared to healthy subjects.
Summary/Conclusions: ET and PV platelets show an increased PLT thrombus formation potential, particularly in patients carrying the JAK2-V617F mutation. On the basis of these results, it is worth to include a dynamic PLT adhesion assay in risk prediction models to evaluate the predictive value of thrombotic events in ET and PV patients. [*Project funded by "AIRC-IG2013" grant Nr. 14505 of the "Italian Association for Cancer Research" (A.I.R.C.)*].

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DOAC ASSOCIATED MAJOR GASTROINTESTINAL BLEEDING: REAL LIFE EXPERIENCE FROM A UNIVERSITY TEACHING HOSPITAL, UK

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Background: Direct acting oral anticoagulants (DOAC) use is increasing amongst patients requiring anticoagulation for AF or VTE. The incidence of major gastrointestinal (GI) bleeding in DOAC patients from clinical trials is reported to be similar or lower than those on warfarin. There is paucity of real life data confirming this important safety measure.
Aims: We investigated our database on patients using DOAC over a 3 year period to identify major GI bleeding events during this period. The objective was to ascertain the incidence of major GI bleeding and identify demographic and clinical risk factors in these patients.
Methods: all patients issued, apixaban, dabigatran or rivaroxaban between April 2013 - July 2016 were identified from the hospital pharmacy records. Hospital admission data was investigated to identify patients who were diagnosed of a major GI bleeding during this period. Records of patients with major G.I. Bleeding were then individually studied for demographic and clinical risk factors. Major bleeding was defined as per the ISTH criteria.
Results: 2611 patients were identified to have received DOAC treatment between April 2013- July 2016. Of these 1601 (61%) received; apixaban, 722 (27%) rivaroxaban and 288 (11%) dabigatran.18 patients (0.7%) suffered from a major GI bleeding equating to annual risk of major GI bleeding of 0.23% per patient/ year. This is a much lower incidence than reported data from clinical trials in patients on DOAC. Patients who suffered from a major GI bleeding did so on average 143 days (range 8-576) after starting the DOAC. Of all patients with major GI bleeding, 14 were taking apixaban (0.8% of all pt on apixaban), 3 (0.4% of all pt on rivaroxaban) rivaroxaban and 1 (0.3% of all pt on dabigatran) on dabigatran). The numbers were too small to identify any statistical difference between the 3 different DOAC drugs.
Summary/Conclusions: The risk of major GI bleeding in our cohort of over 2500 patients over 3 years was noted to be significantly lower than trial data. Since this is a retrospective review from patient hospital database there is a risk of reporting bias and under-reporting of bleeding events. A prospective phase IV study to identify bleeding risk in patients on DOAC is required. Majority of patients with major GI bleeding had other risk factors such as concurrent use of anti- platelets, peptic ulcer disease, alcohol abuse, oesophageal varices, diverticular disease, and bowel malignancy which would increase their bleeding risk on any anticoagulation. Further sub group analysis of this cohort and efforts to improve reporting of anticoagulation associated bleeding is underway.

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Abstract withdrawn.

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INCIDENCE OF VENOUS THROMBOEMBOLISM IN PATIENTS UNDERGOING LOWER LIMB SURGICAL REVASCULARIZATION: IS THROMBOPROPHYLAXIS WARRANTED?

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Background: The incidence of postoperative deep vein thrombosis (DVT) or consequential pulmonary embolism (PE) in patients undergoing lower extremity surgical revascularization procedures is not well studied. The need for routine anticoagulation for DVT/PE prophylaxis after the lower limb surgical revascularization remains controversial.
Aims: The purpose of this study is to retrospectively evaluate the incidence of postoperative DVT/PE in patients undergoing lower limb surgical revascularization.
Methods: Charts for patients undergoing lower limb surgical revascularization, from 01/01/2010 to 12/31/2015, were evaluated for DVT/PE. DVT/PE within three months of the revascularization was considered to be a postoperative DVT/PE. Patients undergoing multiple procedures were counted as different cases if they were on different days. Multiple procedures on a patient on the same day were considered a single case. Patients with hypercoagulable states or previous history of DVT were excluded. Descriptive statistics and t-test was used to analyze incidence of DVT/PE and assess the importance of postoperative thromboprophylaxis.

Table 1.

	DVT/PE within three months from surgery	
Procedure performed	**No**	**Yes**
Balloon angioplasty	15	
Bypass aorta-femoral	18	
Bypass femoral-peroneal	3	
Bypass femoral-femoral	12	1
Bypass femoral-popliteal	252	2
Bypass femoral-tibial	6	
Lower limb embolectomy	3	
Femoral artery exploration	6	
Thrombectomy	39	2
Total	354	5

Results: Between 1/1/2010 to 12/31/2015, 360 patients were found to have undergone lower extremity surgical revascularization. Study population included 200 males and 160 females. Mean patient age was 69.54 years. One patient had a previous history of DVT and was excluded. Overall, of the 359 patients, five (1.4%) were recognized to have a new DVT/PE within 3 months of the surgery. One patient developed DVT in the contralateral limb, and one developed it in the arm. Patients were recognized to have a new DVT/PE, on an average, at 7.6 days after the surgery. A one sided t-test demonstrated that the average

postoperative day for recognition of DVT/PE was significant greater than 3.5 (7.6 *vs* 3.5, t-value=2.17, *p*=0.048). Patients developing DVT/PE did not differ by obesity or age when compared with non-DVT/PE population.
Summary/Conclusions: There have been only a few studies to assess the incidence of DVT/PE in patients undergoing lower limb surgical revascularization. In our study population, 1.4% of patients had evidence of DVT/PE. This constitutes a low risk of venous thromboembolism. The 2012 American College of Chest Physicians (ACCP) guidelines for prevention of venous thromboembolism in nonorthopedic surgical patients (Chest 2012; 141(2)(Suppl):e227s-e277s), recommends the use of pneumatic compression devices (PCDs), over no prophylaxis, to prevent DVT/PE in low risk patients. Since, patients with lower limb surgeries are not a good candidate for PCDs, pharmacological thromboprophylaxis with low dose heparin may be warranted. Given that bleeding is a potential complication in these patient, it might be prudent to start thromboprophylaxis 3.5-4 days after the surgery. Further studies are needed to assess the bleeding risks of postoperative thromboprophylaxis after surgical revascularization procedures.

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THE ROLE OF INFLAMMATION IN THROMBOEMBOLISM IN RESECTABLE RENAL CELL CARCINOMA PATIENTS

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Background: Renal cell carcinoma (RCC) may increase the risk for venous thromboembolism. However, only a few reports have described the clinical features and risk factors for thromboembolism.
Aims: This study aimed to elucidate the clinical features of thromboembolic events and to identify prognosis in patients who experienced thromboembolism events.
Methods: We retrospectively reviewed medical records of patients who underwent nephrectomy at our institution between February 1998 and August 2015. We evaluated the data including pathologic stage, gender, age, smoking history, underline disease, preoperative laboratory findings and survival outcomes.
Results: A total of 3099 patients were included in the study. Among them, 208 thromboembolic events (6.7%) were identified in pathologic and image studies during median follow-up duration of 40 months. Patients who have increased preoperative platelet levels ((≥400x10^{3}uL), neutrophil lymphocyte ratio (NLR) (≥1.86) and c-reactive protein (CRP) (≥0.12mg/dL) experienced significantly more thromboembolic events than those with lower value according to multivariable analysis (hazard ratio [HR], 2.22 [95% CI, 1.01–4.85], *P*=0.047 for platelet levels; HR, 3.39 [95% CI, 1.67–6.90], *P*=0.001 for NLR;HR, 3.38 [95% CI, 1.67–6.80], *P*=0.001 for CRP). Moreover, patients who experienced thromboembolism showed poor overall survival (OS 195 *vs* 67 months HR 1.95, P=0.007).
Summary/Conclusions: Preoperative inflammation markers including NLR, CRP and platelet cout can be the risk factors for venous thromboembolism in RCC patients who experienced nephrectomy. Thromboembolism also has a significant role on the the prognosis of RCC patients.

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GENETIC AND ENVIRONMENTAL RELATIONSHIP BETWEEN VITAMIN B12, FOLATE AND HOMOCYSTEINE AND SUSCEPTIBILITY TO THROMBOSIS IN THE GAIT 2 PROJECT. RESULTS OF A GWAS ANALYSIS

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Background: Venous thromboembolism (VTE) is a common disease involving genetic and environmental risk factors. Earlier studies have estimated that the heritability of the risk of VTE is 60% and we have demonstrated that erythrocyte-related phenotypes are good candidates for risk factor of thrombosis (Genetic Analysis of Idiopathic Thrombophilia 2, GAIT 2, Project).
Aims: To replicate the estimates of the heritability, and to evaluate the role of intermediate phenotypes as VTE risk factors.
Methods: Thirty-five Spanish extended families were recruited in the GAIT 2 Project. The sample consisted of a set of 935 individuals. Inclusion criteria were similar to GAIT 1. Levels of serum vitamin B12 (B12), serum folate (SF), red cell folate (RCF) and homocysteine (HCY) were evaluated using commercial automated methods. Heritability (h2) estimates were obtained using the variance component method. The correlations between a given pair of traits were calculated using a multivariate variance component model. By studying these traits in extended families we estimated both the genetic (ρg), and the environmental (ρe) correlations between traits. All of the statistical analyses were performed employing the Sequential Oligogenic Linkage Analysis Routines (SOLAR, version 6.6.2, official). Then a GWAS analysis was carried out.
Results: The h^2 of VTE was 0.67. All parameters showed significantly high h^2, and environmental (especially in the case of HCY) factors were also related to these parameters (table 1). In addition, VTE was correlated with B12 (0.34. p=0.027). Moreover, B12 was related to autoimmunity (0.5. p= 0.03) and RCF with malignancy (-0.58, p=0.05). The GWAS analysis detected numerous signals (table 2). Some of these signals have been reported (B12 and FUT2, SF and Hcy and MTHFR).

Table 1. Values, heritabilities, household effect and significant covariates effects.

TRAIT	Value	h^2	p value (h^2)	c^2	Covariables
B12 (pmol/l)	441±240 (74-4558)	0.47	2.95×10^{-17}	0.11	Age, contraceptio n, smoking
SF (nmol/l)	21±7.6 (6.2-45.4)	0.27	2.3×10^{-6}	0.07	Sex, contraceptio n, smoking
RCF (nmol/l)	1241±481 (435-3554)	0.42	1.85×10^{-13}	0.06	Age, sex, smoking
HCY (µmol/l)	10.4±5.5 (2.7-97.7)	0.26	3.61×10^{-5}	0.41	Age, sex, smoking

Values expressed as Mean±standard deviation, in brackets maximum and minimum values. B12: serum vitamin B12; SF: Serum folate; RCF: Red cell folate; HCY: Homocysteine.

Table 2. Suggestive signals detected by GWAS.

Variable	Chromosome	Gene and rs	P value
B12	19	FUT 2 rs516246	1.7x10-7
	6	GLYATL3 rs3957334	1.4x10-7
SF	1	MTHFR rs1801133	1.3x10-6
	12	TMEM132C rs1683701	1.4x10-6
RCF	9	Intergenic rs 73651941	1.13x10-6
HCY	1	MTHFR rs1801133	2.5X10-10
	9	Intergenic rs77630217	1.2x10-6
	11	ELF5 rs3824896	2.7x10-7

Summary/Conclusions: In the GAIT2 study, genetic and environmental factors were related to B12, SF, RCF and HCY. Moreover, a relationship was observed between B12 and VTE. In the GWAS analysis some signals were previously reported (FUT2 and B12 or MTHFR with SF and HCY). New signals were found that need to be clarified, especially their possible relationship with susceptibility to thrombosis.

This work was supported by RIC RD12/0042/0032, FIS PI12/00612 and FIS PI 15/0269 grants.

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CELLULAR ORIGIN OF CIRCULATING MICROPARTICLES (MP) ACCORDING TO SOMATIC MUTATIONS IN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS (MPN)

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Background: Essential thrombocythemia (ET) and polycythemia vera (PV) are MPN characterized by a high rate of thrombotic complications. We previously demonstrated increased plasma levels of procoagulant MP in ET (Marchetti *et al.* A.J.H. 2013).
Aims: Aim of this study was to extend the analysis of MP to PV patients and to characterize the cellular origin of plasma MP in both ET and PV patients. The influence of somatic mutations [i.e. JAK2V617F, calreticulin (CalR), thrombopoietin receptor (MPL)] and concomitant cytoreductive or antiplatelet therapies was also evaluated.
Methods: Thirty-seven ET (19 JAK2V617F, 9 CalR and 2 MPL mutation carriers), 35 PV patients (all JAK2V617F carriers) and 36 healthy control subjects were included into the study. Flow cytometry was performed to characterize MP phenotype in platelet free plasma samples. To define MP cellular origin, anti-CD31 (endothelial cell marker), anti-CD41 (platelet marker), anti-CD11b (leukocyte marker), and anti-CD235 (erythrocyte marker) monoclonal antibodies were used. Annexin V (AnnV) staining was used to evaluate the expression of procoagulant phosphatidylserine on MP.
Results: ET and PV patients displayed significantly higher MP levels compared to controls (p<0.05). The majority of circulating MP (90%) were AnnV positive, indicating the expression of phosphatidylserine on their surface. In healthy con-

trols, 71% of MP was positive for platelet (P-MP), 24% for erythrocyte (E-MP), 4% for endothelial cell (EC-MP) and 1% for leukocyte (L-MP) specific markers. In ET and PV patients, the percentage of P-MP was significantly higher (80%; p<0.05), while E-MP level was significantly lower (15%; p<0.05) than controls. L-MP and EC-MP values were comparable between patients and controls. The absolute counts of P-MP and L-MP were higher in both ET and PV *versus* controls. Overall, no significant correlations were found between the levels of MP derived from platelet, leukocytes or erythrocytes and the corresponding cell counts. The analysis according to patient mutations, revealed significantly higher levels (p<0.05) of both P-MP and E-MP concentration in patients carrying JAK2V617F mutation as compared to JAK2V617F negative patients. In addition, ET patients positive for CalR mutation displayed lower levels (p<0.05) of P-MP compared to JAK2V617F carriers. No influence of concomitant therapies on MP levels or composition was observed.
Summary/Conclusions: Our data confirm the presence of high levels of circulating MP in MPN, which support the role in the known hypercoagulable state of these patients. The MP cellular origin has a different distribution profile according to the presence of different mutations. Importantly, the lack of correlation found between the total and subtype-specific MP counts with the corresponding cell of origin counts suggests an active stimulation of MP formation.

Project funded by AIRC-IG2013 N.14505 of the Italian Association for Cancer Research (AIRC).

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ARE WE TESTING APPROPRIATELY FOR THE LUPUS ANTICOAGULANT?

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Background: The diagnosis of antiphospholipid syndrome (APS) requires the presence of thrombosis or defined pregnancy morbidity in addition to the presence of antiphospholipid antibodies on at least 2 occasions. Patients should be tested for antiphospholipid antibodies if they fulfil the required clinical criteria. Lupus anticoagulant may also be tested for when investigating a prolonged activated partial thromboplastin time which does not correct on mixing studies.
Aims: The aim of our study was to examine retrospectively the frequency of lupus anticoagulant (LA) testing in our institution, which we suspected to be high, and the incidence of positive results leading to a diagnosis of APS.
Methods: A total of 914 requests for LA were received over a 5 month period between 1st of May and 30th September 2014. We examined which departments were requesting the tests and the clinical indications for testing.
Results: Over 90% (829) of LA tests were negative. Nine percent (85) of tests demonstrated a positive LA. 33 patient had experienced arterial (11) or venous (22) thrombosis. There were 3 patients who fulfilled the clinical criteria for pregnancy morbidity in APS. A total of 6 patients experienced miscarriage before 10 weeks gestation; however none of these patients had the defined 3 miscarriages. There was one preterm delivery at 25 weeks due to pre-eclampsia. A further 3 patients had a still birth, one of which had an identifiable cause. In total, of the 85 positive results, 12 patients had a confirmed diagnosis of APS; a further 25 patients had the clinical manifestations fitting the clinical criteria for APS. Fourty eight patients had a positive LA but did not fit the clinical criteria for a diagnosis of APS. The clinical specialities requesting the majority of tests were obstetrics and gynaecology (231), rheumatology (179) and clinical haematology (118). Of these, clinical haematology had the highest yield of positive results (16%) compared to 3% in obstetrics and gynaecology.
Summary/Conclusions: Our results highlight a high frequency of LA testing in our institution with a low yield of positive results (9%), resulting in a total of 1% of patients being diagnosed with APS. Our results demonstrate that the majority of tests for LA are not of clinical significance and often requested in patients not fitting the clinical criteria for APS. Further education for all practitioners would help to ensure only appropriate patients are tested. Indeed if a patient fits the clinical criteria for APS they should be tested for all antiphospholipid antibodies namely anti-cardiolipin and anti-β2-glycoprotein I as well as the lupus anticoagulant.

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RESULTS OF USING BRIDGING THERAPY WITH SODIUM BEMIPARIN AT THERAPEUTIC-DOSE

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Background: Bridging therapy consists of the administration of a fast-acting anticoagulant such as the low-molecular-weight heparin (LMWH) during the period of cessation of oral anticoagulant therapy. The decision to continue with anticoagulant therapy or to discontinue the treatment with the establishment of the Bridging therapy have been carried out carefully and on an individual basis. While taking this decision, we have taken into account three factors: the urgency of surgery or invasive process, the risk of bleeding and thrombotic risk for the patient. In recent decades, there have been multiple studies supporting the LMWH treatment, at least as safe and effective and more cost-effective than unfractionated heparin (UFH) in the prophylaxis and treatment of venous thromboembolism (VTE), including deep vein thrombosis (DVT) and pulmonary embolism (PE). Therefore, the LMWH is considered as the drugs of choice in the prevention of venous thromboembolism.
There are several types of commercialized LMWH, with different pharmacological properties, such as molecular weight, anti-Xa/IIa ratio and average life. The sodium bemiparin is the LMWH with greater anti-Xa/IIa ratio, which implies a lower risk of bleeding. In addition, it has shown a low incidence of VTE and bleeding in actual clinical practice.
Aims: There are few published data from bridging therapy at therapeutic doses in patients treated with oral anticoagulants (AVK) and perioperative management. It is intended to assess the efficacy (recurrence of thrombosis) and safe use of sodium bemiparin at anticoagulant doses on the bridging therapy and possible thrombotic and / or hemorrhagic complications (major and minor bleeding) resulting from this use.
Methods: We have analyzed 975 bridging therapies at full dose in our clinic in the last year. They were made to a total of 650 patients (315 men and 335 women) with CHADSVASC >2, aged between 15 and 92, with an average age of 69 years old. The reasons of anticoagulation in our patients were atrial fibrillation, mechanical prostheses, DVT, pulmonary embolism and recurrent thrombosis in patients with thrombophilia. In 70% of the cases, there were comorbidities, such as heart failure, chronic obstructive pulmonary disease, anemia, kidney failure, liver disease and long-term aftereffects of stroke. The bridging therapy has consisted on suspending AVK 4 (acenocumarol) to 6 days (warfarin) before the procedure, and replacing it by sodium bemiparin at full doses <50 kgr: 5,000 IU/24h, 50 to 70 kgr: 7,500 IU/24 h, 70-100 kgr: 10,000 IU/24 h and >100 kgr: 12.500 IU/24 h, and administration of a prophylactic dose of 3,500 IU, 12 hours before the procedure, and another dose 6-12 hours after the procedure, depending on the risk of bleeding of the intervention and the thrombotic risk of the patient's disease. The bridging therapy has been performed in 225 cases of major surgery (orthopedic surgery, ophthalmological procedures, valvular replacements etc), 340 cases of minor surgery (removal of nevus, complex dental extractions, dental implants), 295 cases of invasive procedures (colonoscopies, endoscopies..), 50 cases of bleeding caused by AVK (epistaxis, petechiae and bruises, hemoptysis, menorrhagia and gastrointestinal bleeding), 30 cases of hospitalization with INR decompensation with various causes (infectious endocarditis, pneumonia, uncompensated heart failure...) and 35 cases for thrombophilia study.
Results: As complications of using bemiparin sodium, there have been: 40 cases of hematomas at the needle puncture sites. There was neither cases of major bleeding nor cases of thrombosis.

Table 1.

	Episodes of use of the different doses of bemiparin
Bemiparin 5000 UI/24h	95
Bemiparin 7.500 UI/24h	465
Bemiparin 10.000 UI/24h	376
Bemiparin 12500 UI/24h	30

Summary/Conclusions: Sodium bemiparin administered at therapeutic doses (115 IU/kg/24h) in the perioperative period, according to the scheme described above, it is associated with a low incidence of recurrence of VTE and bleeding. The complications presented in our sample have been very few, in patients with associated co-morbidities. In our study, sodium bemiparin has shown to be safe and effective with minimal bleeding complications. Treatment should be administered on an individual basis according to each patient and factors related to surgery. Further studies will confirm our results.

SIMULTANEOUS SESSIONS III

Targeted therapies in relapsed in chronic lymphocytic leukemia

S769

IBRUTINIB IN PREVIOUSLY TREATED CHRONIC LYMPHOCYTIC LEUKEMIA: UPDATED EFFICACY AND SAFETY OF THE RESONATE STUDY WITH UP TO FOUR YEARS OF FOLLOW-UP

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Background: Ibrutinib is a first-in-class, once-daily oral inhibitor of Bruton's tyrosine kinase. Ibrutinib as a single agent is indicated by the EMEA and US FDA for the treatment of adult patients with CLL and allows for treatment without chemotherapy. The phase 3 RESONATE trial in patients with relapsed CLL showed superior efficacy of ibrutinib compared with ofatumumab (Byrd NEJM 2014).
Aims: We report updated safety and efficacy results of the RESONATE trial with up to 4 years of follow-up.
Methods: Eligibility criteria included ≥1 prior therapy, ineligibility for treatment with a purine analog, and ECOG performance status 0-1. Informed consent was obtained from all patients prior to study initiation. Patients received oral ibrutinib (420 mg once daily) until disease progression or unacceptable toxicity or intravenous ofatumumab (300 mg week 1; 2000 mg weekly for 7 weeks and then every 4 weeks for 16 weeks) for up to 24 weeks. At the interim analysis (median follow-up of 9 months), the data monitoring committee declared superiority of ibrutinib *vs* ofatumumab for progression-free survival (PFS) and overall survival (OS), and access to ibrutinib was recommended for all patients in ofatumumab arm who had disease progression. Long-term follow-up of efficacy endpoints are per investigator assessment. Patients randomized to ofatumumab were censored at crossover for OS.
Results: A total of 391 patients were randomized to receive ibrutinib (n=195) or ofatumumab (n=196). The median age was 67 years, with 40% age ≥70 years, and Rai stage III/IV in 57% of patients. At a median follow-up of 44 months (maximum 53 months) for the ibrutinib arm, PFS was significantly longer for ibrutinib *vs* ofatumumab (median NR *vs* 8 months, [HR 0.133; *P*<0.0001]). The 3-year PFS was 59% for ibrutinib *vs* 3% for ofatumumab. A significant PFS benefit was observed across baseline subgroups. In the ibrutinib arm, PFS for the del11q subgroup trended to have the most favorable outcome; however, PFS outcomes were not statistically different for patients with del17p or del11q or patients without these FISH abnormalities. At time of analysis, with the majority of patients randomized to ofatumumab (68%) crossing over to receive ibrutinib therapy, OS was longer for ibrutinib *vs* ofatumumab (median OS NR for either arm). The 3-year OS rate for ibrutinib was 74%. The ORR for ibrutinib was 91% with a CR/CRi rate that increased over time (currently 9%). Baseline cytopenias improved with extended ibrutinib therapy for hemoglobin (85% of patients), platelet (95% of patients), and absolute neutrophil counts (95% of patients). The adverse event (AE) profile of ibrutinib was consistent with previous reports. During a follow-up of up to 4 years, major hemorrhage occurred in 6%, grade ≥3 atrial fibrillation occurred in 6%, and grade ≥3 hypertension occurred in 8% of patients. The incidence of most grade ≥3 AEs decreased from year 1 *vs* year 2-3: neutropenia: 18% *vs* 8%; pneumonia: 11% *vs* 4%; atrial fibrillation: 4% *vs* 2%, respectively. The most frequent reasons for treatment discontinuation were progressive disease (27%) and AEs (12%). At analysis, 90 patients randomized to ibrutinib (46%) continue to receive ibrutinib.
Summary/Conclusions: In this international phase 3 RESONATE study with median follow-up of up to 4 years, long-term treatment with ibrutinib showed a favorable tolerability profile with sustained PFS and OS benefit regardless of high-risk cytogenetics. The results in relapsed del17p and del11q patients compared favorably to those previously reported in phase 2 studies.

S770

THE INITIAL REPORT OF THE BLOODWISE TAP CLARITY STUDY COMBINING IBRUTINIB AND VENETOCLAX IN RELAPSED, REFRACTORY CLL SHOWS ACCEPTABLE SAFETY AND PROMISING EARLY INDICATIONS OF EFFICACY

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Background: Ibrutinib (IBR) is an oral BTK inhibitor with high response rates in CLL. Venetoclax (VEN) is a potent, highly selective, orally bioavailable small-molecule BCL2 inhibitor. Both IBR and VEN are approved by both the FDA and EMA as single agents for chronic lymphocytic leukaemia (CLL). IBR leads to a rapid nodal response with re-distribution of CLL into the peripheral blood whereas VEN leads to depletion of CLL cells to levels in some patients where they cannot be detected. Two of the key cellular processes that are abnormal in CLL are proliferation and apoptosis. The combination of IBR with VEN is therefore logical as biologically the two drugs would be expected to be synergistic. The eradication of minimal residual disease (MRD) from blood and bone marrow is associated with improved outcome in any treatment of CLL where it has been reported.
Aims: The CLARITY trial (ISCRTN: 13751862) is a feasibility study to investigate the safety and efficacy of IBR combined with VEN in patients with relapsed/refractory CLL. Here we report for the first time the safety of the combination as well as early signs of potent synergy.
Methods: After 8 weeks of IBR monotherapy (420mg/day), VEN was added initially at a dose of 10mg/day with weekly escalations to 20mg, 50mg, 100mg, 200mg to a final dose of 400mg/day. After the initial 3 patients when there was no sign of tumour lysis syndrome (TLS) the starting dose of VEN was amended to 20mg/day. The primary end-point of the trial is MRD eradication (defined as less than 1 CLL cell in 10,000) in the bone marrow after 12 months of IBR+VEN. Key secondary end-points are MRD eradication from the bone marrow after 6 and 24 months of combined IBR and VEN as well as the safety of the combination. Important safety events that were considered critical were the incidence of laboratory and clinical TLS. All patients were given prophylactic treatment with uric acid reducing agents beginning at least 72 hours prior to their initial dose of VEN. Over the first three months of combined therapy the level of CLL in the peripheral blood was monitored weekly during VEN escalation and then monthly thereafter. 50 participants will be treated in total.
Results: A total of 35 patients have been recruited between May 2016 and January 2017. To date 21 patients have completed the dose escalation period of VEN in combination with IBR. To date there has been only a single case of laboratory TLS in a patient whose phosphate (1.21 to 1.48mmol/l) and creatinine (75 to 146 umol/l) both increased when VEN was increased from 100mg to 200mg. Dosing of VEN was interrupted for 7 days (due to the logistics of clinic closure periods over the Christmas break) and IBR for 24 hours. The biochemical abnormalities resolved within 24 hours and the patient subsequently escalated to 400mg/day of VEN with no further TLS. As yet there have been a total of 5 SAEs and 22 AE's of special interest with notably lung infection (n=3) and neutropenia (n=11) occurring on more than one occasion. All SAE's resolved with appropriate management and all patients remained on therapy. No SUSAR's have been reported and no AE's have been fatal. The level of CLL in the peripheral blood increased during the 8 weeks of IBR monotherapy at 420mg/day from a median of 50 x 10^9/l (range: 0 to 330) to 55 x 10^9/l (range: 0 to 237) and then fell during the first 8 weeks of combined IBR with VEN (4 weeks dose escalation followed by 4 weeks at 400mg/day) from a median of 55 x 10^9/l to a median of 0.017 x 10^9/l (range; 0 to 3.1). The rate of fall is rapid in all patients with a median of 3 log reduction in CLL level after 8 weeks of combined therapy.
Summary/Conclusions: The combination of IBR with VEN is well tolerated in relapsed, refractory CLL with to date only a single case of laboratory TLS. The rapid reduction in the peripheral blood CLL level even during the escalation phase of VEN with IBR is promising and suggests a potent synergy between the drugs. The initial bone marrow responses are expected after 6 months of combination therapy.

S771

VENETOCLAX IN RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA WITH 17P DELETION: OUTCOME AND MINIMAL RESIDUAL DISEASE FROM THE FULL POPULATION OF THE PIVOTAL M13-982 TRIAL

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Background: Venetoclax monotherapy in patients (pts) with relapsed/refractory CLL harboring deletion 17p (del17p) resulted in an ORR of 79% with a CR rate of 7% as determined by an independent review committee at the initial analysis of the pivotal M13-982 trial (n=107). Subsequently, 51 additional pts were enrolled in a safety expansion cohort.
Aims: To present results from the full trial, including minimal residual disease (MRD) status by both flow cytometry and next generation sequencing (NGS).
Methods: Pts received venetoclax 400 mg daily after initial standard ramp-up until PD or discontinuation due to other reasons. CT scan was mandatory at week 36, after which disease assessment was by clinical evaluation. MRD assessment was performed beginning with the first clinical assessment of CR or PR with nodes <2 cm and then every 12 weeks until MRD negativity (defined at 10^{-4} sensitivity). MRD was assessed by NGS and multicolor flow cytometry and the best response was reported. Data cutoff date was 10 June 2016.
Results: Pts (N=158) had a median age of 67 (range, 29–85) years; a median of 2 prior therapies (range, 0–10); 32% were fludarabine refractory; 11% had previously received a B-cell receptor signaling inhibitor (BCRi); 48% had nodes ≥5 cm; and 78% had unmutated *IGHV*. The median duration of venetoclax therapy was 16.7 (range 0–34.4) months. Primary reasons for discontinuation (50.6% of pts) were PD (31.0%), adverse events (AEs) (12.6%), withdrawal of consent (2.5%), stem cell transplant (2.5%), and other (1.9%). For all 158 pts, the investigator-assessed ORR was 77% and CR rate was 18%. The 24-month estimates for progression-free survival (PFS) and overall survival (OS) were 52% and 72%, respectively. The safety expansion cohort included 5 pts with previously untreated del(17p) CLL. These pts had an ORR of 80%, CR rate of 40%, and all 5 were alive and progression-free 1 year after the start of study treatment. Among the 18 pts with prior BCRi treatment, ORR was 61% and CR was 11%, with 12-month PFS and OS estimates of 50% and 72%, respectively. The most commonly reported AEs were neutropenia (42%), nausea (37%), diarrhea (37%), anemia (24%), and fatigue (22%). The most common grade 3–4 AEs were neutropenia (39%), thrombocytopenia (15%), and anemia (14%). Infection rate (77% all grades, 22% grade 3–4) and spectrum were consistent with the underlying disease. The rate of laboratory tumor lysis syndrome (TLS) was 5%, with no cases of clinical TLS. Of 101 pts with evaluable blood MRD by flow cytometry, 76 also had MRD data by NGS. From the full trial cohort of 158 pts, 42 (27%) demonstrated blood MRD negativity at 10^{-4} by flow cytometry, and 28 had a contemporaneous NGS sample. MRD negativity (10^{-4} sensitivity) was confirmed by NGS in 20 pts (71%), and 8 pts (29%) were MRD-positive by NGS. MRD negativity by NGS ($\leq 10^{-4}$) was observed in 22 pts in blood; 9 pts were also negative in the marrow although not necessarily at the same time. One pt negative by NGS did not have a matching flow cytometry assessment and 1 pt was positive by flow cytometry (0.008% *vs* 0.02%). Pts who achieved blood MRD-negative CR by flow cytometry (n=19) had a 24-month PFS estimate of 100%, compared with 78.5% pts who had blood MRD-negative PR (n=23). Similar results were obtained when assessed by NGS.
Summary/Conclusions: Venetoclax monotherapy resulted in a high response rate that was durable in this high-risk population, including among pts who had previously received a BCR inhibitor. MRD negativity by either flow cytometry or NGS correlated with outstanding outcomes.

S772

CHEMO-FREE TRIPLET COMBINATION OF TGR-1202, UBLITUXIMAB, AND IBRUTINIB IS WELL TOLERATED AND HIGHLY ACTIVE IN PATIENTS WITH ADVANCED CLL AND NHL

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Background: Novel targeted agents are emerging for B-cell malignancies, but few studies have safely combined these agents. Ublituximab (UTX) is a novel glycoengineered mAb targeting a unique epitope on the CD20 antigen. TGR-1202 is a next generation, once daily, PI3Kδ inhibitor, demonstrating a favorable safety profile compared to prior inhibitors, including in long-term follow up (Burris, 2016).
Aims: This Ph 1 trial evaluates the safety/efficacy of the triplet combination of a novel anti-CD20 mAb+PI3Kδ+BTK inhibitor (ibrutinib) in pts with B-cell malignancies.
Methods: Eligible pts had CLL or rel/ref NHL w/o limit to prior therapies, including those ref to prior PI3Kδ or BTK inhibitors. UTX dosed on D 1, 8, 15 of C 1, D 1 of C 2-6, and C 9 & 12. TGR-1202 dose escalated (400/600/800mg QD), ibrutinib dosed at 420mg (CLL) or 560mg (NHL), both on C1D1.
Results: 38 pts were enrolled: 20 CLL/SLL and 18 NHL, including 6 follicular (FL), 6 DLBCL, 4 mantle cell (MCL) and 2 marginal zone (MZL). Med age 65 yrs (range 32-85); 29 M/9 F; med prior tx=3 (range 0-6). 2 pts ref to prior PI3Kδ /2 prev treated with ibrutinib (1 ref/1 rel). MTD was not reached. Most common (>20%) all causality AE's were fatigue (42%), diarrhea (39%), dizziness (34%), nausea (32%), neutropenia, pyrexia, rash, infusion reaction, insomnia (each at 29%), thrombocytopenia, cough (each at 26%), anemia (24%) and sinusitis (21%). GR 3/4 AE's (all causality) were minimal, the only event >10% was neutropenia (16%). ORR amongst 36 evaluable pts is shown in the following Table 1.

Table 1.

Subtype	N	CR	PR	ORR
CLL/SLL	19	3	16	100%
FL/MZL	7	2	4	86%
DLBCL	6	0	1	17%
MCL	4	1	3	100%

53% of evaluable CLL pts had high-risk cytogenetics and 4/6 DLBCL pts were non-GCB. One CLL pt (17p/11q del) ref to PI3Kδ and ibrutinib achieved a CR. Med time on study is 10 mos (range 1-27 mos). Med DOR not reached (range 3-24 mos).
Summary/Conclusions: This is the first known triplet combination of an anti-CD20 mAb+PI3Kδ+BTK inhibitor. The combination of UTX, TGR-1202, and ibrutinib has been well tolerated with activity observed across heavily pre-treated and high-risk B-cell malignancies. Expansion cohorts at the highest dose (800mg TGR-1202+full dose ibrutinib) are underway. Future trials for the triplet are warranted.

S773

THE DUAL SYK/JAK INHIBITOR CERDULATINIB DEMONSTRATES COMPLETE INHIBITION OF SYK AND JAK AND RAPID TUMOR RESPONSES IN A PHASE 2 STUDY IN PATIENTS WITH RELAPSED/REFRACTORY B CELL MALIGNANCIES

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Background: Subsets of B cell malignancies are addicted to B cell antigen receptor (BCR) signaling for survival. Co-stimulation of the BCR with IL-2 or IL-4 in normal B cells significantly enhances cellular activation relative to BCR or cytokine stimulation alone, and combining SYK selective and JAK selective inhibitors synergize to suppress this response (Coffey et al., 2013). Hence, BCR/SYK and cytokine JAK/STAT signals cooperate to control B cell activation. This cooperation appears to be relevant to B cell malignancies as well. IL-4 promotes the survival of CLL cells in culture via up-regulation of MCL1 and BCL_{XL}, protecting the tumor from death induced by fludarabine and chlorambucil (Steele et al., 2010) and by idelalisib and ibrutinib (Aguilar-Hernandez et al., 2016). Also, unlike ibrutinib, combined SYK and JAK inhibition by cerdulatinib induces apoptosis in primary CLL cells and leads to down-regulation of MCL1 and BCL_{XL} (Blunt et al., 2015) and induces apoptosis in cells from ibrutinib-resistant CLL patients (Guo et al., 2017). It also induces apoptosis in primary DLBCL and DLBCL cell lines that carry BCR pathway mutations resistant to ibrutinib (Ma et al, 2015). Combined SYK/JAK inhibition may therefore represent a powerful strategy to control B cell malignancies. A phase I dose escalation study of cerdulatinib in 43 patients with relapsed/refractory CLL and NHL was recently completed (Hamlin et al., EHA Congress 2016). Inhibition of both BCR/SYK and JAK/STAT signaling pathways by >90% in peripheral blood assays was well tolerated. Durable PRs and 1 CR were observed in CLL and FL, including in patients who had relapsed on prior BCR inhibitor therapy. No consistent hepatic toxicity, anemia, thrombocytopenia or neutropenia was observed. Two grade 3 dose limiting toxicities were observed at 45 mg BID (fatigue, pancreatitis). 35 mg BID was identified as the Phase 2 dose based on Phase 1 data and on PK/PD modeling.
Aims: The primary aim of the study was to understand the safety and activity of cerdulatinib in B-cell malignancies.
Methods: This is an open-label study with 28-day cycles. Twice daily (BID; 30

mg and 35 mg) dosing was evaluated. Pharmacokinetics (PK), pharmacodynamics (PD), and safety were monitored, as well as an assessment of efficacy. Clinical response was assessed by standard criteria. Potency and specificity for SYK and JAK pathway inhibition were measured in whole blood assays by monitoring signaling responses following ligation of the BCR and receptors for IL-4. Serum markers of inflammation, minimal residual disease (MRD) and apoptosis in CLL patients were also measured.

Results: A phase 2 study was initiated in May 2016 to enroll up to 40 patients in each of three cohorts; 1) relapsed/refractory CLL/SLL, 2) relapsed/refractory indolent NHL, and 3) relapsed DLBCL, MCL and transformed FL. As of March 1, 2017, 37 patients have been enrolled, 17 with CLL/SLL, 15 with indolent NHL (10 FL, 4 MZL, 1 WM), and 5 with aggressive NHL (3 DLBCL, 1 MCL, 1 tFL). Median patient age is 70 years (range, 51-93). The median number of prior therapies is 3 (range 1–7). 11 patients had prior BTK or PI3K inhibitor therapy. The safety profile has been similar to what was seen in the Phase 1 study. However, 3 patients at 35 mg BID achieved higher than expected drug concentrations and had SAEs (2 grade 5 infections, 1 grade 3 pancreatitis). The starting dose was reduced to 30 mg BID and a PK monitoring and dose reduction strategy has been implemented. To date, this has resulted in a better safety profile without PK outliers. The most common AEs of any grade have been diarrhea (27%), fatigue (27%) and nausea (24%). Grade 3+ AEs occurring in more than 1 patient are infection (5 patients), abdominal pain (3 patients) and hypertension (3 patients). As seen in phase 1, significant inhibition of SYK and JAK signaling pathways in peripheral blood is observed. Evidence for tumor cell mobilization to peripheral blood in CLL/SLL is consistently observed following one week of therapy. PRs have been seen in all 3 cohorts including 10 of 13 (77%) CLL/SLL and 3 of 6 (50%) FL patients evaluated. Of these 13 PRs, 12 are still on drug with 4 patients in response for greater than 6 months. In addition, PRs have been seen in patients who relapsed on ibrutinib (FL patient, 8+ months) and venetoclax (SLL patient, 7+ months) therapy. As demonstrated preclinically, we have seen evidence of apoptosis (Annexin V+ B-cells) in 6 CLL patients. 5 of these patients had a PR at the end of the 2nd cycle (Figure 1).

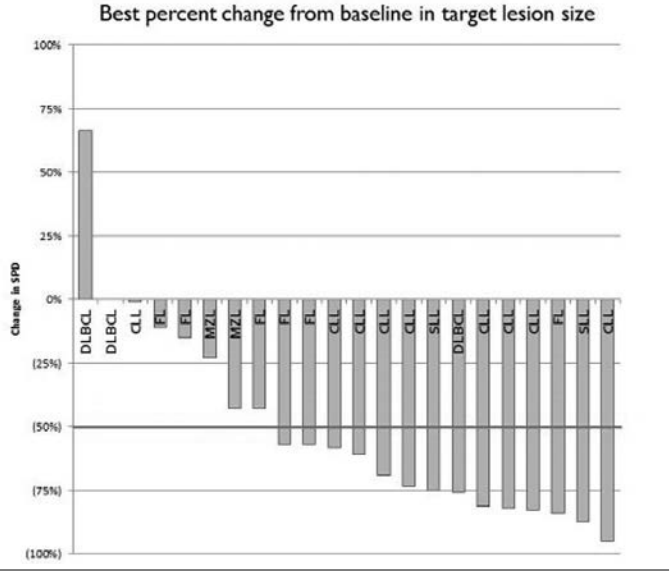

Figure 1.

Summary/Conclusions: Cerdulatinib demonstrates clinical activity in heavily pretreated patients with CLL/B-cell NHL and is generally well tolerated. Consistent activity is seen in patients with CLL and FL. Accrual is proceeding; updated PK/PD, safety and efficacy will be presented.

Follicular lymphoma - Clinical

S774

COMPARISON OF CONTRAST-ENHANCED CT-BASED RESPONSE WITH PET ASSESSMENT AFTER FIRST-LINE THERAPY FOR FOLLICULAR LYMPHOMA IN THE PHASE III GALLIUM STUDY

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Background: Published data show ^{18}F-FDG PET-CT (PET) is predictive after first-line immunochemotherapy in advanced-stage symptomatic FL, and PET is now the recommended modality for response assessment. However, no large-scale prospective comparison of the value of standard contrast-enhanced CT *vs* PET response has been performed.

Aims: To compare CT and PET response assessment for FL pts in the prospective Phase III GALLIUM study, which evaluated chemotherapy plus obinutuzumab (G-chemo) or rituximab (R-chemo) induction followed by maintenance antibody therapy (Marcus 2016).

Methods: PET scans, introduced after an early protocol amendment (July 2011), were performed at baseline and end of induction (EOI; all pts gave informed consent) and assessed by the investigator (INV) and an independent review committee (IRC) comprising two radiologists, with a third adjudicator; final response was determined by a clinician. Response was assessed by CT and PET plus bone marrow biopsy, applying the revised International Working Group (IWG) criteria (Cheson 2007, Juweid 2007). Complete remission (CR) status at EOI for each assessment, CT-CR and PET-CR, was compared with pt characteristics, PFS and OS.

Results: Among 1202 ITT pts with FL enrolled in GALLIUM, IRC-assessed CT showed a CR in 330 pts (27.5%), PR in 747 (62.1%), SD in 20 (1.7%), PD in 35 (2.9%), unavailable (NA) in 48 (4.0%) and unevaluable (NE) in 22 (1.8%). Of 609 pts with a baseline PET scan, 595 had detectable lesions, and 535 also had an evaluable PET at EOI. Baseline demographics and disease characteristics were similar in PET and non-PET populations. Pts with NA (n=52) or NE (n=8) scans were considered non-responders; these pts and those with PD prior to EOI were excluded from landmark PFS analyses. At EOI, 390/595 (65.5%) pts achieved a PET-CR according to IRC, comprising 212/297 (71.4%) G-chemo pts and 178/298 (59.7%) R-chemo pts. However, for these 390 pts, evaluable CT responses were 161 CR (41.3%), 216 PR (55.4%) and 5 SD/PD (1.3%; Table 1). Conversely, PET assessment showed a PET-CR in 161/177 (91.0%) of pts achieving a CT-CR, and PET-PR in only 117/362 (32.3%) of pts with CT-PR. Concordance between CT and PET assessment was 52.6% for IRC and 54.1% for INV. Concordance between INV and IRC evaluation was 71.9% for CT and 68.6% for PET. After a median follow-up of 34.5 mo (range 0–54.5), IRC-PET status was highly predictive of PFS (PET-CR *vs* PET non-CR: HR 0.39; 95% CI 0.25–0.60; p<0.0001) and OS (HR 0.41; 95% CI 0.19–0.86; p=0.018). 2.5-yr PFS from EOI was 87.6% (95% CI 83.5–90.8) for PET-CR pts compared with 70.9% (95% CI 61.3–78.6) for PET non-CR pts; corresponding OS was 96.6% (95% CI 94.1–98.1) *vs* 90.9% (95% CI 84.7–94.6) (Figure 1).

Table 1. CT and PET clinical response assessment by IRC at EOI

PET, n (%)	CT, n (%)					
	CR	PR	SD	PD	NE	NA
CR	161 (27.1)	216 (36.3)	4 (0.7)	1 (0.2)	7 (1.2)	1 (0.2)
PR	7 (1.2)	117 (19.7)	1 (0.2)	1 (0.2)	1 (0.2)	
SD			4 (0.7)			
PD	2 (0.3)	3 (0.5)	1 (0.2)	8 (1.3)		
NE	2 (0.3)	1 (0.2)			5 (0.8)	
NA	5 (0.8)	25 (4.2)	1 (0.2)	2 (0.3)	1 (0.2)	18 (3.0)

Summary/Conclusions: This large prospective analysis confirms EOI PET as an early predictor of PFS and OS in FL, with good concordance between INV

and IRC PET evaluation. Comparison of PFS based on CT-response and re-analysis of PET scans applying the now recommended 5-point scale for PET response assessment will be presented. Pooled analyses of these and data from other studies with longer follow-up may determine PET response as a reliable early surrogate for PFS and OS, providing a platform for study of response-adapted therapy.

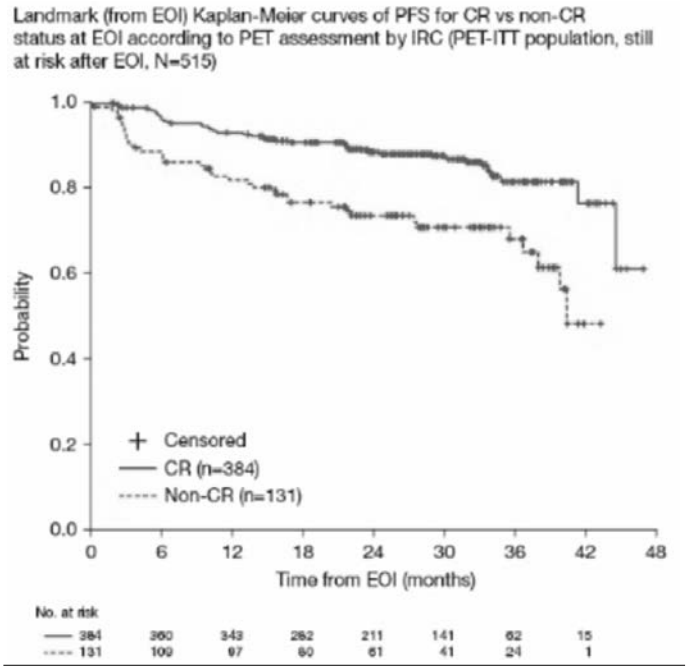

Figure 1.

S775

IMMUNOCHEMOTHERAPY WITH OBINUTUZUMAB OR RITUXIMAB IN PREVIOUSLY UNTREATED FOLLICULAR LYMPHOMA IN THE RANDOMIZED PHASE III GALLIUM STUDY: ANALYSIS BY CHEMOTHERAPY REGIMEN

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Background: The Phase III GALLIUM study (NCT01332968) showed that obinutuzumab (GA101; G) significantly prolonged PFS in previously untreated FL pts relative to rituximab (R) when combined with chemotherapy (chemo; CHOP, CVP or bendamustine [B]). Grade 3–5 and serious AEs were more common with G-chemo.

Aims: To explore outcomes by immunochemotherapy regimen.

Methods: Pts were aged ≥18 yrs with documented, previously untreated FL (grades 1–3a), advanced disease (stage III/IV or stage II with tumor diameter ≥7cm), ECOG PS 0–2, and requiring treatment according to GELF criteria. Chemo regimen was allocated by center. Pts were randomized 1:1 (stratified by chemo, FLIPI-1 group and geographic region) to R 375mg/m2 on day (D) 1 of each cycle (C) or G 1000mg on D1, 8 and 15 of C1 and D1 of C2–8, for 6 or 8 cycles depending on chemo. Pts with CR or PR at EOI (per Cheson 2007) continued to receive R or G every 2 months for 2 yrs or until progression. The cut-off date for this analysis was September 10 2016. All pts gave informed consent.

Results: 1202 FL pts were randomized. Baseline characteristics were generally similar across chemo groups, although B and CVP pts had relatively more comorbidities, *e.g.* GI and vascular disorders, than CHOP pts. After 41.1 months' median follow-up, investigator (INV)-assessed PFS remained superior for G-chemo relative to R-chemo (HR, 0.68; 95% CI 0.54–0.87; p=0.0016) with consistent HRs across chemo groups (Figure 1). HRs for secondary time-to-event endpoints were supportive of the primary analysis. Difference in frequency of grade 3–5 AEs between arms was highest with CHOP and CVP (Table 1). Rates of second neoplasms and grade 3–5 infections were similar in G and R arms for CHOP and CVP but not for B. In all chemo groups, SAEs were more frequent with G than R, and AEs causing treatment discontinuation and fatal AEs were similar. Reductions in T-cell counts were more pronounced and prolonged in the B group than CHOP or CVP groups.

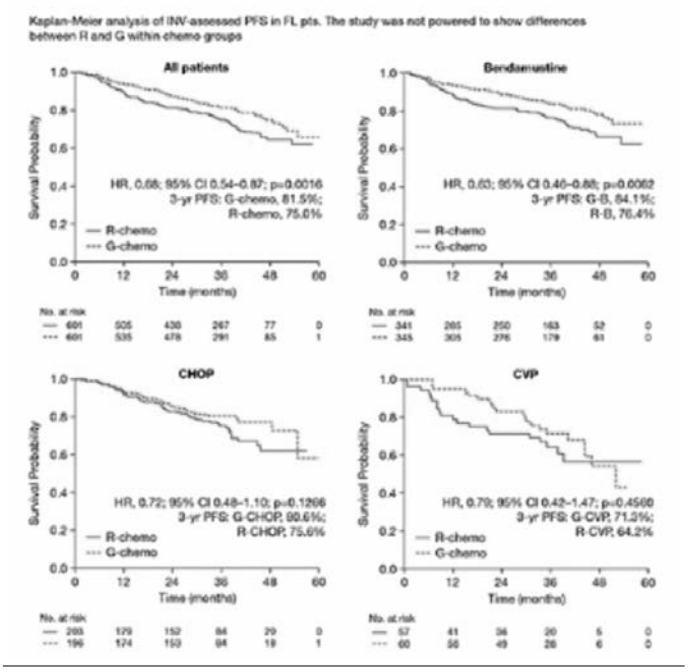

Figure 1.

Table 1. Safety summary (number (%) of FL pts* with ≥1 AE).

	G-B(n=338)	R-B(n=338)	G-CHOP(n=193)	R-CHOP(n=203)	G-CVP(n=61)	R-CVP(n=56)	G-chemo(n=595)	R-chemo(n=597)
AEs	338(100)	331(97.9)	191(99.0)	201(99.0)	61(100)	56(100)	593(99.7)	585(98.0)
Grade 3–5 AEs	233(68.9)	228(67.5)	171(88.6)	151(74.4)	42(68.9)	30(53.6)	449(75.5)	409(68.5)
Neutropenia†	100(29.6)	102(30.2)	137(71.0)	111(54.7)	28(45.9)	13(23.2)	265(44.5)	226(37.9)
Leucopenia†	11(3.3)	15(4.4)	39(20.2)	34(16.7)	1(1.6)	1(1.8)	51(8.6)	50(8.4)
Febrile neutropenia†	18(5.3)	13(3.8)	22(11.4)	14(6.9)	2(3.3)	2(3.6)	42(7.1)	29(4.9)
AEs of special interest by category								
Grade 3–5 Infections‡	89(26.3)	66(19.5)	23(11.9)	24(12.4)	8(13.1)	7(12.5)	121(20.3)	98(16.4)
Second neoplasms§	37(10.9)	23(6.8)	9(4.7)	11(5.4)	1(1.6)	2(3.6)	47(7.9)	36(6.0)
SAEs	176(52.1)	160(47.3)	76(39.4)	67(33.0)	26(42.6)	19(33.9)	281(47.2)	246(41.2)
Fatal AEs	20(5.9)	16(4.7)	3(1.6)	4(2.0)	1(1.6)	1(1.8)	24(4.0)	21(3.5)
AEs causing Tx discontinuation	52(15.4)	48(14.2)	32(16.6)	31(15.3)	11(18.0)	9(16.1)	98(16.5)	88(14.7)

*Pts who received ≥1 dose of study drug. Three pts received G but no chemo; †Occurring in >10% of pts in any group; ‡MedDRA SOC 'Infections and Infestations'; §Malignant or unspecified tumors occurring >6 mo after first study drug intake

Summary/Conclusions: In treatment-naive FL pts, PFS was superior with G-chemo relative to R-chemo with consistent effects across chemo regimens. Some differences were seen in safety profiles between chemo regimens, but comparisons may be confounded by the lack of randomization.

S776

EFFICACY AND SAFETY OF COPANLISIB IN PATIENTS WITH RELAPSED/REFRACTORY FOLLICULAR LYMPHOMA: A SUBSET ANALYSIS OF THE CHRONOS-1 STUDY

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Background: Follicular lymphoma (FL) is the most common indolent non-Hodgkin lymphoma (iNHL) subtype, yet treatment options in the relapsed/refractory setting are limited. Copanlisib is a potent and selective pan-class I PI3K inhibitor with predominant activity against the δ- and α-isoforms.
Aims: We report results from the FL subset of a large phase II study in iNHL patients (NCT01660451, part B).
Methods: Patients with histologically confirmed indolent indolent FL (grade 1-3a) relapsed/refractory to ≥2 prior lines of treatment were treated with copanlisib (60 mg IV infusion) administered on days 1, 8 and 15 of a 28-day cycle until disease progression or unacceptable toxicity. The primary endpoint was objective response rate (ORR) as assessed by independent radiology review according to the response criteria for lymphoma (Cheson et al, JCO 20:579, 2007). Secondary endpoints included progression-free survival (PFS) and duration of response (DOR), safety and tolerability.
Results: A total of 141 patients with iNHL were treated in the phase II study, including 104 patients with FL. The FL subset was characterized as: 52% male, 83% white, median age 62 yrs, 62% ECOG 0, 63% refractory to last therapy, median time from most recent progression 8 wks (range 1-73) and median prior lines of therapy 3 (range 2-8). At the time of primary analysis the ORR was 58.7%, comprising 15 patients (14.4%) with complete response and 46 (44.2%) with partial response. Stable disease was observed in 35 (33.7%) patients and progression of disease as best response in 2 patients. The median duration of response was 370 days (range 0-687), with 43 responders censored at data cut-off. Median duration of treatment was 22 wks (range 1-105); 33 (32%) patients remained on treatment. Per investigator assessment, 87 of 96 evaluable patients (91%) had some degree of tumor shrinkage as best response, and 58/96 (60%) had >50% tumor shrinkage (Figure 1). For all patients in the phase II study, the most common treatment-emergent AEs occurring in >25% of patients included (all grade/grade 3+): diarrhea (34%/5%), reduced neutrophil count (30%/24%), fatigue (30%/2%), and fever (25%/4%). Hyperglycemia (50%/41%) and hypertension (30%/24%) were transient. The incidence of pneumonitis (8%/1.4%), hepatic enzymopathy (AST 28%/1.4%; ALT 23%/1.4%), opportunistic infection (1.4%) and colitis (0.7%) were low. Six deaths were observed, 3 of which were attributed to copanlisib: one lung infection, one respiratory failure, and one thromboembolic event.

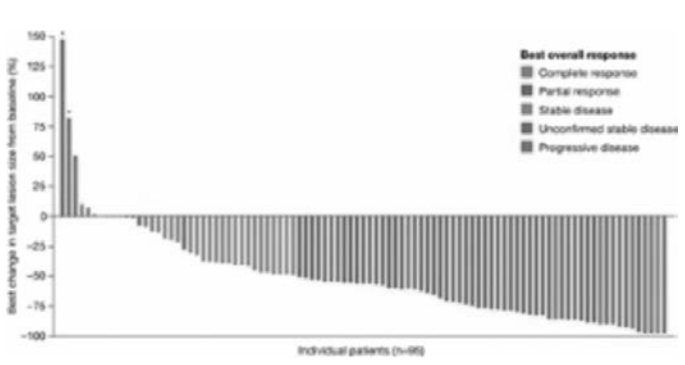

Figure 1.

Summary/Conclusions: Copanlisib was highly active as a single agent in heavily pretreated relapsed/refractory FL patients and resulted in responses in the majority of patients, with a median duration of response exceeding one year. Toxicities were manageable, with a low incidence of severe AEs associated with other PI3K inhibitors, especially hepatic enzymopathy, opportunistic infections, and colitis.

S777

DYNAMO: A PHASE 2 STUDY DEMONSTRATING THE CLINICAL ACTIVITY OF DUVELISIB IN PATIENTS WITH DOUBLE-REFRACTORY FOLLICULAR LYMPHOMA

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Background: Duvelisib is an oral, dual inhibitor of PI3K-δ,γ in development for the treatment of hematologic malignancies. DYNAMO is a Phase 2 study to evaluate the safety and efficacy of duvelisib monotherapy in a double refractory iNHL population, which included a majority of patients (pts) with follicular lymphoma (FL).
Aims: The primary objective was to evaluate the antitumor activity of duvelisib monotherapy in pts whose disease is refractory to rituximab and to either chemotherapy or RIT, with an additional objective to further characterize the safety of duvelisib.
Methods: DYNAMO is an open-label, single-arm, safety, and efficacy study in patients (pts) with FL, small lymphocytic lymphoma (SLL), or marginal zone lymphoma (MZL), whose disease is double-refractory to rituximab (monotherapy or in combination) and to chemotherapy or radioimmunotherapy. Pts received duvelisib 25 mg BID in 28-day treatment cycles until disease progression or unacceptable toxicity. The primary endpoint is overall response rate (ORR) as assessed by an independent review committee (IRC) per revised IWG criteria. Secondary endpoints include duration of response (DoR), progression-free survival (PFS), overall survival (OS), time to response (TTR), adverse events (AEs) and other safety parameters. *Pneumocystis jiroveci* pneumonia (PJP) prophylaxis was mandated for all pts.
Results: 129 pts with iNHL were treated on study. Of these, 83 pts with FL received duvelisib with a median duration of exposure of 6 mo. (range: 0.4 - 24). Median age was 64 years; 68% were male. Most FL pts had an ECOG performance status score at baseline of 0 (51%), followed by 1 (42%) and 2 (7%). Most FL pts (65%) had a FLIPI score at baseline ≥3, and most had either Stage 3 (46%) or Stage 4 (39%) disease. Median time from last anticancer therapy to first dose of duvelisib was 3.2 months. FL pts received a median of 3 prior anticancer regimens (range: 1 - 10); 65% of pts received ≥3 prior regimens, 17% ≥6 prior regimens. The ORR was 41% (CI: 30, 52), all of which were PRs. 36% of pts had SD as best response, and 17% had PD as best response. 6% of pts were not evaluable, as they only had a baseline scan. Median TTR was 1.9 mo. (range: 1.6 - 11.7). 80% of FL pts experienced a reduction in nodal target lesions after treatment with duvelisib. Among the 34 FL pts with a response per IRC, the median DoR was 9.2 months. The median PFS for all FL pts was 8.3 months, while the median OS was 11.1 months. Among all pts treated on study (n=129), AEs were mostly Gr 1-2. Most common ≥Gr 3 AEs were transient cytopenias [neutropenia (23%), anemia (12%), and thrombocytopenia (10%)] and diarrhoea (15%). 17% of pts discontinued duvelisib due to an AE. Opportunistic infections occurred in <5% of pts, none were fatal, and included 1 pt (0.8%) with *pneumocystis* and 3 pts (2.3%) with CMV infections. Six pts had an AE with a fatal outcome.
Summary/Conclusions: In DYNAMO, duvelisib showed clinical activity in a double-refractory FL population (41% ORR, median DoR 9.2 mo., 80% with reduction in target lesions). Duvelisib was generally well tolerated, with a manageable safety profile with appropriate risk mitigation. Duvelisib monotherapy has a favorable benefit-risk profile in double-refractory iNHL, and may represent an important treatment option. Updated clinical data will be available at the time of presentation.

S778

13-YR FOLLOW UP OF MULTICENTER RANDOMIZED CHOP-R VS R-HDS TRIAL IN HIGH RISK FOLLICULAR LYMPHOMA PATIENTS: PROLONGED SURVIVAL AND HIGH RATE OF LONG-TERM DISEASE FREE SURVIVORS

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Background: Between March 2000 and May 2005 a multicenter randomized trial comparing frontline use of CHOP-R *vs* R-HDS with autograft has been performed on 134 Follicular Lymphoma (FL) patients, selected for age less than 60 yrs. and poor prognostic features according to age-adjusted IPI (2-3) and IIL-score (3 or greater). Results at 4-yr follow-up were previously published (Ladetto M et al, Blood 2008), showing superior disease control with R-HDS without any survival advantage.
Aims: We have recently performed a long term update and the results at a median follow-up of 13 yrs are here presented.
Methods: The long-term outcome has been updated for 119 out of the original 134 randomized patients (56 CHOP-R and 63 R-HDS arms). Main features of the updated patients included: median age 51 yrs. (22-60), M/F ratio 68/51, aaIPI 2-3 90%, high LDH 43%, bulky disease 60%, B-symptoms 46%, BM involvement 86%; no significant differences were observed in clinical presentation between the two arms, as previously reported. Treatment schedule consisted of: i. CHOP-R arm: 6 courses of cyclophosphamide/doxorubicin/vincristine/prednisone followed by 4-weekly rituximab courses; ii. experimental R-HDS arm: rituximab with high-dose sequential chemotherapy followed by autografting. The analysis was intention to treat with event-free survival as the primary endpoint. Minimal residual disease (MRD) was evaluated post treatment in 56 patients with a bcl-2/IgH MBR or mcr translocation confirmed at diagnosis by nested PCR. The trial was registered at www.clinicaltrials.gov, no. NCT00435955. The long-term outcome has been updated in January 2017 by 27 out of 30 participating Centers, on 119 patients (88% of the whole series).
Results: Complete remission (CR) was achieved by 86 (72%) patients, including 32 (57%) with CHOP-R and 54 (85%) with R-HDS (p <.001); Molecular Remission (MR) was achieved in 37 out of 56 (66%) evaluable patients. At a median follow-up of 13 yrs., 74 patients (63%) are alive. Overall, 22 patients died for lymphoma progression (13 CHOP-R, 9 R-HDS), 12 died for secondary malignancy (3 in the CHOP-R, 9 in the R-HDS arms), 11 patients died for other causes, including four early toxic deaths. The overall survival (OS) for the whole series is 63% at 13 yrs, as shown in Figure 1A. No significant differences in the long-term OS were observed between the two arms, with 13-yr survival of 65% and 61% for CHOP-R and R-HDS, resppectively (p=0.51). At 13 years, the event free survival is 35%, whereas the disease-free survival (DFS) is of 53%, as shown in Figure 1B. Response to induction therapy had a major impact on the OS, with 13 yr survival of 75% for patients achieving CR *vs* 33% for those with less than CR (p <.001). Similarly, Molecular Remission (MR) achievement was associated with prolonged OS, with 13 yr survival of 81% for patients in MR on BM cells, and of 47% for those with positive MRD (p=.02) (Figure 1C).

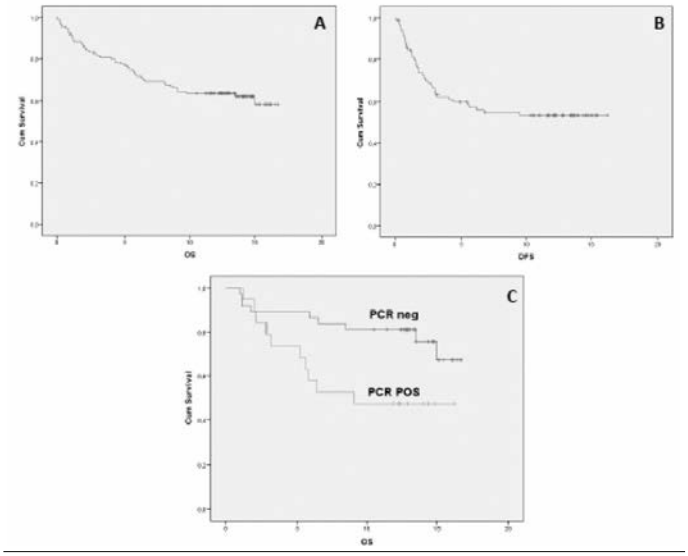

Figure 1.

Summary/Conclusions: i. poor risk FL may have a prolonged survival, with 63% of patients alive at 13 yrs.; ii. no survival differences between CHOP-R and R-HDS can be detected even at 13 yrs of follow-up; iii. achieving CR is still crucial for the long-term survival; iv. the MRD analysis has a prognostic impact not only on progression-free but also on OS; v. lymphoma progression remains the major cause of death, while secondary neoplasms represent the second cause of treatment failure; vi. a subgroup of advanced-stage FL may experience a prolonged DFS lasting at least 13 yrs: this raises the issue of the potential curability of FL.

Changing the strategy of therapy in multiple myeloma

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PHASE II TRIAL OF COMBINATION OF ELOTUZUMAB, LENALIDOMIDE, AND DEXAMETHASONE IN HIGH-RISK SMOLDERING MULTIPLE MYELOMA

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Background: This study aimed to determine the benefit of early therapeutic intervention with the combination of elotuzumab, lenalidomide, and dexamethasone in patients with high-risk smoldering multiple myeloma (SMM).
Aims: The overarching objective of this trial is to determine progression free survival to symptomatic myeloma (MM). Furthermore, the study examined whether genomic studies can help in determining patients who would benefit the most from this early therapeutic intervention.
Methods: Patients enrolled on study met eligibility for high-risk SMM based on the newly defined criteria proposed by Rajkumar et al, Blood 2014. Patients were administered weekly elotuzumab (10 mg/kg) on days 1, 8, 15, and 22 for the first two 28-day cycles while receiving lenalidomide on days 1-21. For cycles 3-8, patients were administered elotuzumab infusions on days 1, 8, and 15. Dexamethasone (40mg) was given on days 1, 8 and 15 for 40 of the 50 patients enrolled. After 8 cycles or best response, patients were given the option to mobilize with either cyclophosphamide or plerixafor and collect stem cells for future transplant. Patients were then allowed to continue on maintenance therapy where they were administered elotuzumab (20 mg/kg) on day 1, in combination with lenalidomide days 1-21 of a 28 day cycle. Bone marrow samples of 33 patients were obtained before starting therapy for baseline assessment and whole exome sequencing (WES) of plasma cells.
Results: In total, 50 patients were enrolled on this study from January 2015 to date, with the participation of eight sites. The median age of patients enrolled was 62 years (range 29 to 79) with 18 males (36%) and 32 females (64%). Interphase fluorescence *in situ* hybridization (iFISH) detected high risk cytogenetics in 20 patients. The median number of cycles completed is 12 (range 1 to 24). Therapy related grade 3 toxicities included hypophosphatemia (30%), neutropenia (14%), infection (12%), anemia (2%), pulmonary embolism (2%), rash (4%), and diarrhea (2%). Therapy related grade 4 toxicities included thrombocytopenia (2%), neutropenia (2%) and one instance of cholecystitis (2%). Stem cell collection was successful in all patients collected to date. Of the 31 evaluable patients that completed the first 8 cycles of therapy, the overall response rate was 84%, including 2 complete responses (7%), 11 very good partial responses (36%) and 13 partial responses (42%), and a clinical benefit rate of 100%. None of the patients showed progression to overt MM to date. WES was performed on 25 samples at the time of initiation of therapy. Recurrent mutations in the MAPK pathway (*KRAS*, *NRAS*) and tumor suppressor gene, *TP53*, were detected in 32% of the cases (16% each), while mutations in NF-KB and plasma cell differentiation pathways were present in 10% of patients. *CCND1* gene mutation was seen in 1 patient and was associated with t(11:14) as reported in the previous WES studies of MM. CNAs were called based on WES: 1q amplification, 13q, 17p, and 1p deletions were identified in 28, 20, 16, and 12% of cases, respectively. Interestingly, in 6 patients, high-risk CNAs were not reported in iFISH but were detected by WES. Finally, we assessed the correlation between neoantigen load and clinical response.
Summary/Conclusions: The combination of elotuzumab, lenalidomide, and dexamethasone is well tolerated and demonstrates a high response rates with no progression to overt MM to date. Correlation with genomic studies can help define patients who benefit the most from this early therapeutic intervention.

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TWICE-WEEKLY IXAZOMIB PLUS LENALIDOMIDE-DEXAMETHASONE IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA: LONG-TERM FOLLOW-UP DATA FOR PATIENTS WHO DID NOT UNDERGO STEM CELL TRANSPLANTATION

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Background: Addition of a proteasome inhibitor to a doublet backbone therapy has been shown to improve efficacy in newly diagnosed multiple myeloma (NDMM) patients (San Miguel et al, N Engl J Med 2008, Durie et al, Lancet 2017). Data from two phase 1/2 studies indicate that the combination of ixazomib plus lenalidomide-dexamethasone (IRd) is feasible and active in patients with NDMM, with weekly and twice-weekly ixazomib dosing having been investigated (Kumar et al, Lancet Oncol 2014; Richardson et al, Blood 2013).
Aims: This phase 1/2 study (NCT01383928) evaluated twice-weekly ixazomib plus Rd as induction therapy, followed by maintenance therapy with single-agent ixazomib. We report long-term efficacy and safety data in patients who did not withdraw from the study in order to receive SCT.
Methods: Patients with NDMM (SCT-eligible or SCT-ineligible) received twice-weekly oral ixazomib (3.0 or 3.7 mg on days 1, 4, 8, and 11) plus lenalidomide (25 mg on days 1–14) and dexamethasone (20 mg [10 mg in cycles 9–16] on days 1, 2, 4, 5, 8, 9, 11, and 12) for up to sixteen 21-day cycles, followed by maintenance therapy with single-agent twice-weekly ixazomib. Patients received therapy until disease progression or toxicity. Those who proceeded to SCT did not receive further ixazomib therapy. Response/progression was assessed per IMWG criteria after cycles 1, 2, 3, 4, and then every 2 cycles during induction and maintenance.
Results: Of the 64 enrolled patients, 40 continued on study treatment without early withdrawal for SCT; long-term follow-up of these 40 patients is reported here. The median age of patients was 66 years (range 34–82), and 45%/38%/18% of patients had ISS disease stage I/II/III. At a median follow-up of 47.0 months, the overall response rate (ORR; ≥partial response [PR]) in the response-evaluable population was 95%, the complete plus very good partial response (CR+VGPR) rate was 68%, and the CR rate was 32%. Median time to first response was approximately 1 cycle (0.72 months). Median time to a best response of ≥CR was 4.2 months. Patients received a median (range) of 14 (1–75) treatment cycles. Median progression-free survival (PFS) for patients not proceeding to SCT was 24.9 months. Median overall survival (OS) was not estimable; the 2-year Kaplan-Meier estimate for OS was 92%. A total of 78% of patients had grade ≥3 treatment-related adverse events (AEs); the most common treatment-related grade ≥3 AEs and serious AEs are shown in the Table 1. After completing induction therapy with IRd, 18 patients went on to receive maintenance with single-agent ixazomib on a twice-weekly dosing schedule. Patients who went on to maintenance received a median (range) of 31.5 (17–75) treatment cycles. Among the patients who received maintenance treatment, the ORR (≥PR) was 94%, the CR+VGPR rate was 89%, and the CR rate was 44%. Two (11%) patients improved their responses during maintenance (1 VGPR to stringent CR, and 1 VGPR to near-CR). Five (28%) patients who received maintenance therapy had an onset of a grade ≥3 treatment-related AE in cycle 17 or beyond. Rash (aggregate term) was infrequent with single-agent ixazomib during maintenance (1 patient, 6%).

Table 1.

Common grade ≥3 treatment-related AEs (occurring in ≥5% of patients), treatment-related serious AEs, and AEs resulting in any study drug dose reductions.

AE, n (%)	All patients who did not withdraw to receive SCT, n=40	Maintenance subset (n=18); AEs with onset during induction (cycles 1–16)	Maintenance subset (n=18); AEs with onset during maintenance (cycle ≥17)
Grade ≥3 treatment-related AEs of clinical importance			
Rash*	7 (18)	4 (22)	1 (6)
Hyperglycemia	4 (10)	2 (11)	0
Peripheral neuropathy*	4 (10)	1 (6)	1 (6)
Pneumonia	3 (8)	3 (17)	0
Hyponatremia	3 (8)	0	0
Neutropenia#	4 (10)	2 (11)	1 (6)
Serious treatment-related AEs			
All	17 (43)	8 (44)	2 (11)
AEs resulting in any study drug dose reductions†			
All	27 (68)	13 (72)	6 (33)

*MedDRA high-level term. #Pooled terms. †Reduction of at least one of the three drugs in the triplet regimen during the induction period and/or of ixazomib during the maintenance period

Summary/Conclusions: In patients with NDMM, twice-weekly ixazomib plus Rd resulted in exceptional response rates in patients who did not receive a SCT and who received maintenance therapy. The responses were deep and durable, with long PFS and a high 2-year OS estimate. The majority of AEs had an onset during induction, and the incidence of AEs during maintenance was infrequent.

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LENALIDOMIDE INDUCTION AND MAINTENANCE THERAPY FOR TRANSPLANT ELIGIBLE MYELOMA PATIENTS: RESULTS OF THE MYELOMA XI STUDY

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Background: Immunomodulatory agents are effective therapies for multiple myeloma (MM) acting via the modulation of cereblon. Lenalidomide (Len) has fewer side effects than Thalidomide (Thal), whilst retaining the benefits of oral administration, enabling long-term treatment that has been associated with better disease control. Combinations of agents induce deeper, longer remissions by targeting different clonal populations, with triplets outperforming doublets. The optimum immunomodulatory-based induction combinations and maintenance regimens are unknown.
Aims: The UK NCRI Myeloma XI study compared triplet induction regimens of Len *vs* Thal and examined the role of post-ASCT maintenance Len *vs* observation, enabling us to explore the interaction of Len induction with Len maintenance.
Methods: Myeloma XI is a multicenter, open-label, parallel group, randomised controlled trial for newly diagnosed MM patients of all ages, with pathways for transplant eligible (TE) and non-eligible patients. For TE patients the induction question compared Len or Thal plus cyclophosphamide and dexamethasone (CRD *vs* CTD) continued for a minimum of 4 cycles and to maximum response. For patients with a suboptimal response there was a subsequent randomization to a proteasome inhibitor containing triplet or no further therapy, prior to high-dose melphalan and ASCT. A maintenance randomisation at 3 months post ASCT compared Len till disease progression with observation. High risk disease was defined as the presence of at least one of t(4;14), t(14;16), del(17p) or gain(1q). 2042 TE patients underwent the induction randomization (CRD 1021, CTD 1021). After a median follow up of 36.3 months, 965 PFS and 415 OS primary endpoint events had occurred. Secondary endpoints include response and toxicity.
Results: In TE patients, CRD induction was associated with deeper responses than CTD (≥VGPR: CRD 60% *vs* CTD 53%), a finding which persisted post ASCT (≥VGPR CRD 82% *vs* CTD 77%). This was associated with a significantly improved median PFS. Patients receiving CRD achieved a median PFS of 35.9 months compared to 32.9 for those who received CTD (HR 0.85, 95%CI [0.75, 0.96], p=0.0116). This also translated into an overall survival benefit, 3 year OS: CRD 82.9% *vs* CTD 77.0% (HR 0.77, 95%CI [0.63, 0.93], p=0.0072). There were higher rates of PN and constipation with CTD *vs* haematological toxicity with CRD. Maintenance therapy with Len was associated with a significantly longer median PFS compared to observation (TE HR 0.47, 95%CI 0.38, 0.60). This finding persisted across all subgroups including patients with high-risk disease. Exploratory analysis across the TE pathway suggested that CRD induction with Len maintenance was optimum: 60 month PFS CRD-R 50.2%, CTD-R 39.1%, CRD-obs 18.5%, CTD-obs 23.4%.
Summary/Conclusions: In TE patients CRD was associated with deeper responses than CTD and with a PFS and OS benefit. The best outcomes were associated with Len induction plus Len maintenance. Our findings support continuing Len therapy through induction until disease progression.
On behalf of the UK NCRI Haemato-oncology CSG.

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COMPARISON OF DENOSUMAB WITH ZOLEDRONIC ACID FOR THE TREATMENT OF BONE DISEASE IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA; AN INTERNATIONAL, RANDOMIZED, DOUBLE BLIND TRIAL

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Background: Multiple myeloma is characterized by osteolytic bone disease, with up to 80% of pts presenting with detectable lesions. Myeloma bone disease is mediated by osteoclast activating factors such as RANKL, increasing the risk of skeletal-related events (SREs) and impacting morbidity and mortality. DMB, a human monoclonal antibody that targets and binds to RANKL, can be administered subcutaneously (SC) to pts regardless of renal function.
Aims: This study evaluates the efficacy and safety of DMB compared with ZA in newly diagnosed myeloma pts.
Methods: Adult pts were randomized 1:1 to DMB 120mg SC Q4W or ZA 4mg IV (adjusted) Q4W along with anti-myeloma therapy. Key stratification factors included type of first-line therapy (novel or non-novel) and previous SRE. Pts with renal insufficiency were excluded if baseline creatinine clearance (CrCl)<30mL/min. The primary endpoint was non-inferiority of DMB to ZA with respect to time to first on-study SRE. Secondary endpoints included superiority of DMB for time to first on-study SRE and first-and-subsequent on-study SRE, and overall survival (OS). Progression-free survival (PFS) was an exploratory endpoint. Safety was also assessed.
Results: A total of 1718 pts were randomized, 859 to each arm. Baseline demographics and disease characteristics were balanced, with 66.0% of DMB and 67.2% of ZA pts reporting prior SRE history; CrCl≤60mL/min was reported in 26.7% of pts. During the primary blinded treatment period (median follow-up 17.4 months [m]), 43.8% DMB pts and 44.6% ZA pts had a first on-study SRE. The median time to first on-study SRE was similar between DMB (22.83 m) and ZA (23.98 m) pts. DMB was non-inferior to ZA (P=0.01) in delaying time to first on-study SRE (HR[95%CI]=0.98[0.85,1.14]). Superiority was not demonstrated for time to first on-study SRE (P=0.82) and time to first-and-subsequent on-study SRE (P=0.84). In this high-risk study population the effect of antiresorptive therapy may only be evident later in the treatment course. A post-hoc, landmark analysis at 15 m for time to first SRE demonstrated a HR(95%CI)=0.66(0.44,0.98),P=0.039 (Figure 1) between DMB and ZA. OS was similar between DMB and ZA (HR[95%CI]=0.90[0.70,1.16],P=0.41), with fewer deaths with DMB (121[14.1%]) than ZA (129[15.0%]). PFS yielded a HR(95%CI)=0.82(0.68,0.99), descriptive P=0.036, with median times of 46.09m (95%CI:34.3,NE) for DMB and 35.38m (95%CI:30.19,NE) for ZA. The most common TEAEs(>25%) for DMB and ZA were diarrhea and nausea. The rates of SAEs (DMB,ZA [%];46.0,47.3), hypocalcemia (16.9,12.4; serious:0.9,0.2), and positively adjudicated ONJ (4.1,2.8) were comparable to known safety profiles. Fewer DMB pts (%) compared with ZA pts had AEs potentially related to renal toxicity (10.0,17.1;P<0.001), most notably in pts with baseline CrCl≤60mL/min (12.9,26.4). TEAEs led to IP discontinuation in 12.2% of all pts (12.9,11.5).

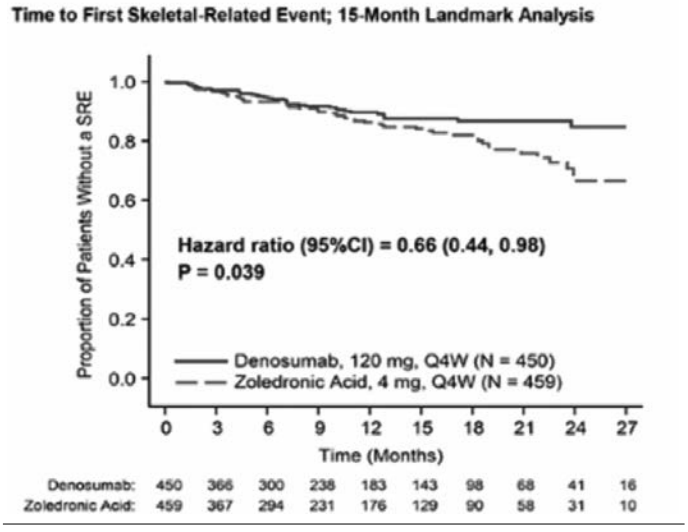

Figure 1.

Summary/Conclusions: DMB demonstrated non-inferiority to ZA in delaying time to first on-study SRE in myeloma pts, meeting the primary endpoint of the study. A landmark analysis at 15 m suggests a significant benefit for DMB with respect to time to first SRE. The rates of renal AEs were significantly lower in DMB pts while the overall rates of AEs, including hypocalcemia and ONJ, were consistent with the known DMB safety profile. The results of the landmark analysis and possible prolongation of PFS with DMB therapy is promising.

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PEMBROLIZUMAB PLUS LENALIDOMIDE AND LOW-DOSE DEXAMETHASONE FOR PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA: EFFICACY AND BIOMARKER RESULTS FROM THE PHASE 1 KEYNOTE-023 STUDY

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Background: Pembrolizumab (pembro) is a humanized, highly selective, high-affinity IgG4/κ antibody that blocks the interaction between programmed death 1 (PD-1) and its ligands PD-L1 and PD-L2, activating antitumor immunity. Pembro plus lenalidomide (len) and low-dose dexamethasone (dex) may provide synergistic antitumor activity in relapsed/refractory multiple myeloma (RRMM). Biomarkers indicative of response, pharmacodynamic activity, and/or mechanism of action to combination therapies are also needed.
Aims: To determine the maximum tolerated dose (MTD) and safety and tolerability of pembro plus len and low-dose dex in patients with RRMM. Additionally PD-L1 and PD-L2 expression in bone marrow (BM), immune profiles in circulating lymphocytes, and gene expression in blood were evaluated.
Methods: This open-label, phase 1 KEYNOTE-023 (NCT02036502) study of pembro plus len and low-dose dex enrolled patients with RRMM previously treated with ≥2 prior therapies, including both a proteasome inhibitor and an immunomodulatory drug. Patients received pembro 200 mg IV every 2 weeks (Q2W), len 25 mg orally on days 1-21, and dex 40 mg orally weekly on each 28-day cycle. Primary end points were safety and determination of the MTD. ORR was assessed by IMWG 2006. Exploratory biomarker analyses included analysis of PD-L1 and PD-L2 on $CD38^+CD138^+$ cells in BM aspirate samples obtained at screening, or before the first dose of study drug. Absolute and/or relative numbers of circulating lymphocytes (by flow cytometry [FC]) and gene expression profile (GEP) (by Nanostring) were evaluated in predose; cycle 1, day 1 (C1D1); and cycle 2, day 1 (C2D1) blood samples.
Results: MTD was determined as pembro 200 mg IV Q2W plus len 25 mg and dex 40 mg. Median (range) age was 61 years (46-77); median (range) number of prior lines of therapy was 4 (1-10); 38 (75%) patients were len-refractory, and 27 (53%) were double refractory. Most common grade ≥3 treatment-related AEs (TRAEs) were neutropenia (33%), thrombocytopenia (18%), and anemia (12%). 2 patients (4%) died because of TRAEs (hepatic failure, ischemic stroke). Immune-related AEs occurred in 5 (10%) patients. No pneumonitis was reported. ORR in the efficacy population was 20/40 (50%) (1 sCR, 5 VGPR, 14 PR); 1 patient had progressive disease. ORR was 38% (11/29) and 33% (6/18) for len- and double-refractory patients, respectively. The disease control rate (sCR+CR+ VGPR+PR+SD) was 39/40 (98%) in the efficacy population and 28/29 (97%) in the len-refractory population. 35/40 (88%) patients had a reduction in M protein or free light chains. In 16/32 patients with FC-evaluable BM aspirate with >100 $CD38^+CD138^+$ cells, all were $PD\text{-}L1^+$, while PD-L2 expression was variable. At C2D1, proportion of circulating $HLA\text{-}DR^+$, central ($CD45RO^+CCR7^-$), and effector memory ($CD45O^+CCR7^+$) $CD8^+$ T cells significantly increased and naive ($CD45RA^+$) $CD8^+$ T cells significantly decreased; all with multiplicity adjusted *P* values ≤0.01.
Summary/Conclusions: The combination of pembro, len, and low-dose dex has an acceptable safety profile and antitumor activity in patients with heavily pretreated RRMM, including len-refractory and double-refractory patients. PD-L1 was expressed in all patients evaluated by FC, whereas PD-L2 expression was variable. Pembro plus len and low-dose dex induced immune activation in the periphery and a phenotypic shift in effector $CD8^+$ T cells among the circulating T-cell pool in blood.

Old and new drugs in MPN

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RUXOLITINIB FOR THE TREATMENT OF INADEQUATELY CONTROLLED POLYCYTHEMIA VERA WITHOUT SPLENOMEGALY: 80-WEEK FOLLOW-UP FROM THE RESPONSE-2 TRIAL

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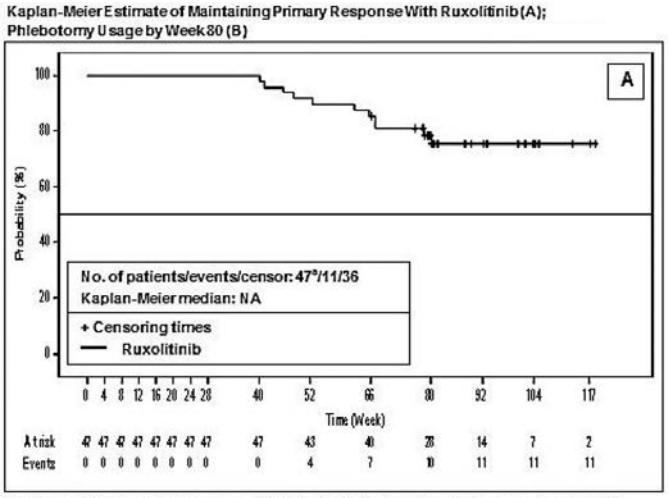
Background: Polycythemia vera (PV) is characterized by hyperproliferation of erythroid/myeloid/megakaryocytic components in the bone marrow, cardiovascular complications, and high symptom burden. Treatment (Tx) in patients (pts) with PV is focused on maintaining hematocrit (HCT) level <45%. RESPONSE-2 study evaluated the efficacy and safety of ruxolitinib (RUX) *vs* best available therapy (BAT) in hydroxyurea (HU)-resistant/intolerant pts with PV ≥18 years without splenomegaly and with phlebotomy [PBT] requirement to control HCT. At week (wk) 28 (primary analysis), HCT control was reported in 46/74 pts in the RUX arm *vs* 14/75 pts in the BAT arm.
Aims: This preplanned analysis of RESPONSE-2 evaluated the durability of efficacy and safety of RUX *vs* BAT, after all pts reached 80 wk into the study or discontinued the study.
Methods: Pts were randomized 1:1 to RUX 10 mg twice daily or BAT. Primary end point was the proportion of pts who achieved HCT control at wk 28 (absence of PBT eligibility [HCT >45%, ie, ≥3 percentage points from baseline, or HCT >48%] from wk 8 to 28, with ≤1 PBT eligibility from wk 0 to 8). Key secondary end point was the proportion of pts who achieved complete hematologic remission at wk 28 (CHR: HCT <45%, WBC ≤10 x 10^9/L, platelet count ≤400 x 10^9/L). Durability of HCT, CHR, and safety was evaluated at wk 80 (data cutoff, September 26, 2016). Additional end points included assessment of patient-reported outcomes (MPN-SAF TSS) and change in *JAK2V617F* allele burden over time. BAT pts could cross over to RUX from wk 28.
Results: Baseline demographics were comparable among RUX (N=74) and BAT (N=75) arms. When last pt reached wk 80 time point, 69 pts randomized to RUX were still receiving Tx; while 5 pts discontinued Tx (adverse events [AEs]=3 pts, physician's decision/pt withdrew consent=1 pt, each). In BAT arm, 58 pts crossed over to RUX (crossover data to be included in presentation) with remaining pts either ongoing follow-up (f/u [n=5]) or having discontinued Tx (completed f/u per protocol, n=7; death, n=1; other reasons, n=4). Median exposure was 93.6 wk in RUX *vs* 28.4 wk in the BAT arm. At wk 80, durable HCT control was achieved in 35 pts (47%) in RUX *vs* 2 pts (3%) in BAT arm. Of those who achieved a HCT response at wk 28, Kaplan-Meier estimate of maintaining response up to wk 80 was 78.37% in the RUX arm. Durable CHR was achieved in 18 pts (24%) in RUX *vs* 2 pts (3%) in the BAT arm. Total number of PBT was higher in the BAT arm *vs* RUX arm (Figure 1). At wk 80, 45% of pts randomized to RUX continued to achieve a ≥50% of reduction in the MPN-SAF TSS. At wk 80, mean percentage change from baseline in *JAK2V617F* allele burden was −9.7% in the RUX (n=65) *vs* +0.3% in the BAT arm (n=3). AEs observed were consistent with those generally reported with RUX (primarily low grade [G]). Most common AEs (all G, exposure-adjusted rate per 100 pt-years) were anemia (14.3), weight increase (10.6), arthralgia (9.1), and pruritus (9.1) in the RUX arm *vs* pruritus (37.5), headache (16.9), and thrombocytopenia (15.0) in the BAT arm. Rate of thromboembolic events (Standardized MedDRA Query, exposure-adjusted) was RUX (1.5) *vs* BAT arm (1.9). No pt in the RUX arm had disease progression *vs* 2 pts in the BAT arm. No deaths were reported in the RUX arm *vs* 3 pts in the BAT arm (septic shock/disease progression/study indication=1 pt, each).
Summary/Conclusions: RUX provided durable HCT control, durable CHR, reduction in PBT requirement, improved symptom burden, and was generally well tolerated with >90% of pts still receiving Tx at wk 80. RUX Tx provided a modest reduction in allele burden over time. Findings from both RESPONSE studies suggest that RUX should be considered as a standard of care for second-line Tx in this inadequately controlled pt population with PV.



[a]During week 80 data review, 1 more ruxolitinib-treated patient was found to be responding at week 28 (compared to original analysis done with week 28 data cut); updating the number of primary responders to 47.

Phlebotomy usage by week 80 (B)		
Phlebotomy frequency	Ruxolitinib, N=74, n (%)	BAT, N=75, n (%)
0	54 (72.9)	27 (36.0)
1-2	15 (20.3)	29 (38.7)
3-4	5 (6.8)	16 (21.3)
>4	0 (0)	3 (4.0)
Total number of phlebotomies	36	106

Figure 1.

S785

PHASE 3 RANDOMIZED TRIAL OF MOMELOTINIB *VERSUS* RUXOLITINIB IN JAK INHIBITOR NAIVE PATIENTS WITH MYELOFIBROSIS: RESULTS OF THE SIMPLIFY-1 STUDY

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Background: Momelotinib (MMB), an investigational oral JAK inhibitor (JAKi), has been shown in early trials to reduce spleen volume, improve disease associated symptoms and improve red blood cell (RBC) transfusion requirements in patients with myelofibrosis (MF).
Aims: To test the non-inferiority of MMB *vs* ruxolitinib (RUX) in splenic volume reduction and symptom amelioration, and superiority in transfusion requirement, in JAKi naïve patients with primary myelofibrosis, and post-polycythemia vera or post-essential thrombocythemia myelofibrosis.
Methods: Eligibility included primary myelofibrosis or post-polycythemia vera/essential thrombocythemia myelofibrosis; International Prognostic Scoring System (IPSS) high risk, intermediate-2 risk, or intermediate-1 risk associated with symptomatic splenomegaly; palpable spleen ≥5cm; platelets ≥50 K/µl; no Grade ≥2 peripheral neuropathy. Informed consent was obtained. Stratification was by transfusion dependency and platelets (<100K, 100K-200K, and >200K/µl). Patients were randomized 1:1 to 24 weeks of MMB 200 mg qd+RUX placebo or RUX 20 mg bid (or modified per label)+MMB placebo, after which all patients could receive open label MMB. Assessments: spleen volume by MRI, and patient reported symptoms using a daily eDiary of modified MPN-SAF Total Symptom Score (TSS). The primary endpoint was splenic response rate (SRR; ≥35% reduction in volume from baseline) at 24 weeks. Secondary endpoints, evaluated sequentially at 24 weeks, were rates of TSS response (≥50% reduction from baseline), RBC transfusion independence and RBC transfusion dependence, and rate of RBC transfusion.
Results: 175 of 215 (81%) and 201 of 217 (93%) patients randomized to MMB and RUX, respectively, completed the 24 week double blind phase. Efficacy results are shown in the Table 1. The most common Grade ≥3 adverse events in the double blind phase with MMB were thrombocytopenia (7%) and anemia (6%), and with RUX were anemia (23%), thrombocytopenia (5%) and neutropenia (5%). Grade ≥3 infections occurred in 7% of MMB and 3% of RUX patients. Treatment emergent peripheral neuropathy occurred in 22 (10%) of MMB (all Grade ≤2) and 10 (5%) of RUX (9 Grade ≤2, 1 Grade 3) patients in the double blind phase, none discontinuing study drug for this problem. Overall, adverse events led to study drug discontinuation in 13% of MMB and 6% of RUX patients in double blind phase.

Table 1.

Endpoints	MMB	RUX	*p*-value
Spleen response rate, %	26.5	29.0	0.011[a]
TSS response rate, %	28.4	42.2	0.98[a]
Transfusion independence rate, %	66.5	49.3	<0.001[b]
Transfusion dependence rate, %	30.2	40.1	0.019[b]
Transfusion rate (units/month), median	0.0	0.4	<0.001[b]

[a]*Test for non-inferiority;* [b]*Test for superiority, all values nominally significant.*

Summary/Conclusions: In patients with JAKi naive myelofibrosis, 24 weeks of momelotinib is non-inferior to ruxolitinib for spleen response but not for symptom response. Momelotinib treatment is associated with a reduced transfusion requirement. NCT01969838

S786

PHASE 3 RANDOMIZED TRIAL OF MOMELOTINIB *VERSUS* BEST AVAILABLE THERAPY IN PATIENTS WITH MYELOFIBROSIS PREVIOUSLY TREATED WITH RUXOLITINIB: RESULTS OF THE SIMPLIFY-2 STUDY

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Background: Momelotinib (MMB), an investigational oral JAK inhibitor, has been shown in early trials to reduce spleen volume, improve disease associated symptoms and improve red blood cell (RBC) transfusion requirements in patients with myelofibrosis.
Aims: Test the superiority of MMB *versus* best available therapy (BAT) in splenic volume reduction, symptom amelioration, and transfusion requirement at 24 weeks in patients with primary myelofibrosis (PMF), post-polycythemia vera or post-essential thrombocythemia myelofibrosis (Post-PV/ET MF) who were previously treated with ruxolitinib.
Methods: Eligibility included PMF or post-PV/ET MF; Dynamic International Prognostic Scoring System (DIPSS) high risk or intermediate-2 risk, or intermediate-1 risk associated with symptomatic splenomegaly; currently or previously treated with ruxolitinib for at least 28 days who either required transfusions or dose reduction to <20 mg BID with at least one of Grade ≥3 thrombocytopenia, anemia, or bleed; palpable spleen ≥5cm; and no Grade ≥2 peripheral neuropathy. Informed consent was obtained. Stratification was by transfusion dependency and baseline TSS (modified MPN-SAF Total Symptom Score) <18 or ≥18. Patients were randomized 2:1 to 24 weeks of open-label MMB 200 mg QD or BAT. Assessments included spleen volume by MRI, and patient-reported symptoms using a daily eDiary for TSS. Primary endpoint was splenic response rate at 24 weeks (SRR24; ≥35% reduction in volume from baseline). Secondary endpoints, evaluated sequentially, were rates of TSS response (TSS RR; ≥50% reduction from baseline), RBC transfusion, RBC transfusion independence (TI) and RBC transfusion dependence (TD).
Results: 73 of 104 (70%) and 40 of 52 (77%) patients receiving MMB or BAT, respectively, completed the 24 week randomized treatment phase. BAT for 88% of patients included ruxolitinib, and 27% of patients were on ruxolitinib in combination with other drugs. Efficacy results are in Table 1. The most common treatment-emergent adverse events in MMB patients were diarrhea (33%), asthenia (19%), nausea (19%), and cough (17%), and in BAT patients, asthenia (21%), fatigue (19%), anemia (15%), diarrhea (15%), and abdominal pain (15%); the most common Grade ≥3 adverse events in MMB patients were anemia (13%) and thrombocytopenia (7%), and in BAT patients, anemia (13%), thrombocytopenia (6%) and abdominal pain (6%). Treatment emergent peripheral neuropathy occurred in 11 (11%) of MMB (1 Grade 3) and in no BAT patients; MMB was discontinued in 3 patients due to neuropathy.

Table 1.

Endpoints	MMB	BAT	*p*-value
SRR, %	6.7	5.8	0.90
TSS RR, %	26.2	5.9	<0.001[a]
Transfusion rate (units/month), median	0.5	1.2	0.39
TI rate, %	43.3	21.2	0.001[a]
TD rate, %	50.0	63.5	0.10

[a]*p-values nominally significant.*

Summary/Conclusions: In previously ruxolitinib-treated patients with myelofibrosis, 24 weeks of momelotinib was not superior to best available therapy for splenic response, but significantly better in improving disease related symptoms and transfusion independence. *NCT02101268.*

S787

MOLECULAR RESPONSE TO HYDROXYUREA AND ROPEGINTERFERON ALFA-2B IN THE PROUD-PV RANDOMIZED PHASE 3 TRIAL

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Background: Interferon alfa (IFNa) has been successfully used to treat myeloproliferative neoplasms (MPN) for many years and several phase 2 studies have independently shown high rates of hematological and molecular responses assessed by the quantification of mutant JAK2 allele burden (%JAK2V617F) in peripheral blood. However, direct *in vivo* studies investigating the impact of IFNa treatment on proliferation of bone marrow (BM) normal and malignant hematopoietic progenitors are lacking.
Aims: We took advantage of the randomized controlled phase III trial (PROUD-PV) comparing the novel, long-acting Ropeginterferon alfa-2b (AOP2014) with hydroxyurea (HU) in polycythemia vera (PV) patients (pts) to assess correlation between evolution of %JAK2V617F in peripheral blood and the impact of therapy on malignant clones by functional assays testing mutant BM hematopoietic progenitors in the French study population.
Methods: Randomized, controlled, multicenter phase 3 trial comparing efficacy, safety and tolerability of hydroxyurea and Ropeginterferon Alfa-2b in PV pts (NCT01949805). The primary endpoint was non-inferiority of AOP2014 *vs* HU at 12 months (mos) of therapy in terms of complete hematological response (CHR) according to ELN criteria and normal spleen size. As important secondary endpoint the effect of treatment on %JAK2V617F was assessed as rate of complete and partial molecular response (C/PMR) according to modified ELN criteria. In the group of pts enrolled in France, we could study BM progenitors clonogenic potential by cultures with or without Erythropoietin (EPO) at baseline and after 12 months of therapy. The presence of colonies without EPO, namely Endogenous Erythroid Colonies (EECs) is a hallmark of PV. After 14 days, erythroid colonies were enumerated and picked for molecular analyses.
Results: A total of 257 pts were randomized in 13 European countries including 13 pts in France. Non-inferiority of AOP2014 *versus* HU regarding CHR could be demonstrated in the whole study population (43.1 *vs* 45.6%). In the subgroup of French pts (54% males, mean age 55 years) CHR at 12 mos was 40% in pts receiving AOP2014 (n=5) and and 50%, in those receiving HU (n=8). %JAK2V617F at baseline in the AOP2014 and HU arms were 39.4% and 46.5%, respectively, reduced to 29.1% and 25.8% after 6 mos, and 13.8% and 33.2% at 12 mos. No complete MR was achieved at 12 mos, but PMR was observed in 40% and 25% of pts in AOP2014 and HU arms (p= ns), respectively. BM progenitors could be studied in 10/13 French pts, 3 treated with AOP2014 and 7 with HU. AOP2014 treatment induced an important decrease of the proportion of EEC (median decrease 64%) between samples collected at baseline and after 12 months of therapy compared to HU (median decrease 25%). In addition, clonal architecture studies showed that the % of JAK2V617F mutant colonies before and after treatment profoundly decreased in all AOP2014-treated pts (mean ratio of mutant *vs* wild type JAK2 colonies decreased from 96% at baseline to 46% at 12 mos). Among HU-treated pts, only 1 experienced a decrease in the % of mutated colonies while mean ratio of mutant *vs* wild type JAK2 colonies didn't significantly decrease (from 87% at baseline to 79% after 12 mos).
Summary/Conclusions: In this phase 3 trial comparing Ropeginterferon alfa-2b *versus* HU, we found a different impact of both drugs on hematopoietic cells. Although both treatment induced a decrease of JAK2 mutant allele burden at 12 mos in peripheral blood, BM clonogenic assays suggest that AOP2014 is able to specifically target JAK2 mutant progenitors, an effect not seen in HU treated pts. Such targeted impact of AOP2014 may account for the strikingly different kinetics in allele burden reduction and suggests that sustained long-term molecular response may only be achieved with IFNa based treatment.

S788

POOLED SURVIVAL ANALYSIS OF MIDOSTAURIN CLINICAL STUDY DATA (D2201+A2213) IN PATIENTS WITH ADVANCED SYSTEMIC MASTOCYTOSIS COMPARED WITH HISTORICAL CONTROLS

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Background: AdvSM (ie, aggressive SM [ASM], SM with an associated hematologic neoplasm [SM-AHN], and mast cell leukemia [MCL]) comprises rare hematologic neoplasms with a poor prognosis. KIT D816V mutations occur in a majority of patients with advSM. Midostaurin is a multitargeted kinase inhibitor that blocks wild-type and D816V-mutated KIT. Two single-arm phase 2 studies (D2201+A2213) evaluated the safety and efficacy of midostaurin in advSM. Overall, 60% and 69% of patients in D2201 and A2213, respectively, achieved the primary endpoint of partial or complete normalization of SM-related organ damage.
Aims: We compared pooled data from these studies with data from a patient registry to determine the effects of midostaurin on overall survival (OS).
Methods: Data from the midostaurin studies, in which patients received midostaurin 100 mg twice daily until progression or toxicity, were pooled. Historical control data were obtained from a contemporary patient registry based at University Medical Centre Mannheim, Germany. Although the primary analysis did not include matching for patient selection, subgroup analyses, and multivariate analyses were performed to assess whether baseline patient characteristics affected OS and estimated HR. Propensity scoring was used for supportive analyses to match the patients in the registry. Patients were evaluated for OS based on time from diagnosis to death; patients in the pooled analysis with known dates of diagnosis were included in the primary analysis. A sensitivity analysis to compensate for potential bias in patient selection was conducted using the start date of last treatment to death.
Results: The primary analysis of OS in patients with advSM included 89 patients from the midostaurin pooled analysis for whom the date of diagnosis was available (77% of the entire pooled cohort) and all 46 patients from the German registry who had not been treated with midostaurin. SM subtypes among patients from the pooled analysis and registry were similar; 66% of patients in the pooled cohort and 63% in the registry had an AHN (Table 1).*KIT* D816 mutations were present in 82% of patients in the pooled analysis and 96% in the registry. More patients in the registry (67%) *vs* the pooled analysis (42%) were aged >65 y. Median follow-up (time from diagnosis to data cutoff for the analyses) was similar for the 2 patient groups: registry, 54.9 (range, 1.9-150.4) mo and midostaurin, 53.6 (range, 31.6-215) mo. Patients in the midostaurin pooled analysis had a clinically relevant improvement in OS *vs* historical controls (HR=0.62 [95% CI, 0.39-0.98]; P=.0204; Figure 1). Median OS was 42.6 (95% CI, 31.0-53.9) mo in the pooled analysis *vs* 24.0 (95% CI, 13.0-39.5) mo in the registry. Multivariate Cox regression analysis after adjusting for covariates was consistent with the primary analysis: HR=0.51 (95% CI, 0.30-0.88); P=.0147. Data using propensity score for matched pairs (n=44) were also consistent (HR=0.381 [95% CI, 0.169-0.960]; P=.101). Subgroup analyses of OS showed HR in favor of midostaurin for all subgroups analyzed (age [≤65 y *vs* >65 y], *KIT* D816V status, number of prior therapies [≤1 *vs* >1], and SM subtype) except MCL. Subgroup analysis data should be interpreted with caution due to the small patient numbers in the German registry. Sensitivity analysis of OS from date last treatment received (pooled analysis, n=115; registry, n=42) was consistent with the main analyses (HR from the multivariate analysis=0.38 [95% CI, 0.22-0.65]; P=.0004).

Table 1.

Patient characteristics

	Pooled Analysis: Midostaurin-Treated Patients (N=89)	Registry (N=46)
Age at diagnosis, n (%)		
≤65 y	52 (58)	15 (33)
>65 y	37 (42)	31 (67)
KIT D816 mutation status, n (%)		
Not mutated	15 (17)	2 (4)
Mutated	73 (82)[a]	44 (96)[b]
Unknown	1 (1)	0
SM subtype, n (%)		
ASM	16 (18)	12 (26)
SM-AHN	59 (66)	29 (63)
MCL	14 (16)	5 (11)
Median time from diagnosis to start of last therapy (interquartile range), mo	2.2 (0.5-7.8)	5.5 (0.8-21.0)
Median no. of prior therapies (range)	2 (1-5)	2 (0-5)
No. of prior therapies, n (%)		
0	0	4 (9)
1-2	68 (76)	27 (59)
≥3	21 (24)	15 (33)

[a] Includes 67 patients with D816V mutations, 1 with D816Y, and 5 with missing/undetermined D816 mutations.
[b] All 44 patients had D816V mutations.

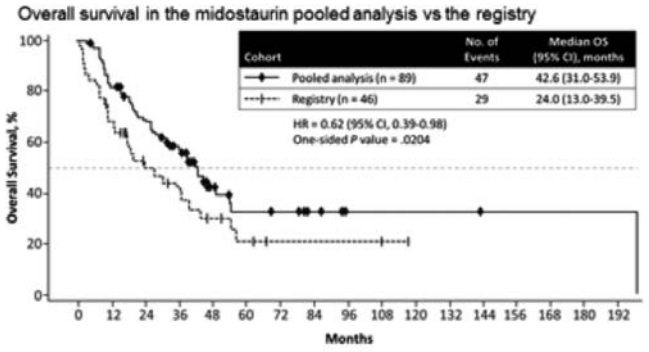

Figure 1.

Summary/Conclusions: Midostaurin was associated with a 38% lower risk of death *vs* historical controls. Benefit was generally consistent across key subgroups.

Childhood and more intensive treatment of AML

S789

LOW-DOSE CYTARABINE TREATMENT IN CHILDREN WITH DOWN SYNDROME AND TRANSIENT MYELOPROLIFERATIVE DISORDER TO PREVENT ML-DS: AML-BFM TMD PREVENTION 2007 STUDY

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Background: Approximately 10% of the children with Down syndrome are diagnosed with transient myeloproliferative disorder (TMD) within the first days of life. Previous studies have shown that TMD patients face an around 20% risk of early death and a 20% to 30% risk to develop myeloid leukemia during the first 4 years of life (ML-DS).
Aims: The aim of the AML-BFM TMD Prevention 2007 trial was to analyze the outcome of patients diagnosed with TMD and to evaluate whether the application of a low-dose cytarabin treatment can prevent the progression to ML-DS.
Methods: The AML-BFM TMD Prevention 2007 trial is a multi-center, non-randomized, historically controlled study. Patients with TMD were prospectively enrolled. They received a low-dose cytarabin treatment (1.5 mg/kg i.v./s.c. daily) for one week respectively if they met the following criteria: TMD-related symptoms (*e.g.* hyperleucocytosis, hepatopathy) at diagnosis, MRD-positivity (FACS≥10^{-3} or qPCR≥10^{-4}) eight weeks after diagnosis. Patients could receive cytarabin-treatment up to three weeks in case of failure to respond to the cytarabin-treatment (morphologic detection of blasts between week four and eight after diagnosis and/or MRD-positivity after treatment in week ten after diagnosis).
Results: Here we report a cohort of 108 patients (male: 60, female:48) diagnosed with TMD. The median age at diagnosis was 4 days. As common in children with Down syndrome, many of the patients presented with comorbidities (cardiac defects: 68%, other malformations: 15%); 36% were delivered preterm. 45 patients received low-dose cytarabin treatment, 57 patients did not receive this treatment. Overall, patients in this trial do not show a significantly better event-free survival (EFS; 72±4% *vs* 63±4%, p=0.15) and overall survival (OS; 91±3% *vs* 85±3%, p=0.15) than the historic control group (n=146). The cumulative incidence (CI) of death was lower, (8±3% *vs* 15±3%) albeit not significantly (p=0.09). The CI of ML-DS was also similar (19±4% *vs* 22±4%, p=0.88). Patients that presented with TMD-related clinical symptoms (n=43; symptoms: hyperleucocytosis [WBC>100,000], hepatopathy, ascites, hydrops fetalis) had a tendency for a better EFS (59±8% *vs* 44±8%, p=0.097), OS (80±6% *vs* 67±7% p=0.10) and CI of death (20±7% *vs* 33±7%, p=0.10) than patients with those symptoms in the historic control group (n=45). For the progression to ML-DS there is no significant difference between the two groups (21±7% *vs* 23%±7%, p=0.91). For patients that do not show any of the TMD-related symptoms (n=59), no significant differences were observed regarding EFS (81±5% *vs* 71±5%, p=0.27), OS (98% *vs* 93±3%, p=0.16) and CI of ML-DS (19±6% *vs* 22±4%, p=0.95) compared to patients without these symptoms in the historic control (n=101).
Summary/Conclusions: The consequent treatment with low-dose cytarabine of symptomatic patients results in a trend towards reduced CI of death as compared to the historic control. However, progression to ML-DS remained unchanged suggesting that the treatment with low-dose chemotherapy does not seem to prevent the development of subsequent leukemia in TMD-patients. Therefore, a general preventive chemotherapeutic treatment of children diagnosed with TMD cannot be recommended. However, children with TMD-related symptoms should receive low-dose cytarabine to reduce disease-related mortality.

S790

FINAL RESULTS OF THE CETLAM LAM-2003 TRIAL FOR THE TREATMENT OF PRIMARY AML UP TO THE AGE OF 70

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Background: AML is a heterogeneous disease based on genetic characteristics with impact on prognosis. So, it becomes necessary to treat patients according to risk-adapted therapies.
Aims: To analyze the results of intensive induction and post-remission treatment in 868 patients with the novo AML enrolled into the CETLAM-03 trial between 2003 and 2012 with a prolonged follow-up (results reported at 10 years).
Methods: Patients received 1 or 2 induction chemotherapy courses of IDICE-G (idarubicin, intermediate cytarabine (IDC), VP-16 and priming with G-CSF) followed by mitoxantrone and IDC as consolidation therapy. Further treatment was assigned according to the CETLAM risk groups as follows: Favorable risk (FR) defined as favorable cytogenetics according to MRC: autologous stem cell transplantation (ASCT) if leukocyte index [LI=leucocytes x (BM blasts/100)] ≥20 or high dose cytarabine (HDAC) (one course) if LI <20. Intermediate risk (IR), defined as patients in CR after a single induction course, <50x10E9/l white blood cells at diagnosis, normal karyotype and absence of FLT3 internal tandem duplication (FIT3-ITDwt) and no MLL rearrangement: ASCT . Adverse risk (AR), patients not included in FP or IP: ASCT or allogeneic stem cell transplantation (allo-SCT) depending on donor availability (HLA-identical sibling or unrelated donor if high risk of relapse).
Results: There were enrolled 868 patients. Median age was 53 years-old (16-70). According to MRC cytogenetics, available in 802 patients, 99 belonged to the favorable (12%), 581 (73%) to the intermediate and 122 (15%) to the adverse groups. 66 patients with no metaphases. FLT3-ITD was present in 128 patients with normal karyotype (36%). Four patients died before treatment and 864 patients received induction therapy. 77% of patients achieved a CR (88% with a single course), 11% were refractory and 12% died during induction. CR rate was 92% in CBF leukemia, 91% in NPM1 mutation without FLT3-ITD, 77% in intermediate cytogenetic and no mutations, 74% if FLT3-ITD, 70% in adverse cytogenetics and 62% if monosomal karyotype was present (p<0.001). The multivariate analysis showed that mutational status (adverse cytogenetics, FLT3-ITD and absence of NPM1 mutation) had an adverse impact on CR achievement. Overall survival (OS), event free survival (EFS) and cumulative incidence of relapse (CIR) of the whole series at 10 years were: 36±2%, 29±2% and 44±5% respectively. Post-remission results of OS, EFS and CIR according to the different CETLAM risk groups at 10 years follow up were: FR (n=85, 14%): 85±4%, 70±6% and 22±1%; IR (n=99, 17%): 64±6%, 51±5% and 47±2%; AR (n=417, 69%): 41±3%, 33±3% and 52±16% respectively. In FR there were no differences in OS, EFS and CIR depending if intention to treat was HDAC or ASCT. In AR statistical differences were observed at 10 years in EFS and CIR when comparing ASCT *vs* allo-SCT (27±4% *vs* 39±4%, p=0.026 and 66±6% *vs* 39±1%, p<0.001). In IR intention to treat was ASCT, but in 21% mobilization failed and most of them received HDAC. Forty-nine patients received an ASCT and 21 relapsed, 9 of them were rescued with an allo-SCT.
Summary/Conclusions: In this large cooperative experience CR rate was above 75%, in most cases after a single course. In patients with favorable MRC cytogenetics, the adverse impact of high LI observed in our previous protocol was abrogated with autologous transplantation. In IR group, a remarkable proportion of patients allocated to ASCT had mobilization failure. In HR group, allo-SCT improves the outcome compared to ASCT. In our experience, molecular characterization and MRD studies are helpful to decide post-remission therapy.

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MOLECULAR PREDICTORS OF RESPONSE TO AZACITIDINE THERAPY: THE RESULTS OF THE UK TRIALS ACCELERATION PROGRAMME RAVVA STUDY

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Background: Azacitidine (AZA) represents an important therapeutic advance in patients with acute myeloid leukaemia (AML) and high risk myelodysplasia (MDS) ineligible for intensive chemotherapy. However disease progression appears inevitable and a number of strategies aimed at improving outcome, including co-administration of histone deacetylase inhibitors such as vorinostat (VOR), have been proposed. Leukaemic stem/progenitor cells (LSC) have been postulated to represent a reservoir of resistant disease but the impact of AZA based therapy on LSC numbers has not been studied. An additional factor limiting the rational use of AZA based therapy in AML and MDS is imprecision in the identification of patients likely to achieve a significant clinical benefit and molecular predictors of outcome would improve the rational utilisation of thsi important new agent.
Aims: We wished to study the impact of AZA based therapy on LSC numbers aswell as identify molecular predictors of outcome in patients treated on the recently completed UK Trials Acceleration Programme RAVVA randomised Phase II trial which compared AZA monotherapy with AZA/VOR combination therapy.
Methods: The RAVVA trial randomized 259 adults with AML (n=217) and MDS (n=42) to receive AZA monotherapy (AZA (75 mg/m^2) x 7 days every 28 days) or AZA combined with VOR (300 mg bd days 3-9) po for a minimum of 6 cycles. Next generation sequencing was performed on 42 genes commonly mutated in AML and MDS in 250 patients treated on the RAVVA trial and correlated with response. Separately serial immunophenotypic quantitation of leukaemic stem/progenitor cells (LSC) was performed in 44 patients.
Results: Co-administration of VOR did not increase overall survival (OS) (1 year OS AZA 43% *versus* 41% p=0.32) as previously reported (Blood 2016 Absract No 1065). The mean number of mutations per patient in the 250 genotyped patients was 3.4. The presence of mutations in CDKN2A (p=0.0001), IDH1 (p=0.004) and TP53 (p=0.003), NPM1 (p=0.037) and FLT3-ITD (p=0.04) were associated with reduced OS in univariate analysis. In multivariate analysis adjusted for all clinical variables mutations in CDKN2A, IDH1 and TP53 remained predictive of decreased OS. No mutations were associated with improved OS. The presence of ASXL1 (p=0.035) and ETV6 (p=0.033) mutations were found to be associated with a reduced duration of response. AZA based therapy had no significant impact on LSC numbers in patients who failed to achieve a CR. LSC numbers were reduced but not eradicated in patients achieving a CR and observed to expand at relapse.
Summary/Conclusions: In this, the largest such study reported to date, the demonstration that mutations in CDKN2A, IDH1 and TP53 are associated with a decreased OS in patients treated with AZA not only can inform patient risk stratification but also provides insights into the mechanism of action of AZA. Specifically, the observation that mutations in the cell cycle regulator CDKN2A was associated with a markedly decreased overall survival is consistent with the hypothesis that induction of cell cycle arrest represents at least one of the mechanisms by which AZA exerts an anti-tumour activity. Furthermore our data identify serial quantitation of LSC populations as a potentially important biomarker of response to AZA based therapies which may assist in the evaluation of novel treatment combinations.

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SORAFENIB MAINTENANCE IN FLT3-ITD MUTATED ACUTE MYELOID LEUKEMIA AFTER ALLOGENEIC STEM CELL TRANSPLANT

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Background: The fms-like tyrosine kinase 3 internal tandem duplication (FLT3-ITD) mutation is a genetic alteration found in approximately 30% of patients with acute myeloid leukemia (AML). Although patients with FLT3-ITD AML achieve remission rates similar to those with FLT3 wildtype status with induction chemotherapy regimens; patients with FLT3-ITD have significantly shorter remission durations and increased rates of relapse. Even though allogeneic SCT improves outcomes, patients still have higher rates of relapse comparatively with poor prognosis post relapse. Sorafenib(SFB) is a TKI with activity against RAF, VEGF and FLT3-ITD and its use as maintenance therapy after allogeneic SCT has been shown as a promising approach to decrease relapse. Several studies report that SFB maintenance post SCT provides durable complete responses; however, there are also descriptions of sorafenib post SCT triggering acute GVHD, cytopenias, rash and diarrhea.
Aims: To assess the outcomes, including progression free survival (PFS) and overall survival (OS), in patients with FLT3-ITD mutated AML who receive SFB maintenance after allogeneic SCT.
Methods: We analyzed adult patients (age≥18) with a diagnosis of FLT3-ITD mutated AML who received an allogeneic SCT between 1/1/2010 and 10/28/16 at our institution. Using a case control analysis and matching patients who received maintenance SFB (maintenance group) with control patients, FLT3-ITD mutated AML who did not receive maintenance post SCT(control group); we matched each case to two control patients accounting for disease status, type of conditioning, donor type, cytogenetic risk factors and age. To be considered as maintenance, SFB had to be started within 101 days of the SCT. To reduce bias from disease risks and transplant-related mortality (TRM), all patients were required to be in complete remission (CR) at study entry - defined as the date of SFB initiation for cases and the same time point after SCT for their matched controls without maintenance. Actuarial OS and PFS were estimated from study entry using Kaplan-Meier method. OS and PFS were compared between cases and controls using log rank test and cox proportional hazards regression analysis. Patient-, transplant- and disease characteristics were compared between cases and controls using chi square and Fisher exact tests.
Results: Among the 214 AML patients with FLT3-ITD mutation that underwent SCT during study period, we identified 13 cases (maintenance) and 26 controls (no maintenance). Median follow-up of survivors were 12 months and 30 months for maintenance and control group respectively. Disease and transplant

characteristics were comparable between groups as presented in Figure 1. Patients were classified by the European Leukemia Net (ELN) classification and 23% in both groups were categorized as adverse risk while 77% were intermediate risk. All patients received myeloablative conditioning and diseases status at SCT was first/second complete remission (CR1/2) with or without count recovery (Cri/p) in 69% while it was active disease in 31%. PFS at 24 months post SCT was 82% in the maintenance and 45% in control group HR 0.3; 95% CI (0.1-1.3) p=0.1. Overall survival at 24 months was also higher in SFB cases as 100% compared with 60% in control group p=0.035. Only, 2 patients relapsed post SCT on SFB maintenance, one with new TP53 mutation at relapse, and other received only <30 days of SFB. However, more than half the patients had disease progression within the control period. The most commonly administered dose was 400 mg daily (5 patients) for 28 days cycle; only 2 patients tolerated higher doses and 6 patients received SBF as 300mg daily or less. There were delays in subsequent cycles in 10 of 12 patients, and the most common reasons for delays included cytopenias, liver function test abnormalities, and fatigue.

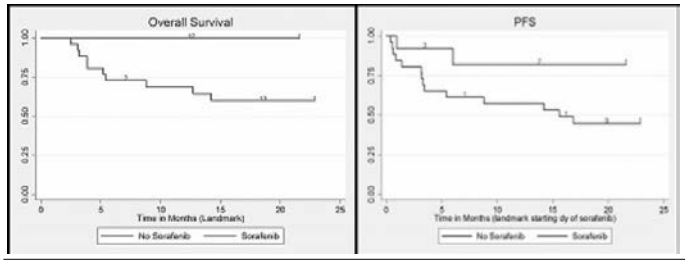

Figure 1.

Summary/Conclusions: Sorafenib maintenance is safe and can produce long term durable remissions after allogeneic stem cell transplant in a high risk population with FLT3-ITD mutated AML.

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A PHASE 1B STUDY OF THE COMBINATION OF VADASTUXIMAB TALIRINE AND 7+3 INDUCTION THERAPY FOR PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA

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Background: For patients <65 yrs with newly diagnosed AML, standard induction treatment is continuous infusion of cytarabine for 7 days and an anthracycline for 3 days (7+3). Although a high percentage of patients achieve a CR by morphologic criteria, some requiring a 2nd induction, many are resistant to treatment or achieve a morphologic CR with evidence of minimal residual disease (MRD). Vadastuximab talirine (SGN-CD33A; 33A) is a CD33-directed antibody conjugated to 2 molecules of a pyrrolobenzodiazepine dimer. Combining 33A with 7+3 could result in enhanced and deeper (MRD negative) remissions, resulting in reduced relapse rates and improved OS.
Aims: This phase 1b study (NCT02326584) evaluated the safety and antileukemic activity of escalating doses of 33A on 2 schedules: split dose (D1 and 4) or single dose (D1) with 7+3 induction therapy (cytarabine 100 mg/m^2 and daunorubicin 60 mg/m^2).
Methods: AML patients must be eligible for induction therapy. Response assessments occur on D15 and 28. Second induction and post-remission therapies were per investigator choice and did not include additional 33A. MRD was assessed centrally by bone marrow exam by a multiparametric flow at D15 and D28.
Results: *Split-dose cohort*: 42 patients (median age 45.5 yrs [range, 18-65]) were treated with 33A on D1 and 4 (10+10 [n=4] or 20+10 [n=38] mcg/kg) with 7+3. Most patients had intermediate (50%) or adverse (36%) cytogenetic risk. 19% had secondary AML. 2 patients had hematologic DLTs (lack of recovery of platelets [25K] and/or ANC [500] by D42) and 20+10 mcg/kg was determined to be MTD. The median time to count recovery from D1 of therapy in patients who achieved CR/CRi was 4.9 wks for ANC (≥1K) and 5.1 wks for platelets (≥100K). No non-hematologic TEAEs ≥G3 were reported in >10% of patients; non-hematologic TEAEs of any grade occurring in ≥25% of patients were nausea (62%), diarrhea, and constipation (38% each). Of the 42 efficacy evaluable (EE) patients, best responses included 25 CR (60%), 7 CRi (17%), and 5 morphologic leukemia-free state (mLFS; 12%) with a CR+CRi (CRc) rate of 76%; 23 of 25 (94%) responses were achieved in the 1st cycle. Of the patients with blast clearance (CR+CRi+mLFS), 73% (27/37) achieved MRD negative status. *Single-dose cohort*: To date, 25 patients (median age 58 yrs [range, 38-65]) were treated with 33A on D1 only (30 [n=14] or 40 [n=11] mcg/kg) with 7+3. Patients had intermediate (48%) or adverse (36%) cytogenetic risk. 16% had secondary AML. The median time to count recovery from D1 of therapy was 4.1 wks for ANC (≥1K) and 5.9 wks for platelets (≥100K) in patients who achieved CR/CRi. Four patients had hematologic DLTs, 1 at 30 and 3 at 40 mcg/kg. Non-hematologic TEAEs were consistent with those seen in the D1 and 4 schedule. Of the 24 EE patients, best responses included 12 CR (50%), 6 CRi (25%), and 3 mLFS (13%) with a CRc rate of 75%, achieved in 1st cycle. Of the evaluable patients with blast clearance, 89% (17/19) achieved MRD negative status. The CRc rate at the 40 mcg/kg dose level was 91% (10/11); all 11 patients had blast clearance and 90% (9/10) of evaluable patients achieved MRD negative status. Across schedules (N=67), the CRc rate was 76%; 79% (44/56) of evaluable patients with blast clearance achieved MRD negativity. The 30- and 60-day mortality rates were 1% and 7%, respectively. Median OS is not reached for either schedule and 52 patients (78%) were alive at the time of analysis.
Summary/Conclusions: 33A can be safely combined with 7+3 with acceptable count recovery in this population at the doses and schedules studied. Extramedullary AEs, including hepatotoxicity, and induction mortality rates were similar to reported rates for 7+3 alone in this AML population. A high remission rate with the 1st induction cycle was observed, the majority of which were MRD negative.

Stem cell transplantation - Clinical 2

S794

21-COLOR FLOW CYTOMETRY REVEALS IMMUNOPHENOTYPES ASSOCIATED WITH RESPONSE IN ACUTE GRAFT-*VERSUS*-HOST DISEASE PATIENTS TREATED WITH THE JANUS KINASE INHIBITOR INCB039110

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Background: Although ~50% aGVHD patients respond to steroids, no consensus second-line treatment exists. Recent preclinical models, retrospective studies, and this prospective trial have demonstrated safety and efficacy of JAK inhibitors (*e.g.* ruxolitinib, INCB039110) in steroid-refractory aGVHD.
Aims: Here, we present 21-marker FACS analysis of blood from patients enrolled in a prospective, randomized, parallel-cohort, open-label phase 1 trial of the potent and selective JAK1 inhibitor INCB039110 for aGVHD (NCT02614612). Preliminary results were previously presented at ASH 2016 (Schroeder et al).
Methods: Patients (n=30) were >18 years old undergoing first alloHSCT from any source with steroid-refractory or treatment-naïve grades IIB-IVD aGVHD, randomized 1:1 to 200 or 300 mg oral daily INCB039110 combined with corticosteroids. Peripheral blood, obtained at treatment days 7, 14, 28, 56, 100, and 180, was analyzed by 21-color FACS quantifying >30 cell types, including B, CD4+ and CD8+ T, memory T, T regulatory (Treg), Th1, Th2, Th17, T follicular helper (Tfh), Th9, Th22, ThGM-CSF cells, granulocytes, monocytes, monocyte-derived suppressor cells (MDSCs), natural killer cells (NKs), and monocytic and plasmacytoid dendritic cells (DCs). Patients were stratified by treatment response (*e.g.* complete response (CR), partial response (PR), mixed response (MR)).
Results: During INCB039110 treatment, overall B, T, and myeloid proportions did not correlate with response. However, the CR group showed increased NK cells (CD3-CD20-CD14-HLADR-CD56+), mDCs (CD3-CD20-CD14-HLADR+CD11c+), and memory CD4+ T cells (CD3+CD4+CD45RA-). Among CD4+ memory cells, the CR group showed significant or trend-toward-significant increases in Tfh (CCR10-CXCR5+), Th1 (CXCR5-CCR6-CCR10-CXCR3+), Th2, Th17 (CXCR5-CCR6+CCR4+CXCR3-CCR10-), ThGM-CSF (CXCR5-CCR6-CCR10+CXCR3-), and Th22 (CXCR5-CCR6+CCR4+CXCR3-CCR10+) cells. Tregs (CD4+CD25+CD127-) trended toward a ~2-fold increase in the CR group. Within the monocyte subgroup (CD3-CD20-CD14+), the CR group skewed toward classical monocytes (HLADR+CD16-) (64.7% *vs* 36.0%, CR *vs* PR/MR, p=0.0078) and away from MDSCs (HLADR-CD16-) (30.0% *vs* 58.4%, CR *vs* PR/MR, p=0.0139) during treatment. Interestingly, the NK-to-MDSC ratio was a sensitive and specific predictor of CR *vs* all other responses, a finding consistent for both CD16+ and CD16- NK cells (Figure 1 a, b). Before treatment, decreased naïve CD8+ T cells (CD45RA+CCR7+) predicted CR *versus* PR/MR (12.6% *vs* 32.3% of CD8+ cells, CR *vs* PR/MR, p=0.0047) with a similar trend toward decreased naïve CD4+ T cells (13% *vs* 24.4% of CD4+ cells, CR *vs* PR/MR, p=0.0749). While naïve T cells did not correlate with pre-treatment aGVHD grade, grades III-IV aGVHD demonstrated increased Th2 cells (CD45RA-CXCR5-CCR6-CCR10-CXCR3-) and activated CD8+ cells (CD38+HLADR+) as compared to grade II aGVHD. Further correlation with serum cytokines, JAK-STAT signaling, and pharmacology will be available at time of presentation.

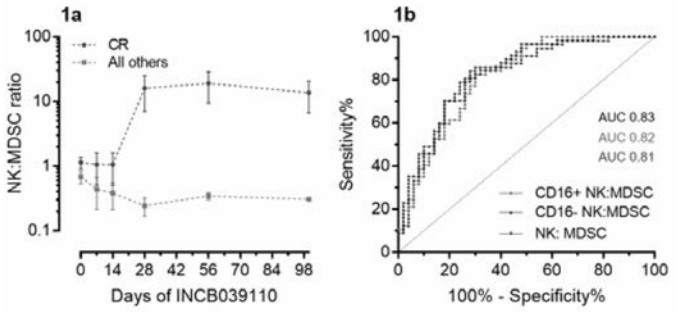

Figure 1.

Summary/Conclusions: Decreased pre-treatment naïve T cells may predict better outcomes in INCB039110-treated aGVHD. During treatment, increased DCs, NKs, and memory T cell subsets correlated with better response. Surprisingly, increased MDSCs associated with poorer response, suggesting MDSC expansion during persistent inflammation. The NK-to-MDSC ratio may be an important clinical marker to track treatment progress. Finally, this study establishes a novel FACS-based 21-marker immunophenotyping method with superior throughput, sample preservation, and flexibility as compared to cytometry time of flight (CyTOF) methods.

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GUT COLONIZATION BY MULTI-DRUG RESISTANT BACTERIA IS AN INDEPENDENT RISK FACTOR FOR DEVELOPMENT OF INTESTINAL ACUTE GRAFT-*VERSUS*-HOST DISEASE

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Background: Research has recently highlighted the importance of healthy gut microbiota in the prevention of graft-*versus*-host disease (GVHD). Gut decontamination and the use of broad-spectrum antibiotics have led to the loss of natural microbiota diversity and the overgrowth of opportunistic pathogens with emerging antimicrobial resistence. However, the role of multi-drug resistant (MDR) bacteria in development of GVHD needs to be elucidated.
Aims: Our aim was to evaluate the impact of gut colonization with MDR bacteria on the acute GVHD and related outcome.
Methods: Retrospectively we evaluated 145 adult patients who consecutively underwent allogeneic stem cell transplantation (allo-SCT) in our institution between 2011 and 2014. All patients were weekly screened by cultivating stool specimens for gut colonization by the following MDR bacteria: vancomycin-resistant *Enterococcus* (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant Gram-negative bacilli (MDR-GNB). Univariate and multivariable proportional hazards models using the Fine and Gray approach were considered to evaluate the variables for acute GVHD, treating death as competing event.
Results: Our study population included 88 male and 57 female patients who underwent allo-SCT at a median age of 46 years (range 18-64). Among them, most patiens were treated for myeloid malignancies (70%), while the rest had lymphoproliferative disorders and one patient had aplastic anemia. The donors were unrelated in 74 cases, related in 67 patients and haploidentical in 4 patients. Most of the patients (70%) received peripheral blood stem cells after a reduced-intensity conditioning regimen (56%). At the time of allo-SCT 37% patients were colonized with MDR bacteria, while another 19% became colonized in the early postransplantation period. Among colonized patients, 12% patients were colonized by VRE, 1% by MRSA, 43% by extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, 27% by carbapenem-resistant Enterobacteriaceae (CRE), 9% by MDR *Acinetobacter baumannii* and 58% by MDR *Pseudomonas aeruginosa*. 36% patients were colonized with more than one MDR pathogen. The cumulative incidence (CI) of severe (grade III-IV) acute GVHD was significantly higher in patients colonized with MDR-GNB (27%, 95% CI, 19-39%) than in non-colonized patients (14%, 95% CI, 7-23%) (p=0.04). Moreover, MDR-GNB colonized patients had significantly more gastrointestinal (GI) GVHD CI as opposed to non-colonized patients (28% (95% CI, 20-41%) *vs* 14% (95% CI, 7-23%), p=0.02) and more acute GVHD-related mortality (16%, (95%CI, 9-26%) *vs* 7% (95%CI, 3-15%), p=0.10). A substantial and independent role of gut colonization with MDR-GNB on the development of GI GVHD was confirmed by multivariate analysis using time-dependent covariate functions for high risk disease, myeloablative conditioning, peripheral blood stem cells, unrelated donor (hazard ratio 2.14; 95%CI, 0.99-4.68, P=0.05), older age (hazard ratio 2.15; 95%CI, 1.00-4.59, P=0.04) and MDR-GNB gut colonization (hazard ratio 2.26;95% CI, 1.05-4.83, P=0.03).
Summary/Conclusions: In summary, this report shows a significant role of MDR-GNB in the pathogenesis of severe acute GVHD. To our knowledge, we are the first to show that gut colonization with MDR-GNB represents an independent risk factor for GI GVHD. With growing resistance and lack of efficient antibiotics, decolonization strategies as fecal microbiota transplantation become an attractive strategy for restoration of healthy gut flora and prevention of severe acute GVHD.

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IMPACT OF HLA DISPARITY ON OUTCOME IN HLA-HAPLOIDENTICAL BONE MARROW TRANSPLANTATION FOLLOWED BY HIGH DOSE POST-TRANSPLANT CYCLOPHOSPHAMIDE

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Background: By definition “haplo-identical” donors share genotipically 4/8 anti-

gens with recipients. However, casual phenotypical homozygosity in the non-shared haplotype makes the real degree of disparity less than 4/8 in a few donor/recipient couples.
Aims: Since 2010, patients who lacked a HLA-identical donor have been transplanted from a haploidentical donor in our two Italian institutions. In this large series of patients we aim to verify the real degree of antigen disparity between donors and recipients and whether it impacts on transplantation outcome.
Methods: All haplotransplants performed in two Italian istitutions from August 2010 to July 2016 (n =318) were included. All patients received a myeloablative regimen (MA) followed by unmanipulated bone marrow and high dose post-transplant cyclophosphamide (PT-CY), combined with cyclosporine and mycophenolate. Donors and recipients were typed, until 2015, using DNA method (SSO and SBT) for HLA A, B, C, DRB1, DQ and DPB at a high resolution level, as defined by EFI standards and by NGS at allelic level in 2016 for the same loci. When applicable (72,3% of patients) members of the immediate family where typed to definitively establish HLA genotype and haplotype identity. Differences at loci A, B, C, DRB1 in both GvH and HvG direction were evaluated. We evaluated overall survival (OS) and non-relapse mortality (NRM) according to the amount of overall mismatches; also, we analyzed cumulative incidence of grade II – IV aGVHD, moderate-severe chronic GvHD and relapse (CIR) according to the degree of HLA mismatches in the GVH direction and graft rejection rate according to the degree of HLA mismatches in the HVG direction. For analysis purpose, the whole patient population was divided into 2 groups: 0-1-2 antigen mismatches *versus* 3-4 antigen mismatches. The same distinction was mantained when analyzing only GVH or HVG directed mismatches. Acute GvHD was calculated at day 100, the other parameters were calculated at the second year of follow up. OS was estimated using the Kaplan-Meier approach while cumulative incidence was calculated for aGvHD, cGvhD, relapse and NRM.
Results: Median age of patients was 48 years (17-74). Diagnoses included acute myeloid leukemia (130), acute lymphoblastic leukemia (64), lymphoid and plasma cell malignancies (43), myeloproliferative neoplasms (48) and myelodisplastic syndrome (33). 144 patients (45%) were transplanted in advanced phase of disease. With a median follow up of 562 days (range 6-2241 days), 2-year OS was 55,7%. Concerning the proportion of "true" haploidentical D/R pairs, 231 out of 318 (72%) couples showed 4/8 mismatches at HLA A, HLA B, HLA C and HLA DRB1 loci. Neither OS nor NRM showed significant correlation with the degree of overall mismatches at 2 years (0-2 mm: 54.2% *vs* 3-4 mm:58.8%, p=0.58 and 0-2 mm:18.2% *vs* 3-4 mm:19.1%, p=0.93, respectively). Considering only GVH directed mismatches, no difference was highlighted between low or high HLA mismatch burden in cumulative incidence of aGVHD (12.6% *vs* 23.9%, p=0.13), cGVHD at 1 year (12.2% *vs* 14.8%, p=0.84) and relapse (33.3% *vs* 24%, p=0.26). In this series graft rejection rate was 6.6%; no correlation was observed with the amount of HLA mismatch in the HVG direction.
Summary/Conclusions: In this series, about one third of haploidentical donor/recipient pairs differ for less than 4 /8 HLA antigens. Furthermore, in the setting of a MA conditioning with PT-CY the real degree of HLA mismatching observed had no impact on OS, NRM, CI of Relapse and acute and chronic GvHD.

S797

CYCLOPHOSPHAMIDE *VERSUS* ETOPOSIDE IN COMBINATION WITH TOTAL BODY IRRADIATION AS CONDITIONING FOR ADULTS WITH PH(-) ALL UNDERGOING ALLO-HCT. A STUDY FROM THE ACUTE LEUKEMIA WORKING PARTY OF THE EBMT

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Background: Allogeneic hematopoietic stem cell transplantation (alloHCT) is widely used for the treatment of adults with acute lymphoblastic leukemia (ALL). Although the choice of pre transplantation conditioning has never been a subject of a randomized trial, results of many retrospective studies suggested advantage of total body irradiation (TBI)-based over chemotherapy-based regimens. TBI is most frequently administered in combination with either cyclophosphamide (Cy/TBI) or etoposide (Vep/TBI).
Aims: The goal of this study was to retrospectively compare the outcome of alloHCT using Cy/TBI or Vep/TBI as conditioning.
Methods: Adult patients with Ph-negative ALL (n=1498) treated with alloHCT from either HLA-identical sibling (n=696) or unrelated donor (n=802), in CR1 (n=1186) or CR2 (n=312), between year 2000 – 2015, were included in the analysis. Peripheral blood was used as a source of stem cells in 62% of the patients, while bone marrow in 38% of the patients, respectively. Conditioning was myeloablative in all cases (the median TBI dose was 12Gy); 1346 patients were treated with Cy/TBI while 152 patients with Vep/TBI. Patients in the Vep/TBI group were younger (median 28 y. *vs* 30 y., p=0.04), treated in more recent period (median year of HCT: 2009 *vs* 2007, p=0.009) and treated more frequently in CR1 (87% *vs* 78%, p=0.001).
Results: In a univariate analysis, as compared to Cy/TBI, the use of Vep/TBI was associated with significantly reduced incidence of relapse (17% *vs* 30% at 5 years, p=0.007), increased rate of leukemia-free survival (LFS, 60% *vs* 50%, p=0.04) as well as improved "GVHD and relapse-free survival" (GRFS, 43% *vs* 33%, p=0.04). No significant effect could be observed in terms of the incidence of non-relapse mortality, acute or chronic GVHD. In a multivariate model the use of Vep/TBI was associated with reduced risk of relapse (HR=0.62, p=0.04) while the effect on other study end-points was no longer significant. Among other factors, recipient age (HR=1.17 per every 10 years, p=<0.0001), year of alloHCT (HR=0.97 per every year, p=0.001) and disease stage (HR=2.14 for CR2, p<0.0001) had significant influence on the risk of treatment failure, either relapse or non-relapse mortality. The risk of relapse was additionally increased for sibling *vs* unrelated donor transplants (HR=1.47, p=0.01) and donor/recipient gender combination other than female/male (HR=1.37, p=0.04).
Summary/Conclusions: Conditioning regimen based on etoposide combined with TBI appears more effective than the cyclophosphamide TBI combination for adult patients with Ph-negative ALL treated with alloHCT. Further, prospective studies are needed to confirm our observation and potentially discriminate subgroup of patients who are most likely to benefit from the use of etoposide.

S798

ALLOGENEIC STEM CELL TRANSPLANTATION FOR ACUTE MYELOID LEUKEMIA WITH DELETION 5Q OR MONOSOMY 5: A STUDY FROM THE ACUTE LEUKEMIA WORKING PARTY OF THE EBMT

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Background: High-risk acute myeloid leukemia (AML) is mainly defined by the presence of determined poor-risk cytogenetic abnormalities and is a standard indication for allogenetic stem cell transplantation (SCT). Nevertheless, high-risk AML is a very heterogeneous group including several abnormalities with different levels of prognostic impact. Deletion 5q or monosomy 5 (-5/5q-) has been part of the high-risk group of AML for many years. SCT seems to improve their outcomes but the additive effects of other high-risk cytogenetic features on survival have never been thoroughly studied.
Aims: To evaluate the role of SCT in -5/5q- AML with additional cytogenetic abnormalities such as complex karyotype (CK), monosomal karyotype (MK), monosomy 7 (-7), or 17p abnormalities (abn(17p)).
Methods: We included adult patients (pts) with AML with -5/5q- reported to the EBMT registry as having their first SCT between 2000 and 2015.
Results: Five hundred and one pts, 21% of them with secondary AML, have been allocated. Median age at SCT was 55 year-old (range, 18-75) and median follow-up was 21 months (range, 2-173). At time of SCT, 338 pts (67%) were in first remission (CR1), 21 pts (4%) were in subsequent remission and 142 (28%) had active disease. Two hundred seventy-seven pts (55%) were transplanted from an unrelated donor (UD) and 224 from a sibling donor. A myeloablative conditioning (MAC) was administered in 45% of the pts and a reduced-intensity conditioning (RIC) in 55% of them. The 2-year probabilities of overall survival (OS) and leukemia-free survival (LFS) were 27% and 20%, respectively. The 2-year probality of treatment-related mortality (TRM) was 20%. The cumulative incidence of grade II-IV acute graft-*versus*-host disease (GvHD) was 29% and the 2-year cumulative incidence of chronic GvHD was

27%. The main cause of death was disease-related. In multivariate analysis, active disease correlated strongly with worse OS, LFS and NRM. The other factors influencing outcomes were UD with increased NRM, and age with decreased OS and LFS.
Based on the frequencies of the different additional cytogenetic abnormalities, we identified 4 groups within our cohort. Group 1 (None) included 47 pts with -5/5q- but without CK, MK or abn(17p). Group 2 (CK) included pts with -5/5q- and CK but no MK or abn(17p) (N=90). Group 3 (MK) included 169 pts with -5/5q- and MK but no abn(17p). Finally, group 4 (17p) included pts with -5/5q- and abn(17p) (N=193). The 4 groups were quite similar in term of characteristics. The 2-year probablity of LFS was 39% for group 1, 25% for group 2, 20% for group 3 and only 13% for group 4 (p<0.001). OS decreased also significantly from group 1 to group 4 (p<0.001). NRM was similar across the groups. In multivariate analysis, factors associated with worse OS and LFS were active disease, age, MK and abn(17p). The corresponding 2-year probability of GvHD and relapse-free survival was 27% for group 1, 17% for group 2, 14% for group 3 and 7% for group 4 (Figure 1).

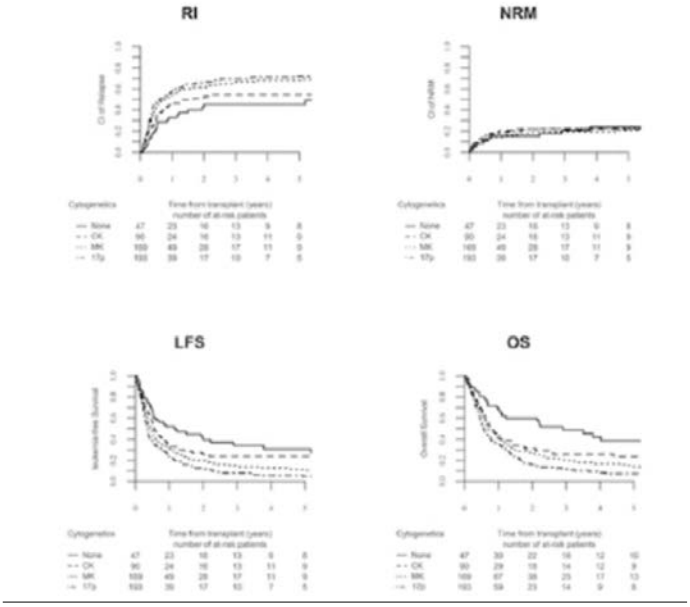

Figure 1.

Summary/Conclusions: SCT in -5/5q- AML provides a durable response for approximately 20% of pts. Active disease at time of transplantation was the most powerful predictor of an inferior outcome. The presence of -5/5q- without CK, MK or abn(17p) was associated with a significant better survival and the addition of MK or abn(17p) translated into worse outcomes. We confirmed the deleterious effect of the combination of -5/5q- and abn(17p) on SCT outcome. Future efforts should be focused on this subgroup in order to improve their outcome.

Biomarkers in ALL

S799

IDENTIFICATIONS OF NOVEL RECURRENT PU.1 FUSIONS WITH HIGHLY AGGRESSIVE PHENOTYPE IN PEDIATRIC T CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: T-cell acute lymphoblastic leukemia/lymphoma (T-ALL/LBL) accounts for 10% to 15% of newly diagnosed cases of childhood acute lymphoblastic leukemia (ALL), arising from the malignant transformation of hematopoietic progenitors primed toward T cell development, as result of a multistep oncogenic process. However, since the prognostic significance of these genetic alterations in pediatric T-ALL is not clear, genetic basis which contributes aggressive phenotype or progression of pediatric T-ALL is still to be elucidated.
Aims: To discover driver genetic events, which involved in the aggressive phenotype of pediatric T-ALL and to identify its novel prognostic markers, we performed integrated genetic analysis in a large cohort of T-ALL case.
Methods: Our cohorts included samples from Tokyo Children's Cancer Study Group (TCCSG) and Japan Association of Childhood Leukemia Study (JACLS). Whole transcriptome sequencing (WTS) was performed in 123 cases. Whole transcriptome sequencing (WTS) was performed in 123 cases.
Results: Representative recurrent fusion genes were as follows, *SIL-TAL1* (n=25), *MLL-ENL* (n=5), *PICALM-MLLT10* (n=5), and *NUP214-ABL1* (n=2). Intriguingly, novel recurrent in-frame *PU.1* fusions (*STMN1-PU.1* n=2; *TCF7-PU.1* n=5) were detected, and RT-PCR analysis in additional 60 cases revealed other 2 *TCF7-PU.1* fusions. Thus, *PU.1* fusions accounted for 4% of pediatric T-ALL/LBL. Expression data of WTS revealed cases with *PU.1* fusion showed significantly higher expression of PU.1 compared to cases without *PU.1* fusion, implicating that aberrant high expression of *PU.1* involved in leukemogenesis. Using consecutive two-step unsupervised consensus clustering, we obtained 5 stable clusters. Among these, 4 clusters largely recapitulated distinct T-ALL subtypes characterized in previous studies by an early T-cell precursor (ETP) signature (ETP-ALL), 2 clusters of high *TAL1* expression (TAL1-RA and -RB -ALL), and mutually exclusive expression of, *TLX1*, and *TLX*3 (TLX-related-ALL). However, the remaining one was newly identified and exclusively consisted of the 7 *PU.1* fusion-positive cases. Compared to ETP-ALL, these *PU.1* fusion cases typically showed a reduced expression of the phase I genes implicated in early T-cell development**,** except for *PU.1*, which was ectopically upregulated by the relevant gene fusions. All cases with *PU.1* fusion were grouped into *PU.1* high cluster. Moreover, *PU.1* high cluster had distinct genetic features with mutations of transcription factors, such as *GATA3*, *RUNX1*, and *EVT6.* Of note, significant poor outcome was confirmed by multivariate analysis in cases with *PU.1* high cluster (p=0.048). Consistently, we defined *PU.1* overexpression cases as outliers of *PU.1* expression, which resulting in extremely poor prognosis (3-year OS 21%, log-rank p=6.9 $\times 10^{-7}$).
Summary/Conclusions: *PU.1* fusions expressing cells expanded and they remained at an immature stage, implicating a potential leukemogenic activity of these fusions. Not only the cases with *PU.1* fusions, but also the cases with

high *PU.1* expression without fusions showed extremely poor prognosis, suggesting the prognostic value of aberrant *PU.1* expression in pediatric T-ALL. Although it remains unclear, why cases with *PU.1* fusions/high *PU.1* expression have a poor prognosis, our results indicate that these cases are genetically distinct subgroup from other pediatric T-ALL.

S800

PROGNOSTIC IMPACT OF ADDITIONAL MOLECULAR LESIONS IN PH+ ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: The outcome of Ph+ acute lymphoblastic leukemia (Ph+ ALL) has drastically improved since the introduction of tyrosine kinase inhibitors (TKI). At present however, well-defined prognostic markers, beyond the monitoring of minimal residual disease (MRD) during follow-up and to a lesser extent *IKZF1* deletions, are lacking.
Aims: To identify genomic lesions of prognostic value, we evaluated copy number aberrations (CNA) by SNP arrays, confirmed them by multiplex ligation-dependent probe amplification (MLPA) and we set up a droplet digital PCR (ddPCR) assays for additional lesions. Furthermore, we correlated the lesions identified with MRD monitoring, outcome and biological features, such as the type of fusion protein (p190 or p210). Finally, in a subset of patients gene expression profiling (GEP) was carried out.
Methods: Genomic DNA of 116 newly diagnosed adult Ph+ ALL patients enrolled in 4 consecutive GIMEMA trials, namely 0201B, 0904, 1205 and 1509, was evaluated. All the trials were based on an induction with steroids and TKI, the first 2 with imatinib and the remainders with dasatinib. For CNA, the Cytoscan HD Arrays (Affymetrix, Santa Clara, CA) were used. The lesions were confirmed by MLPA on all samples using the Salsa MLPA P335-A3 ALL-*IKZF1* kit (MRC-Holland, Amsterdam, The Netherlands). ddPCR was used to validate lesions targeting *MEF2C*. In 42 cases, GEP experiments were performed using the HGU133 Plus 2.0 gene chips (Affymetrix, Santa Clara, CA).
Results: We found a similar load and type of lesions across the 4 trials, one of which included elderly. The majority of lesions targeted *IKZF1* (84%), *PAX5* (36%) and *CDKN2A/B* (32%). In our cohort, *IKZF1* deletions alone did not affect complete molecular response (CMR) achievement or disease-free survival (DFS), while patients harboring *CDKN2A/B* and *PAX5* deletions had a significant inferior outcome (p=0.004, p=0.003 respectively). In line with this, a worse DFS was observed for the so-called "*IKZF1* plus" cases, *i.e.* concomitant deletions of *IKZF1* and *CDKN2A/B* and/or PAX5 (46% *vs* 24% at 36 months, p=0.005). MLPA confirmed the incidence of these deletions and allowed the study of *IKZF1* isoforms. Among *IKZF1* deleted cases, patients carrying the Δ4-7 isoform (25%) had a worse DFS (p=0.02) than patients harboring other *IKZF1* isoforms. Importantly, SNP arrays highlighted novel genomic lesions targeting *MEF2C* in 13% of cases, which were associated to the achievement of a CMR (p=0.05) and had a significant impact on DFS (62% *vs* 32% at 36 months, p=0.02). The association with CMR was not affected by the trial (p=0.76) or the TKI used (p=0.57). This result was confirmed by ddPCR. Unsupervised hierarchical clustering of GEP experiments identified 3 subgroups: the first comprised mainly patients who reached a CMR, the second one patients who had *IKZF1* alone, and the last one comprised "*IKZF1 plus"* patients. ANOVA analysis showed an overexpression of genes involved in cell communication and protein modification process in *PAX5* deleted cases, suggesting that these genes could be contributing factors in BCR/ABL1-driven leukemogenesis.
Summary/Conclusions: In adult Ph+ ALL, *IKZF1* deletions have a prognostic impact only if associated with other lesions. Among *IKZF1* deletions, only the Δ4-7 deletion has a deleterious effect. *MEF2C* lesions carry prognostic implications, being significantly associated with a better prognosis. This study paves the way to design a prognostic model for adult Ph+ ALL that includes these findings and more conventional features, in order to better stratify patients at diagnosis and to further optimize treatment.

S801

MULTI-CENTER VALIDATION OF STANDARDIZED NGS ASSAYS FOR REARRANGED IG / TR MARKER DETECTION IN ACUTE LYMPHOBLASTIC LEUKEMIA – A REPORT OF THE EUROCLONALITY-NGS CONSORTIUM

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Background: Amplicon-based next generation sequencing (NGS) of immunoglobulin (IG) and T-cell receptor (TR) gene rearrangements can be used to identify suitable markers for subsequent quantification of minimal residual disease (MRD) in acute lymphoblastic leukemia (ALL). Within the EuroClonality-NGS Consortium we established and validated a standardized quality controlled amplicon-based NGS application to detect clonally rearranged IGH, IGK, TRB, TRG and TRD genes in lymphoid disorders.
Aims: 1) to test EuroClonality-NGS IG / TR NGS assays within an international multi-laboratory pilot for their suitability to identify clonal markers in ALL at diagnosis, and 2) to compare these NGS results with conventional Sanger sequencing (SS) of Genescan or Heteroduplex peaks/bands local multiplex PCRs
Methods: Within the EuroClonality-NGS Consortium, V, D, and J gene-specific primers tailed with universal and T7-linker sequences, were designed to amplify complete and incomplete IGH, IGK, TRB, TRG or TRD gene rearrangements employing eight different multiplex PCR assays. PCR protocols were standardized in a common NGS workflow for all targets. NGS assays were tested in a European multi-laboratory pilot run in five institutes (Kiel, Bristol, Prague, Monza, Paris). Diagnostic DNA (100ng) from 10 ALL patients per centre were amplified in each institute using the EuroClonality NGS primer sets, and subsequently sequenced on the Illumina MiSeq (2×250bp v2 kit). Defined copy numbers of clonal reference DNAs were spiked into all samples for calculation of clonotype copy numbers. A standard polytarget quality control (QC) DNA was used to monitor inter- and intra-lab NGS reproducibility. Results of NGS based marker identification were compared to results of routine SS results.
Results: Fifty ALLs (29 BCP-ALL and 21 T-ALL) were analysed. A total of 480 libraries were deep sequenced, leading to 47 M high quality reads (≈ 9.2 M/lab). Pre-processing, identification and immunogenetic annotation of target sequences, quality control and copy number calculations, were performed with ARResT/Interrogate, using IMGT germline sequences – further analyses and verifications were performed with Vidjil and IMGT/V-QUEST. Overall, 339 clonal IG / TR sequences were identified, with a mean of 6.3 (2-13)/sample using NGS *vs* 5.0 (2-13)/sample using SS. A total of 228/339 (68%) clonal sequences were concordant between both methods. NGS identified additional clonal rearrangements in 88 (26%) libraries, whereas 23 (7%) clonal markers were only detected by SS. NGS primers covered possible IG /TR rearrangement types more completely compared to local multiplex PCR sets and enabled sequencing of bi-allelic rearrangements and weak PCR products. Currently discrepant cases are analysed in more detail using allele-specific PCR assays. Assay performance was analysed by standardized evaluation of QC samples and showed high intra- and inter-lab consistency without statistically significant differences between the labs.
Summary/Conclusions: The IG / TR NGS panel, as established by the EuroClonality-NGS Consortium, allows for quality controlled high-throughput detection of clonal IG /TR rearrangements in ALL. Compared to low throughput methods more clonal MRD markers are identified, sensitivity is increased, processing time is reduced and labour intensive conventional methods to resolve mixed sequences in case of bi-allelic rearrangements or background are avoided.

S802

POST-INDUCTION MRD PREDICTS HIGH RELAPSE RISK FOLLOWING REDUCED INTENSITY CONDITIONED ALLOGENEIC STEM CELL TRANSPLANTATION: A PROSPECTIVE STUDY OF ADULT ALL (UKALL14,ISRCTN 66541317)

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Background: Reduced intensity conditioned allogeneic haematopoietic stem cell transplant (RICalloHCT) enables HCT to be performed in older patients. The UK NCRI UKALL14 study of adult acute lymphoblastic leukaemia (ALL) considers patients ≥41 years "high risk" and recommends a RICalloHCT where there are high quality donors. Other "high risk" factors are high WBC at presentation, t(9;22), t(4,11), hypodiploidy/near triploidy, complex karyotype and positive minimal residual disease (MRD) after completing induction therapy. The presence of MRD at this time-point predicts poor outcome after conventional chemotherapy. There is evidence that myeloablative alloHCT can overcome this risk, but the benefit of RICalloHCT is uncertain.
Aims: To determine whether RICalloHCT mitigates the high relapse risk predicted by MRD positivity after induction therapy.
Methods: Protocol treatment: patients receive a steroid pre-phase before 2 cycles of induction chemotherapy. At the end of induction, patients are assigned subsequent therapy on the basis of risk. All patients over 41 years are allocated RICalloHCT, conditioned with fludarabine, melphalan and alemtuzumab. Post HCT, escalating doses of donor lymphocyte infusions were given for T-cell mixed chimerism +/- MRD persistence or relapse. MRD assessment: *BCR/ABL1* or Ig/TCR MRD was assessed and analysed per EuroMRD guidelines. MRD is negative (undetectable with an assay quantitative range of $1x10^{-4}$ or less), positive (≥$1x10^{-4}$), positive outside quantitative range (POQR)(<$1x10^{-4}$) or indeterminate (undetectable but assay quantitative range ≥$5x10^{-4}$). Patients with indeterminate MRD were excluded from this analysis.
Results: There are 736 patients randomised to date, of whom 184 received a RICalloHCT; of these, 115 had analysable MRD. The following Table 1 shows patient characteristics.

Table 1.

Patient characteristics	n=115	Disease characteristics	n=115
Age at randomisation median (range)	49 (30-65)	B-ALL	100 (87)
Presenting WBC median (range)	8.6 (0.1-557)	T-ALL	15 (13)
Sex N (%)		**High-risk cytogenetics** N (%)	
Male	61 (53)	*BCR/ABL1* N (%)	39 (33.9)
Female	54 (47)	t(4,11)	6 (5.2)
Donor type N (%)		Hypodiploidy/near triploidy	7 (6.1)
Sibling	40 (34.8)	Complex karyotype	3 (2.6)
Matched unrelated	75 (65.2)	**UKALL14 cytogenetic risk group** N (%)	
Post induction MRD N (%)		Standard	42 (36.5)
Negative/POQR	77 (67)	High risk	53 (46.1)
Positive	38 (33)	Unknown	20 (17.4)

At 2 years post transplant, overall survival (OS) was 63.1% in the 115 patients with evaluable MRD and 62.7% in the 184 patients receiving RICalloHCT; event free survival (EFS) was 55.2% and 55.9% respectively. By contrast, in the 38 of 115 patients with positive MRD after induction, OS and EFS were 40.6% and 28.4% respectively. Twenty eight of the 115 patients relapsed, with a 2 year actuarial relapse risk of 31.5% (22.2-43.5). We assessed the association of the following factors; age, sex, immunophenotype, presenting WBC, *BCR/ABL1*, other cytogenetics, post-induction MRD and donor type with the risk of relapse. Among this population of high risk patients, post-induction MRD was the only independent prognostic factor for relapse (univariable HR: 3.82 (1.59–9.16), p 0.001 (see Figure 1) and multivariable HR: 4.14 (1.61-10.65), p= 0.003). The relapse rate of the MRD+ patients was 57.2% at 2 years post HCT.

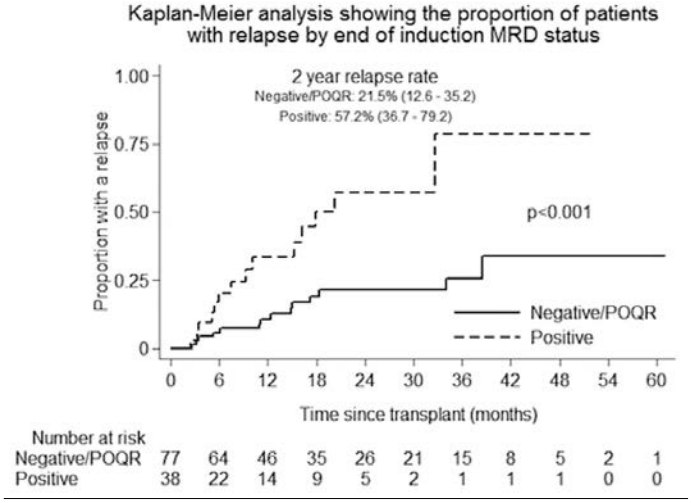

Figure 1.

Summary/Conclusions: The 2-year OS of 62.5% in UKALL14 participants over 41 years old after RICalloHCT is greater than would be expected with chemotherapy alone. However, MRD positivity after induction is associated with significantly lower OS, EFS and a higher risk of relapse, which is not abrogated by RICalloHCT.

S803

T-CELL RECEPTOR B REPERTOIRE CHARACTERISTICS IN RELAPSED/REFRACTORY B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA ON BLINATUMOMAB TREATMENT

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Background: Blinatumomab (Blin) is a bispecific monoclonal antibody, activating autologous effector T-cells and redirecting them against CD19-positive malignant cells. This leads to polyclonal effector T-cell expansion which is the necessary component of its antitumour mechanism. Recent reports indicated promising antitumour activity of Blin in relapsed/refractory (r/r) B-cell precursor acute lymphoblastic leukemia (BCP-ALL). However, approximately half of these patients do not achieve minimal residual disease (MRD) response.
Thanks to recent advances in next generation sequencing (NGS) of immunoglobulin and T-cell receptor gene rearrangements the deep and comprehensive evaluation of expanded T-cell repertoire on Blin treatment is now possible.
Aims: To compare the differrences in TRB repertoire diversity and composition between two groups of patients with r/r ALL: 1) responders: reaching MRD negativity at the latest at day 29 of 1. Blin cycle (C1D29), and 2) persisters: with quantifiable MRD positivity (>0.01%) at C1D29, or with MRD >1% at cycle 1 day 15 (C1D15) if C1D29 sample is not available.
Methods: We used NGS to investigate TRB repertoire in bone marrow samples (114× at time of screening (scr), 74× C1D15, 98× C1D29) of 114 r/r Ph-negative BCP-ALL patients (median age: persisters 47; responders 42; p-value=0.81). Patients received Blinatumomab within the phase II trial (MT103-211). Sequencing libraries were prepared using 100ng of DNA via 2-step PCR and sequenced on the Illumina MiSeq (2 x 250bp) with a median coverage of 117,563 reads (range 59.512 - 447.767 reads) per sample. In the first PCR virtually all TRB rearrangements present in the investigated sample were amplified using universal V(D)- and J-regions primers. In the second step, sequencing adaptors and sample-specific barcodes were added. Annotation of V (D)- and J-regions of TRB sequences was performed using ARResT/Interrogate (Bystry, Bioinformatics, 2016). Diversity of TRB repertoire within patient groups and time points was expressed as the Shannon index, using the R-package *vegan*. Analysis of variance was employed to assess statistically significant differences in diversity between groups and time.
Results: Diversity of TRB repertoire (Figure 1) was significantly higher in responders at time of scr (p=0.02) and at C1D29 (p=5.47E-6). Patients in the persisters group had significantly higher blast counts, which is in accordance with previously published data (Topp, The Lancet Oncology, 2015). The increase of diversity between scr and C1D29 of Blinatumomab treatment was sharp and highly significant in responders (p=3.96E-6), but not statistically significant in persisters (p=0.4).

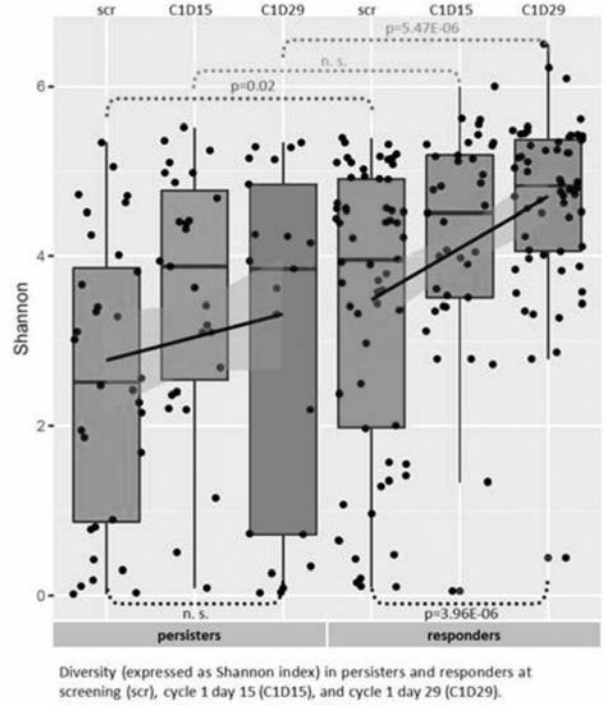

Figure 1.

Summary/Conclusions: We showed that Blin responders have significantly higher TRB repertoire diversity at scr compared to persisters and that the repertoire expansion during Blin treatment is sharper in responders. Other repertoire characteristics did not differ significantly between groups. Further studies on larger patient cohorts are necessary in order to elucidate whether the response to treatment can be predicted by repertoire diversity at scr.
Amplicon NGS is a useful tool for monitoring of T-cell repertoire. Development, standardization, and validation of TRB primer sets is in progress within Euro-Clonality-NGS Consortium.
Research Support: Amgen.

Infectious diseases, supportive care

S804

DISCONTINUING ANTIBACTERIAL THERAPY AFTER APYREXIA AND CLINICAL STABILITY REGARDLESS OF NEUTROPHIL COUNT IN FEBRIL NEUTROPENIA IS SAFE AND REDUCES EXPOSITION TO ANTIBIOTICS (HOWLONG RANDOMIZED TRIAL)

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Background: In neutropenic patients with unexplained fever the classical approach is maintaining the empirical antibacterial therapy (EAT) until neutrophil recovery. This strategy may result in unnecessarily prolonged EAT favoring bacterial resistance, organ toxicity and damage to microbiota. Nevertheless, the available scientific evidence supporting the alternative approach of stopping EAT before neutrofile recovery is moderate.
Aims: To investigate if a clinical approach (based on apyrexia and clinical recovery) is better than and as safe as the standard criteria (recovery from neutropenia) to decide the discontinuation of EAT.
Methods: After local Ethical Committee approval, a randomized, controlled, multicenter, open-labeled phase IV clinical trial was performed (EudraCT: 2011-005152-34). Study period: May-2012 to May-2016. Inclusion criteria: a) Adult patients (≥18 years); b) Hematologic malignancy or autologous or allogeneic hematopoietic stem cell transplantation (SCT) recipients; c) High risk febrile neutropenia (FN) d) Informed consent signed. Exclusion criteria: etiological diagnosis of FN. Patients were randomized 72 hours after fever onset to: 1. Experimental group (EG): EAT discontinuation if a) apyrexia ≥72 h, plus b) clinical recovery ≥72 h (independently of neutrophil count) or 2. Control group (CG): EAT discontinuation if a) apyrexia ≥72 h, plus b) clinical recovery ≥72 h, plus c) >0.5x106/L neutrophils. Follow-up: 28 days from EAT. Primary (efficacy) end-point was number of EAT-free days. Secondary (safety) end-points were total days of fever and crude mortality.
Results: One hundred and fifty seven patients were included (EG 78 and CG 79). There were no differences in baseline characteristics or clinical presentation between groups. The most frequent underlying conditions were induction/re-induction chemotherapy for acute leukemia (n=42, 26,7%), autologous SCT (n=42, 45,8%), and allogeneic SCT (n=14, 8,9%). The most frequent clinical presentation was non-focused FN (n=63, 40,1%), abdominal focused FN (n=34, 21,6%) and mucositis (n=31, 19,7%). Days with fever, and neutropenia duration and EAT-free days difference between groups are detailed in Table 1. Recurrent fever frequency was 14,3% (EG) and 17,9% (CG) (p=ns) and crude mortality was 1,3% (EG) and 3,8% (CG) (p=ns).

Table 1.

Neutropenia, fever duration and EAT-free days.

Variables	Median (IQ range)	Median (IQ range)	
ITT population	EG (n=78)	CG (n=79)	*P*
Days of neutropenia	14 (9,5-24)	11 (8-21)	*p*=ns
Days of fever	4 (2-8)	4 (2-8)	*p*=ns
EAT-free days*	18 (12,5-21,5)	16 (9,7-20,2)	*p*=0,047
Per protocol population	EG (n=66)	CG (n=66)	
Days of fever	4 (1-14)	5 (2-8,2)	*p*=ns
EAT-free days*	19 (14-22)	14,5 (8,7-20)	*p*=0,02
Modified per protocol population [#]	EG (n=36)	CG (n=30)	
Days of fever	3 (1-7,2)	3 (1-5,7)	*p*=ns
EAT free-days*	20 (11,2-23)	11,5 (5-16,7)	*P*<0,001

ITT: Intention to treat; EAT: empirical antimicrobial therapy; EG: experimental group; CG: control group. IQ range: interquartile range.
*EAT free-days: days of follow-up (28) – days of EAT.
[#]Patients in which clinical recovery and neutropenia recovery did not match.

Summary/Conclusions: In hematological patients with febrile neutropenia of unknown origin the discontinuation of empirical antibacterial therapy after 72 hours of apyrexia and clinical recovery regardless of neutrophils count is safe and reduces unnecessary exposure to antibiotics.

S805

CONJUGATED PNEUMOCOCCAL VACCINE TRIGGERS A BETTER IMMUNE RESPONSE THAN POLYSACCHARIDE PNEUMOCOCCAL VACCINE IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA A RANDOMIZED STUDY BY THE SWEDISH CLL GROUP

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Background: Patients with CLL have an increased risk for infection and *Streptococcus pneumoniae* is one of the most common pathogens with high morbidity. Patients with CLL are known to respond poorly to the traditionally used polysaccharide vaccines. Conjugation of polysaccharide to protein carriers renders a thymus-dependent, memory-inducing and more immunogenic vaccine. In patients with CLL, there is no consensus on a recommendation for pneumococcal vaccination, due to a lack of comparative studies.
Aims: To determine if patients with untreated chronic lymphocytic leukemia (CLL) benefit from vaccination with a 13-valent conjugated pneumococcal vaccine (PCV13), Prevenar13®, compared with a 23-valent capsular polysaccharide vaccine (PPSV23), Pneumovax®, in terms of immune response.
Methods: 128 treatment naïve CLL patients from eight hematology clinics in Sweden were randomized to vaccination with PCV13 (n=63) or PPSV23 (n=65) after stratification by IgG levels and CLL clinical stage (Rai). Blood samples for evaluation of immune response were obtained at baseline, at one and at six months after vaccination. Analyses for each of the 12 pneumococcal serotypes common for PCV13 and PPSV23 were performed by opsonophagocytic assay (OPA) and enzyme-linked immunosorbent assay (ELISA).
Results: PCV13 elicited a superior immune response than PPSV23 in 10/12 serotypes one month after vaccination and in 5/12 serotypes six months after vaccination, measured as OPA geometric mean titers (GMTs). Geometric mean concentrations of serotype-specific IgG antibodies elicited by PCV13 as measured by ELISA, were higher than those elicited by PPSV23 in half of the common serotypes, both after one and six months. The proportion of patients with good response (defined as response in 8 of 12 common serotypes according to predefined response criteria) was higher in PCV13 recipients than in PPSV23 recipients after one month (40% *vs* 22%, p=0.034) as well as after six months (33% *vs* 17%, p=0.041). Never did PPSV23 trigger a better immune response for any of the serotypes, than PCV13, regardless of analysis. For two of the serotypes, OPA GMTs were lower at the six months than at the one-month follow up. Negative predictive factors for vaccination response were hypogammaglobulinemia and long disease duration. Both vaccines were well tolerated.
Summary/Conclusions: In patients with previously untreated CLL, the efficacy of PCV13 in terms of immune response is superior to PPSV23 for many serotypes common for the two vaccines. PCV13 should be considered as a part in vaccination programs against *Streptococcus pneumoniae* for these patients and administered as early as possible during the course of the disease.

S806

INFECTION-RELATED MORTALITY AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: AGE, CMV AND PRE-TRANSPLANT LEVELS OF IGA/IGM PREDICT IRM IN A NEW CLINICO-BIOLOGICAL SCORING SYSTEM

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Background: Infection-related mortality (IRM) is a major challenge after allogeneic hematopoietic stem cell transplantation (allo-HSCT).
Aims: The aim of this study was to develop a scoring system predicting IRM based exclusively on pre-transplant data.
Methods: A total of 589 adult patients receiving allo-HSCT were studied (Jan 2009 - Nov 2016). In a training set of patients (n=273, Jan 2012-May 2015) the ROC curve analysis defined the optimal cut-offs predicting 100-day IRM for continuous data. All clinical and biochemical variables were challenged in a multivariate analysis and a 3-tiered weighted score was elaborated and tested firstly in a retrospective validation set (n=219, Jan 2009-Dec 2011) and then in a prospective validation set (n=97, Jun 2016-Nov 2016).
Results: Median follow-up was 43 months (range 1-85). Acute leukemia was the main indication to transplant, accounting for 60% (n=356) of patients. The majority of the patients received an alternative-donor transplant (44% a HLA-haploidentical, 37% a matched unrelated donor). Forty-seven percent (n=277) of patients had advanced diseases. Multivariate analysis revealed age >60 yrs (P=0.003), CMV host/donor serostatus different from negative/negative (P<0.001) and pre-transplant levels of IgA <1.11 g/L (P=0.004) and IgM <0.305 g/L (P=0.028) as the only independent predictors of increased IRM. Noticeably, these associations were independent from disease type or status, donor type, intensity of conditioning, *in vivo* T or B-cell depletion or from previous colonization by multidrug-resistant bacteria. According to the proposed IRM score, patients were divided into 3 classes: low (<10.17 points), intermediate (10.17-11.11 points) or high-risk (>11.11 points). In the training set, 100-day and 2-yrs IRM were 5% (95% CI 2-10) and 9% (95% CI 4-16) for low-risk, 11% (95% CI 5-18) and 23% (95% CI 14-33) for intermediate-risk, and 16% (95% CI 16-37) and 41% (95% CI 28-53) for high-risk patients, respectively (P=0.001). In the retrospective validation set, 100-day and 2-yrs IRM were 7% (95% CI 3-14) and 14% (95% CI 8-22) for low-risk, 17% (95% CI 10-26) and 23% (95% CI 15-33) for intermediate, and 28% (95% CI 15-42) and 33% (95% CI 19-4) for high-risk patients, respectively (P= 0.044), with a c-index of 0.608 (Figure 1). In the prospective validation set, only 100-day IRM was calculated due to a shorter follow-up, being of 0%, 3% (95% CI 0-13) and of 14% (95% CI 3-33) for low, intermediate and high-risk patients (P=0.003). Additionally, in both training and retrospective validation sets (n=492), the 2-yrs OS was different among the 3 groups, being 59% (95%CI 52-67), 50% (95%CI 43-59) and 37% (95%CI 29-48) for low, intermediate and high-risk groups, respectively (P=0.0001). In the prospective validation set, only 100-day OS was evaluated, being of 95% (95%CI 88-100), 91% (95%CI 82-100) and 80% (95%CI 65-100), respectively (P=0.03). Out of a total of 129 infection-related deaths, 94/129 (73%) were attributed to bacteria, 22/129 (17%) to viruses, 11/129 (8%) to fungi and 2/129 8 (2%) to parasites.

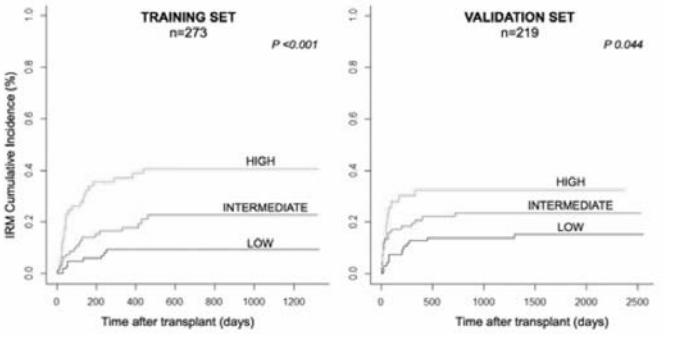

Figure 1.

Summary/Conclusions: This new clinic-biological score based on age, CMV serostatus and levels of IgA and IgM, may contribute to the prompt identification of patients at higher risk of fatal infections prior to allo-HSCT, thus promoting post-transplant personalized intensive active surveillance strategies and immune-intervention approaches to improve the overall outcome of transplant. A multicentric Italian study in currently on the way for the external validation of these results

S807

LETERMOVIR FOR PREVENTION OF CYTOMEGALOVIRUS INFECTION IN ADULT CMV-SEROPOSITIVE RECIPIENTS OF ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

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Background: CMV remains a common complication of HCT, yet no antiviral drug suitable for prophylaxis is available in HCT. LET is a first-in-class drug

that inhibits the CMV terminase complex. A dose-escalation phase 2 trial showed that LET prophylaxis for up to 12 weeks post-HCT was effective with a safety profile similar to placebo.

Aims: To compare LET prophylaxis to placebo for the prevention of clinically significant CMV infection (CS-CMV), defined as CMV disease or CMV viremia leading to preemptive treatment (PET) in a Phase III randomized, double-blind, placebo-controlled trial.

Methods: CMV seropositive HCT recipients 18 years or older who had undetectable plasma CMV DNA within 5 days of randomization were eligible (full eligibility at clinicaltrials.gov, NCT02137772). Subjects had to start treatment by Day+28 post-HCT. Subjects were randomized 2:1 to receive LET or placebo PO or IV through Week 14 (Day +100) post-HCT, stratified by study site and high or low CMV disease risk. LET was dosed at 480 mg/d (or 240 mg/d if on cyclosporine due to drug-drug interaction). Subjects were assessed weekly through Week 14, biweekly through Week 24, and every other month through Week 48 after HCT. Plasma obtained at each visit was assayed for CMV DNA in a central laboratory. Subjects who developed CS-CMV discontinued study drug and received anti- CMV treatment. Local CMV assay results could be used to start PET. The primary endpoint was the stratum-adjusted proportion of subjects with CS-CMV through Week 24 post-HCT among subjects with undetectable CMV DNA at randomization; subjects who discontinued the study for any reason or with missing data at Week 24 were considered failures. All adverse events (AEs) were analyzed through 14 days after the last dose of study drug.

Results: From June 2014 to March 2016, 565 randomized subjects received study treatment; 31% were at high CMV disease risk. 50% subjects received myeloablative conditioning, 35% received ATG. Donors included 14% mismatched unrelated, 13% haploidentical and 4% cord blood. Study arms were balanced. Subjects began study drug a median of 9 days post-HCT; 37% had engrafted prior to start. Of 495 treated subjects with undetectable CMV DNA at randomization, fewer subjects developed CS-CMV or were considered failures in the LET arm (122/325, 38%) compared to placebo (103/170, 61%; p<0.0001) by Week 24 post-HCT. Figure 1 shows the time to CS-CMV analysis. The most common AEs (LET, placebo) were GVHD (39%, 39%), diarrhea (26%, 25%), and nausea (27%, 23%). More frequent vomiting (19%, 14%), edema (15%, 9%), atrial arrhythmias (10%, 5%), and ALT levels >5xULN (4%, 2%) was noted in LET-treated subjects; no increased myelotoxicity or nephrotoxicity was observed. The Week 24 all-cause mortality was 10% for LET recipients and 15% for placebo recipients.

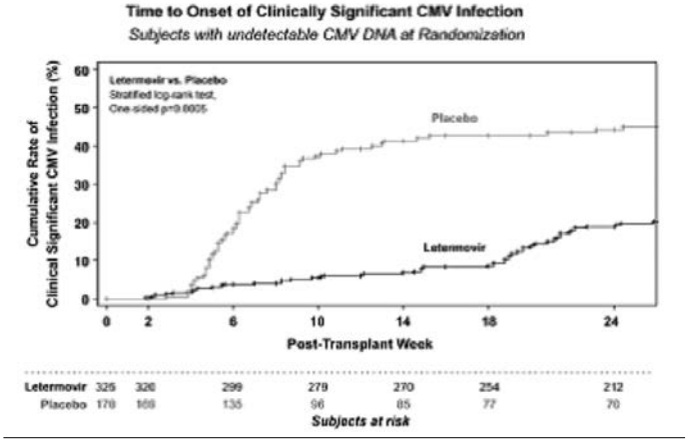

Figure 1.

Summary/Conclusions: Letermovir prophylaxis was effective in reducing clinically significant CMV infection, was overall well tolerated, and provides a new approach to CMV prevention after HCT.

S808

EFFICACY AND SAFETY OF DEFIBROTIDE TO TREAT HEPATIC VENO-OCCLUSIVE DISEASE/SINUSOIDAL OBSTRUCTION SYNDROME POST-CHEMOTHERAPY: A POST HOC ANALYSIS OF FINAL DATA OF AN EXPANDED-ACCESS PROTOCOL

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Background: Hepatic VOD/SOS, which may be unpredictable and potentially life-threatening, is typically considered a complication of hematopoietic stem cell transplantation (HSCT), and VOD/SOS with multi-organ dysfunction (MOD) may be associated with >80% mortality. Defibrotide is approved to treat severe hepatic VOD/SOS post-HSCT in the European Union and to treat hepatic VOD/SOS with renal or pulmonary dysfunction post-HSCT in the United States. However, VOD/SOS can occur after chemotherapy without HSCT.

Aims: To perform a *post hoc* analysis of final data on safety and response to defibrotide in patients developing VOD/SOS after primary chemotherapy without HSCT (off label).

Methods: In an expanded-access protocol for patients with VOD/SOS post-HSCT or chemotherapy, with or without MOD (renal and/or pulmonary dysfunction), defibrotide 25 mg/kg/d (4 divided doses of 6.25 mg/kg) was given a recommended ≥21 days after patients provided informed consent. Post-chemotherapy subgroup survival was analyzed *post hoc* from the day defibrotide was started (days 0–30 after start of chemotherapy) for 70 days (because follow-up data were collected for 100 days post-chemotherapy).

Results: Of 1154 VOD/SOS patients receiving defibrotide, 137 (12%) developed VOD/SOS post-chemotherapy without HSCT. Among the 82 patients (38 with MOD) treated with DF by day 30 after start of chemotherapy, median age was 7.5 years (range, 0–68 years) and 66 (81%) were pediatric patients (≤16 years of age). Among pediatric patients, 15% were age 0-23 months, 74% were 2-11 years and 11% were 12-16 years. Most common primary diseases were acute lymphocytic leukemia (51%), acute myeloid leukemia (13%), and neuroblastoma (6%). Kaplan-Meier estimated survival at Day +70 was 74% overall (95% CI, 63–82%); 66% (49–79%) in patients with MOD and 81% (66–90%) in patients without MOD. By age subgroup, Kaplan-Meier estimated survival at Day +70 was 80% (95% CI, 68–88%) in pediatric patients (Figure 1) and 50% (95% CI, 25–71%) in adults. Adverse events (AEs) were reported in 54/82 patients (66%). Hemorrhagic AEs (≥2%) were pulmonary (6%), epistaxis or mouth (4%), and hematochezia (2%). There were 22 (27%) patients with AEs assessed as being at least possibly related to defibrotide, the most common (≥2%) were pulmonary or mouth hemorrhage (4% each) and hematochezia, nausea, encephalopathy, epistaxis, or hypotension (2% each). Related AEs led to discontinuation in 6 patients and were associated with 1 death (pulmonary hemorrhage, hypotension).

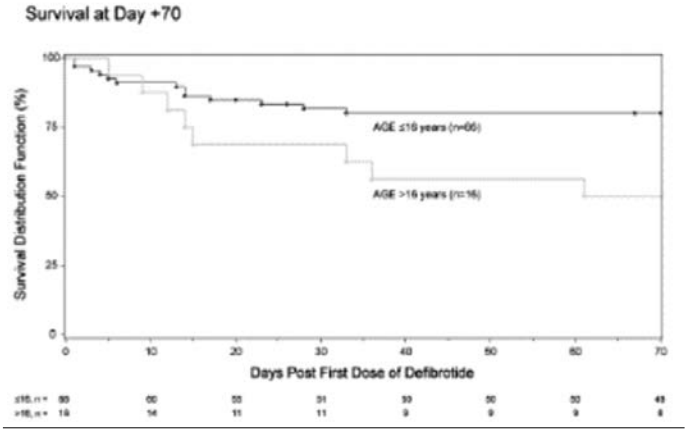

Figure 1.

Summary/Conclusions: The 74% survival rate at Day +70 in patients with VOD/SOS receiving defibrotide within 30 days of starting chemotherapy (81% in patients ≤16 years) is clinically encouraging. Of note is the 66% survival rate in patients with MOD. The defibrotide safety profile was consistent with that previously reported in the overall population of this expanded-access protocol.

Support: Jazz Pharmaceuticals.

Iron: Deficiency and overload

S809

LACK OF THE FERROPTOSIS INHIBITOR GPX4 IN ERYTHROID CELLS CAUSES A BLOCK IN RETICULOCYTE MATURATION AND A HYPOXIC SIGNATURE WITH IMPAIRED HEPCIDIN REGULATION

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Background: GPX4 is a selenoprotein belonging to the family of the glutathione peroxidases, a class of enzymes involved in cellular defence against oxidative stress. This enzyme is essential for life since it is the only peroxidase able to use lipid peroxides as substrate. Mice constitutively lacking GPX4 die at embryonic stage while tissue-specific ablation in neurons and T-cells cause neurodegeneration and impaired immune response. Recent studies have identified GPX4 as the main regulator of ferroptosis, an iron-dependent ROS-mediated form of nonapoptotic cell death. Erythrocytes are highly specialized cells that utilize a large amount of iron to bind and deliver oxygen to all tissues. Being constantly exposed to oxygen, erythroid cells need to continuously fight against oxidative stress by expressing a variety of antioxidant enzymes, including GPX4. Iron availability for erythropoiesis depends on systemic iron levels which are regulated via the hepcidin/ferroportin regulatory system. Hepcidin binding to the iron exporter ferroportin reduces systemic iron export regulating body iron levels. In hypoxic conditions the erythroid hormone ErFe suppresses hepcidin synthesis to provide iron for the elevated erythropoietic demand.
Aims: The aim of this study is to identify how the lack of GPX4 in the hematological compartment affects iron homeostasis.
Methods: Lethally irradiated C57BL/6 female mice were reconstituted with bone marrow cells from $Gpx4^{fl/fl}$; $Rosa26\text{-}Cre^{ERT2}$ or $Gpx4^{wt/wt}$; $Rosa26\text{-}Cre^{ERT2}$ and allowed to recover for 8 to 10 weeks. Gpx4 deletion in the hematopoietic system was induced by feeding tamoxifen citrate for 3 weeks and blood and organs were drawn at 3 and 6 weeks after terminating the tamoxifen-containing diet. Erythroid cells have been analysed in FACS. Serum iron levels have been assessed using the SFBC and UIBC iron kits (Biolabo). Gene expression analysis has been performed using SYBR-green qRT-PCR. Circulating Hepcidin has been measured with a specific murine ELISA kit (Intrinsic Lifesciences). Tissue iron levels have been measured with a colorimetric assay. All animal experiments were approved by and conducted in compliance with institutional guidelines
Results: Compared to $GPX4^{wt/wt}$;Cre^{ERT2} controls, $GPX4^{fl/fl}$;Cre^{ERT2} transplanted mice lacking GPX4 in the haematological compartment show a decrease in the number of red blood cells, haemoglobin and haematocrit. Reticulocyte count measurement revealed a strong increase in this population, suggesting that the erythropenia could be due to a block in the reticulocyte maturation. Reticulocyte FACS characterization revealed a shift towards a more immature population while electron microscopy analysis showed an accumulation of unphagocytosed vesicles containing remnants of mitochondria. Analysis of the spleen revealed extramedullary erythropoiesis. The anemia and the erythropenia trigger a hypoxic signature hallmarked by an increase in circulating EPO and increased ErFe expression. However, both hepatic mRNA analysis and circulating protein measurement failed to show alteration in hepcidin production. Analysis of the liver showed an increase in non-heme iron content and in the lipid peroxidation causing an elevated mRNA and protein expression of heme oxygenase 1. Hepatic ferritin and ferroportin are also increased as a consequence of the increased iron content.
Summary/Conclusions: Our data show for the first time that the presence of GPX4 in the haematological compartment is essential for the proper hepcidin downregulation upon ErFe stimulation. This finding opens new insights in the mechanism that regulate hepcidin during hypoxia.

S810

IDENTIFICATION OF GUANOSINE 5'-DIPHOSPHATE AS POTENTIAL IRON MOBILIZER: PREVENTING THE HEPCIDIN-FERROPORTIN INTERACTION AND MODULATING THE INTERLEUKIN-6/STAT-3 PATHWAY

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Background: Anemia of inflammation (AI) is one of the most common manifestations of iron deficiency in the patients with inflammatory conditions. AI is responsible for hypoferremia, with consequent iron-restricted erythropoiesis with high level of hepcidin, which stimulate the internalization of ferroportin (FPN) transporter. Therefore, inhibiting hepcidin-mediated FPN degradation can be an important strategy to ameliorate AI.
Aims: To increase iron bioavaibility we selected a Novel compound against hepcidin action through natural compound libraries that might provide a new alternative approach to increase iron absorption for prevention of hepcidin-mediated FPN internalization and to ameliorate turpentine-induced anemic state with different *insilico, invitro and invivo* studies.
Methods: A systematic approach involving *in silico*, *in vitro* and *in vivo* studies was employed to identify hepcidin inhibiting agents. To identify a potent hepcidin-binding agent, natural compounds were screened using molecular docking and dynamics simulations and further investigated on cell lines (GFP-FPN, Caco-2, HepG2) using flow cytometry and western blotting. Normal or turpentine induced anemic mice were used in the associated studies.
Results: The virtual screening via molecular modelling showed that GDP as a potent hepcidin-binding agent as shown in the (Figure 1A). *In vitro* studies revealed that GDP significantly increased ferroportin stabilization in GFP-FPN cell lines (Figure 1C) and *In vivo* results showed that co-administration of GDP and ferrous sulphate (FeSO4) significantly improved the turpentine-induced anemic state with increase in haemoglobin level (Figure 1B).

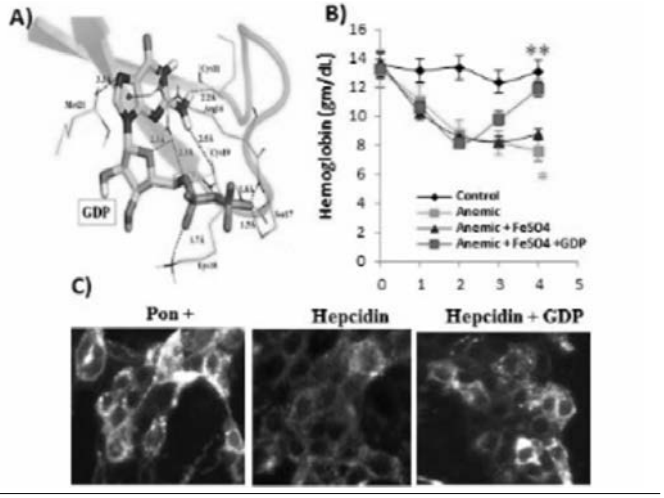

Figure 1.

Summary/Conclusions: AI is a normocytic anemia, common among patients with chronic infection and inflammatory disorders. We found that GDP molecule have higher contribution to the stability of hepcidin-GDP complex and thus blocks its interaction with FPN. The results support the novel hypothesis that GDP along with iron supplement regime can overcome the binding of hepcidin from interaction with FPN that would be an effective treatment for AI.

S811

UNRAVELING THE MOLECULAR PATHOGENESIS OF INEFFECTIVE ERYTHROPOIESIS IN CONGENITAL DYSERYTHROPOIETIC ANEMIA TYPE II: *IN VITRO* EVALUATION OF RAP-011 TREATMENT

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Background: Congenital Dyserythropoietic Anemias (CDAs) are subtypes of bone marrow failure syndromes, hallmarked by ineffective erythropoiesis. The most common form is CDAtype II (CDAII), showing moderate/severe anemia, relative reticulocytopenia, jaundice, and iron overload. It is inherited as autosomal recessive disorderdue to loss-of-function mutations in*SEC23B* gene. More than 300 CDAII cases and 80 causative mutationshave been described so far. Despite this high allelic heterogeneity, two variants, R14W and E109K, represent more than 50% of the mutational events. To date, treatments for CDAII patients consist of supportive therapy, such as erythrocyte transfusions, or bone marrow transplantation or splenectomy in transfusion-dependentcases.Recently, members of TGF-β superfamily have been studied as potential regulators of erythropoiesis,especially the growth differentiation factor 11 (GDF11). Through the binding of specific receptors, GDF11 leads to an inhibited late-stage erythropoiesis. Indeed, two GDF11 inhibitors, ACE-011 and ACE-536,have been associated to an improvement of hematologic parameters. Studies with the mouse counterpart of ACE-011, RAP-011, on mouse model of β-thalassemia showed increased differentiation of erythroid cells, improvement of anemic condition and reduced iron overload in treated mice.

Aims: The main aim of our study is to assess the effects of RAP-011 on different cellular models of CDAII.
Methods: We measured circulating GDF11 levels in CDAII patients and healthy controls(HC) bywestern blot(WB). To assess the effectiveness of RAP-011(provided by Celgene Corporation)*in vitro*, we established two different cellular models of CDAII: (i) K562 cells stably silencedfor *SEC23B* by Sh-RNA carried in GIPZ lentiviral vectors; (ii) K562 stably overexpressing SEC23B-WT and the two variants, R14W and E109K. *In vitro* treatment has been performed at 0, 3, and 6 days of erythroid differentiation by hemin+GDF11 in presence or absence of RAP-011 in K562 cells stably silencedfor *SEC23B*.
Results: WB and subsequent densitometric analysis showed an increase of GDF11 levels in serum samples from 18 CDAII patients compared to 18 HC (p=0.02). Stable silencing of *SEC23B* in K562 cellsled to the establishment of two different clones, Sh-70 and Sh-74, showing amarkedreduction of SEC23Bexpression compared to Sh-CTR (85-90% and 60-65%, respectively). At 3 and 6 days of K562 erythroid differentiation by hemin, we observed an increased expression of pSMAD2 in GDF11-treated cells compared to non-treated ones; interestingly, a reduction of pSMAD2 in RAP-011+GDF11-treated cells was observed.
Summary/Conclusions: We firstly demonstrated the increased levels of GDF11 in CDAII patients. Thus, we used a combined treatment with hemin+GDF11 in SEC23B-silenced K562 stable clones, in order to reproducethe pathologic phenotype of the disease, andto make K562 cells suitable for RAP-011 treatment, as attested by the increased expression of pSMAD2 in GDF11-treated cells. The reduced pSMAD2 in RAP-011+GDF11-treated cells suggests that RAP-011treatment leads to repression of ActRIIA/B pathway, which in turn should increase nuclear levels of GATA1 transcription factor. This action should lead to an increased expression of GATA1-activated genes involved in erythroid development. The evaluation of GATA1 activation is ongoing, as well as the *in vitro* treatment of K562 stably overexpressing SEC23B-WT,SEC23B-R14W and -E109K.

S812

INTRAVENOUS IRON *VERSUS* ORAL IRON *VERSUS* NO IRON WITH OR WITHOUT ERYTHROPOIESISSTIMULATING AGENTS FOR CANCER PATIENTS WITH ANAEMIA: A SYSTEMATIC REVIEW AND NETWORK META-ANALYSIS

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Background: A widely prevalent complication in patients suffering from cancer is the deficiency of haemoglobin-containing red blood cells, referred to as anaemia. While many patients develop anaemia due to an involvement of malignant bone marrow cells, others suffer from so called chemotherapy/radiotherapy-induced anemia. Erythropoiesis-stimulating agents (ESAs) stimulate the production of red blood cells within the bone marrow and have shown to increase Hb levels in anaemic patients. Uncertainties remain regarding the effect of iron supplementation on the fatal consequences of ESA-treatment.
Aims: The aims of this systematic review and network meta-analysis are to evaluate benefits and risks of ESAs and iron for the treatment of disease-related as well as therapy induced anaemia in cancer patients.
Methods: Based on an a-priori Cochrane protocol, we developed sensitive search strategies for Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, databases of ongoing trials and conference proceedings (search date 12/2016). We included only randomized controlled trials (RCTs) including anaemic patients of any age with solid and/or haematological malignancy undergoing chemotherapy, radiotherapy or no anti-cancer therapy. We excluded studies including anaemic cancer-patients as a result of surgery or due to haemolysis. Two authors independently assessed studies for eligibility, extracted data and assessed quality of trials. The primary outcome was on-study mortality. Secondary outcomes included number of red blood cell transfusions and thromboembolic events. For binary outcomes, we used risk ratios (RRs) with corresponding 95% confidence intervals (CIs) to evaluate the treatment effects. We performed a random-effects meta-analysis for direct comparisons. For network meta-analyses, we used the frequentist graph-theoretical approach. Treatment hierarchy was obtained giving P-scores on a scale from 0 (worst) to 1 (best).
Results: We identified a total number of 105 eligible studies, including 25.722 patients. The network analysis of the primary outcome, on-study mortality, included 69 studies and 8 treatments. As the given network was not fully connected, we performed pairwise comparisons on the four subnetworks with 2 treatments each. Statistically significant treatment disadvantages were shown in the direct comparison of ESA plus iron supplementation (given if necessary) compared to placebo/no treatment plus iron supplementation (given if necessary) (RR 1.14 (95% CI 1.03-1.25), including 41 studies). Network meta-analysis on the need for red blood cell (RBC) transfusions showed the treatment of ESA plus iron supplementation to have the most positive effect compared to ESA alone (RR: 0.70 (95% CI 0.53-0.92) P-score: 0.87). No relevant heterogeneity was found within the analysed network of four treatments (I^2=18.4%). Inconsistency could not be tested statistically as no closed loop was included. Thromboembolic events occurred most often in patients treated with ESAs, irrespective of iron supplementation (ESA plus iron *vs* no treatment/placebo plus no iron: RR 1.79 (95% CI 0.74-4.32) P-score: 0.22, ESA plus no iron *vs* no treatment/placebo plus no iron: RR 1.90 (95% CI 0.96-3.75) P-score: 0.16). Subgroup analysis regarding type of iron, as well as route of administration will be presented at the EHA-congress (Figure 1).

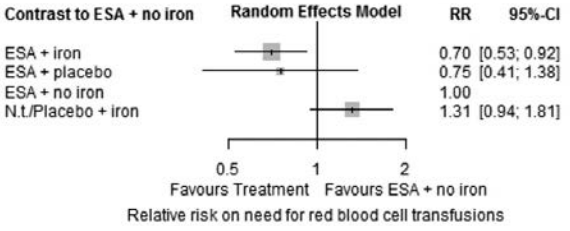

Figure 1.

Summary/Conclusions: While our analyses show that ESA use increases mortality and risk for thromboembolic events, there is no evidence that iron supplementation alters these risks. However, addition of iron to ESA does further decrease the need for RBC-transfusions compared to ESA alone. Further investigation, with regards to iron type and route of administration may yield further distinct results.
This project was funded by the Federal Ministry of Education and Research, grant number: 01KG1405

S813

DIFFERENT IRON SOURCES AND ACQUISITION PATHWAYS SHAPE MACROPHAGES TOWARDS OPPOSING FUNCTIONAL PHENOTYPES

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Background: Iron homeostasis and macrophage biology are closely interconnected. On the one hand, reticulo-endothelial macrophages are central for the regulation of iron homeostasis. The phagocytosis and degradation of senescent red blood cells (RBC) by macrophages enable efficient recycling of iron and the maintenance of systemic iron balance. On the other hand, iron exerts multiple effects on macrophage polarization and functionality. Macrophages exhibit a remarkable functional plasticity, reflected in their capacity to integrate diverse signals from the microenvironment and acquire distinct phenotypes. Macrophage polarization has been shown to dictate the expression of iron-regulated genes and determine cell iron handling.
Aims: Increasing evidence shows that iron availability itself has significant effects on immune effector functions and macrophage polarization. However, it is still unclear how different iron sources and acquisition pathways affect macrophage phenotypes.
Methods: To investigate this aspect, we analyzed both *in vivo* and *in vitro*, and compared the phenotypic switching of macrophages induced by different iron sources, including heme and iron, as well as hemolytic or intact RBCs.
Results: Hemolytic RBCs, free heme and iron-dextran treatment in mice shape macrophage polarization towards an M1-like pro-inflammatory phenotype. Splenic and hepatic macrophages from treated mice show iron deposition and increased expression of iron-related genes (ferroportin, ferritin, HO-1). Moreover, in these cells, the expression of M1 markers such as MHCII, CD86 and pro-inflammatory cytokines (TNFa, IL-6, IL-1b) is strongly increased, whereas the expression of M2 markers such as CD206, Arg-1 and IL-10 was significantly suppressed. Consistent results have been obtained treating bone marrow-derived macrophages with hemolytic RBCs, free heme and Fe-NTA. Importantly, the addition of the heme scavenger hemopexin and the iron carrier transferrin or the chelator deferoxamine fully abolish the ability of free heme and iron to trigger M1 polarization. On the contrary, RBC transfusions in mice shape macrophages towards an M2-like anti-inflammatory phenotype. After three transfusions, serum iron and hepcidin levels significantly rise, and tissues as well as macrophages are heavily iron loaded. Macrophages show a drastic suppression of M1 markers and inflammatory cytokines, and induction of M2 markers. Interestingly, repeated transfusions result in extensive macrophage cell death and new monocytes recruitment in both liver and spleen.
Summary/Conclusions: Collectively, these results suggest that the source and route of iron acquisition have a key role in shaping macrophage phenotype, and demonstrate a dynamic role of iron overload in determining macrophage polarization and function. When iron is provided in the form of free heme or non-transferrin bound iron, it exerts a clear pro-inflammatory effect on macrophages; whereas when provided via a controlled physiological acquisition pathway such as erythrophagocytosis, it dampens macrophage immune effector functions, being its clearance activity more active.

Our findings have potential implications, on one side, for hemolytic diseases, where RBC hemolysis and elevated circulating heme might promote a detrimental chronic inflammatory state, and, on the other one, for infectious diseases, where free heme and iron, released upon cell damage, might boost inflammation and enhance resistance to infections. Conversely, accelerated RBC clearance, by suppressing macrophage pro-inflammatory response, is rather expected to promote infections in transfused individuals.

Gene therapy, cellular immunotherapy and vaccination 2

S814

A PHASE 3 STUDY TO EVALUATE SAFETY AND EFFICACY OF LENTIGLOBIN GENE THERAPY FOR TRANSFUSION-DEPENDENT B-THALASSEMIA IN PATIENTS WITH NON-B0/B0 GENOTYPES: THE NORTHSTAR-2 (HGB-207) TRIAL

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Background: Standard treatment for transfusion-dependent β-thalassemia (TDT) includes regular red blood cell (RBC) transfusions and management of iron overload. Successful allogeneic hematopoietic cell transplantation (HCT) can eliminate RBC transfusions and, eventually, chelation. However, due to transplant-related risks such as graft-*versus*-host disease (GVHD), as well as donor constraints, HCT is rarely an option for TDT patients. By transferring a functioning copy of the β-globin (*HBB*) gene into hematopoietic stem cells (CD34+ cells) and re-infusing the modified cells, gene therapy may be an alternative one-time treatment available to all patients with TDT, without risks of GVHD. LentiGlobin gene therapy is an investigational treatment consisting of autologous CD34+ cells transduced with the BB305 lentiviral vector. The Northstar (HGB-204) phase 1/2 clinical study of LentiGlobin gene therapy for TDT included 18 patients who received LentiGlobin drug product (DP). As of September 2016, all patients in Northstar with non-β^0/β^0 genotypes and at least 12 months of follow-up stopped transfusions (median total hemoglobin [Hb] 11.2 [range 9.4−12.2] g/dL) and there was >60% reduction in transfusions in patients with a β^0/β^0 genotype. The safety profile was consistent with autologous HCT. In this initial study, the average number of therapeutic gene copies per CD34+ cell in the DP (*i.e.* DP vector copy number per diploid genome or DP VCN; median 0.7, range 0.3 to 1.5) correlated with peripheral HbA^{T87Q} (genetically engineered hemoglobin) expression at 6 months (ASH, 2016). In an effort to optimize the proportion of patients able to discontinue blood transfusions to achieve "transfusion independence" in all patients and increase unsupported Hb levels after treatment, the manufacturing process for LentiGlobin DP was modified to increase the DP VCN and the proportion of genetically modified cells. Northstar-2 (HGB-207) is a recently initiated phase 3 study using this new manufacturing process in patients with TDT and a non-β^0/β^0 genotype.

Aims: To evaluate safety and efficacy of autologous HCT with LentiGlobin DP in patients with TDT and a non-β^0/β^0 genotype.

Methods: After providing informed consent, patients 12 to 50 years of age (N=15) will have CD34+ cells collected via mobilization and apheresis. After individualized DP manufacture and satisfaction of release criteria, the patient will receive myeloablative conditioning with single-agent busulfan (starting dose 3.2 mg/kg/day for 4 days, with target AUC 4500 [range 4000−5000] μM*min) followed by infusion of LentiGlobin DP. Patients will be followed for engraftment, safety and efficacy endpoints for 2 years after infusion; patients will then have the option to enroll in a 13-year follow-up study. The primary endpoint is the proportion of patients who achieve transfusion independence after DP infusion, defined as total Hb ≥9g/dL without RBC transfusions for a continuous period of ≥12 months. Secondary endpoints include time to neutrophil engraftment, adverse events, and biological parameters including VCN in peripheral blood and levels of HbA^{T87Q} over time.

Results: As of March 1, 2017, two 20-year-old females with β^0/β^E genotypes have been treated with LentiGlobin DP in the Northstar-2 trial. The DP VCN was 2.9 and 2.4 copies per diploid genome, respectively. Outcomes in all evaluable patients will be presented.

Summary/Conclusions: Results from the Northstar-2 study will provide data on safety and demonstrate the extent to which an increase in LentiGlobin DP VCN yields normalization of total Hb and consistently achieves transfusion independence in patients with TDT of non-β^0/β^0 genotypes. Optimizing DP VCN has the potential to improve outcomes across all TDT genotypes treated by investigational LentiGlobin gene therapy.

S815

CIS IS A POTENT CHECKPOINT IN NK CELL ANTI-LEUKEMIA IMMUNITY

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Background: The detection of leukemia by natural killer (NK) cells is controlled

by the integration of signals from activating and inhibitory ligands and from cytokines such as IL-15.
Aims: We set out to identify the negative regultors of NK cell function in order to understand why immunogenic tumours and leukemia can evade or overcome NK cell detection and killing.
Methods: We used a multidisciplinary approach including RNAseq, Mass Spectrometry, Structual biology, kinase enrichment and activity assays, NK cell *in vitro* analysis, biochemistry and de novo/experimental tumor/leukemia *in vivo* models.
Results: We identified cytokine-inducible SH2-containing protein (CIS, encoded by *Cish*) as a critical negative regulator of IL-15 signaling in NK cells. *Cish* was rapidly induced in response to IL-15, and deletion of *Cish* rendered NK cells hypersensitive to IL-15, as evidenced by enhanced proliferation, survival, IFN-gamma production and cytotoxicity toward tumors. This was associated with increased JAK-STAT signaling in NK cells in which *Cish* was deleted. Correspondingly, CIS interacted with the tyrosine kinase JAK1, inhibiting its enzymatic activity and targeting JAK for proteasomal degradation. *Cish*−/− mice are resistant to leukemia *in vivo*, and this was independent of MHC-I expression.
Summary/Conclusions: Our data uncover a potent intracellular checkpoint in NK cell–mediated tumor immunity and suggest possibilities for new cancer immunotherapies directed at blocking CIS function.

S816

GENERATION OF MEMORY STEM T CELLS MODIFIED WITH A NOVEL OPTIMIZED CD30-SPECIFIC CHIMERIC ANTIGEN RECEPTOR FOR THE TREATMENT OF CD30+ T-CELL MALIGNANCIES

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Background: Peripheral T-cell lymphomas (PTCL) represent the most aggressive form among non-Hodgkin lymphomas with a very poor prognosis (5-year survival of 30%), demanding innovative novel treatment strategies. Adoptive immunotherapy with chimeric antigen receptor (CAR) engineered T cells has demonstrated its therapeutic potential in advanced hematological malignancies. However, its application to PTCL remains a formidable challenge mainly due to a lack of truly tumor-specific antigens that are not expressed on normal T cells. Anaplastic large T-cell lymphomas (ALCL) and several other subtypes of PTCL express CD30, which is expressed by activated normal T cells but no other healthy tissues. Indeed, brentuximab vedotin, an anti-CD30 antibody-drug conjugate, has shown some clinical efficacy in PTCL and ALCL patients although duration of responses is short in the majority of cases. Here, we developed a refined CD30-CAR T-cell approach to target CD30$^+$ PTCL as a potential novel therapeutic strategy. We selected a novel targeting domain that is unaffected by soluble CD30 protein to prevent blockade of the CD30-CAR *in vivo*. Moreover, we optimized the therapy by using memory stem T cells (T_{SCM}) to promote engraftment and persistence of CD30-CAR Tc ells after transfer, and we have included an EGFRt depletion marker as a safety feature.
Aims: We evaluated the antitumor effect of memory stem T cells (T_{SCM}) genetically-modified with a novel CD30-specific CAR that recognizes a membrane-proximal epitope in the CD30 molecule in a CD30$^+$ T-cell lymphoma model.
Methods: A second generation CD30-41BBz-EGFRt CAR was generated using a scFv that recognizes a tumor-cell membrane-proximal epitope of CD30 protein (Nagata S *et al*. Clin Cancer Res, 2002). Naive T cells from healthy donors were activated with anti-CD3/CD28 beads in presence of IL-7, IL-15 and IL-21 during 10 days to obtain a T_{SCM}-enriched population (Alvarez C *et al*. J Transl Med, 2016); on day 2 of culture, cells were transduced with a third-generation lentiviral vector encoding the CD30-CAR. The anaplastic large T-cell lymphoma cell line Karpas 299 was used as tumor model. Cytotoxicity assay was performed at 4 hours at 10:1, 5:1, 1:1 and 1:5 effector/target (E/T) ratios, and the tumor cell death was detected by flow citometry. Cytokines (IFN-γ and IL-2) were analysed at 24 hours in a 5:1 E/T ratio culture using Luminex technology.
Results: T_{SCM} were the most prevalent T-cell subset at day 10 of culture, representing 84 ± 3.1% of total cells, and the CD30-CAR expression in these cells was 76.9 ± 1.0% in CD4$^+$ T_{SCM} and 77.3 ± 2.0% in CD8$^+$ T_{SCM}. Although CD30 protein was detected in a fraction of activated T cells in culture (CD4$^+$ T cells: 32.4 ± 2.1%; CD8$^+$ T cells: 59 ± 4.3%), lentiviral transduction of T_{SCM} with our CD30-CAR did not compromise their *ex vivo* expansion (CD4$^+$ CD30-CAR T_{SCM}: 96.0 ± 3.2 fold expansion; CD8$^+$ CD30-CAR T_{SCM}: 109.0 ± 4.2 fold expansion). CD8$^+$ CD30-CAR T_{SCM} conferred specific cytolytic activity and lysed Karpas 299 cells *in vitro* (tumor cell death 1:1 ratio: 92.6 ± 2.4% *vs* 0% with untransduced T_{SCM}; p<0.001), while control CD30$^-$ target cells (Raji) were not recognized. In addition, CD30-CAR T_{SCM} secreted IFN-γ and IL-2 after stimulation with Karpas 299 cells (IFN-γ: 126.6 ± 18.12 pg/ml *vs* 5.03 ± 0.16 pg/ml with control targets, p=0.002; IL-2: 20.47 ± 2.3 pg/ml *vs* 4.06 ± 0.24 pg/ml, p=0.002, respectively).
Summary/Conclusions: Collectively, our data demonstrate the potential to generate CD30-CAR T cells with enhanced functional attributes against CD30$^+$ PTCL. T_{SCM} cells can be efficiently transduced and *ex vivo* expanded with a novel CD30-CAR and confer potent antitumor efficacy against CD30$^+$ PTCL *in vitro*. Our findings suggest the potential to improve outcome of patients with CD30$^+$ PTCL through adoptive therapy with CD30-CAR modified T cells.

S817

MESENCHYMAL STROMAL CELLS FOR THE TREATMENT OF STEROID-RESISTANT ACUTE GRAFT *VERSUS* HOST DISEASE: FACTORS INFLUENCING CLINICAL RESPONSES

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Background: The immunosuppressive activity of mesenchymal stromal sells (MSC) have been extensively tested for the treatment of steroid-resistant acute graft *versus* host disease (aGvHD). However, the factors affecting clinical responses are poorly understood.
Aims: We assessed the impact of MSC treatment on clinical outcomes and investigate factors influencing the response to MSC.
Methods: Data collected from a cohort of 60 patients treated with MSC between May 2008 and December 2014 in the UK were analyzed. Clinical grade MSC were generated from bone marrow aspirates collected from the iliac crest of healthy donors and expanded using platelet lysate. All patients received MSC for the treatment of steroid-resistant aGvHD, defined as failure to respond to high-dose steroids (2mg/Kg methyl-prednisolone) after 6 days. Informed consent was obtained from all patients in accordance with the local ethics committee requirements. Clinical responses to MSC were assessed 1 week after MSC infusion. Patients were defined as: a) Responders when an improvement of at least 50% in at least one organ affected by aGvHD was observed, or b) Non-Responders if they had stable or progressive disease.
Results: Patient characteristics are summarized in Table 1.

Table 1.

Total (n)	60
Age	
Median (Range)	40yr (4mo-68yr)
Sex	
Male	21
Female	39
Disease	
AML	14
ALL	10
CML	10
MDS/MPNs	7
CLL	1
NHL/HL/MM/Other Lymphomas	11
Others	7
Time from HSCT to MSC treatment	
Median (range, days)	62 (12-929)
Time from GvHD to MSC treatment	
Median (range, days)	20 (1-909)
GvHD treatment before MSC infusion (n° patients)	
Methylprednisolone Alone	13
Methylprednisolone in combination with other drugs	45
Other drug combination not including Methylprednisolone	2
CSA	17
MMF	30
anti-TNFα (Infliximab, Etanercept)	18
Tacrolimus	8
MTX	3
anti-CD25 (Basiliximab, Daclizumab)	1
ECP	3
anti-CD20 (Rituximab)	1
anti-CD52 (Alemtuzumab)	1
ATG	1
GvHD grade	
II	5
III-IV	55
Biopsy Proven	
yes	46
no	14

aGvHD was biopsy proven in 45 patients, while in the remaining patients the diagnosis was clinical and based on the exclusion of alternative causes. 10, 16 and 1 patients had skin, gut and liver involvement only, respectively. 16 patients exhibited gut and skin, 11 skin, gut and liver, 3 skin and liver and 3 gut and liver. 34 patients received 1 dose, while 19, 6 and 1 were treated with two, three and four doses, respectively. No side effects were observed. 36 patients (60%) responded to MSC. Amongst patients who received multiples doses (26), subsequent doses did not change the status after the first dose (24 responded, 1 did not respond), except from one patient who, although respond-

ing to the first dose, failed to respond to the second one. When we evaluated potential factors for response, organ involvement, age at transplant and the cumulative dose of MSC infused were found statistically significant. Response rate was 67% among patients with involvement of gut, skin or both, but only 22% among those with involvement of the liver (alone or in combination with skin and/or gut). Patients younger than 20 years fared better, with 88% of them responding. Conversely, only 30% and 42% of those aged 20-50 years or older than 50 responded, respectively. Lastly, higher cumulative MSC dose (>3.0x10^6/Kg) was associated with a response in 76%, while none of those receiving less than 1.5x10^6/Kg responded. All 3 factors remained significant in multivariate logistic regression analysis. Patient gender, pre-MSC therapy, interval from transplant or aGvHD diagnosis to MSC treatment and grade of aGvHD did not affect response. The impact of achieving a response 1 week after MSC had a profound impact on the overall survival at 18 months accounting for 59% in responders and 17% in non-responders (log-rank test, p<.001).
Summary/Conclusions: In our cohort of patients, MSC treatment was safe and well tolerated. We conclude that the presence of a response at one week highly impacted on the survival of patients with an otherwise very poor prognosis. Importantly, younger age at the transplant, absence of liver aGvHD involvement and use of higher MSC doses were strong predictors of a response.

S818

CARD9 CONTROLS DECTIN-1-INDUCED T-CELL CYTOTOXICITY AND TUMOR GROWTH IN MICE

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Background: Activation of the C-type lectin receptor Dectin-1 by beta-glucans triggers multiple signals within dendritic cells (DCs) that result in activation of innate immunity. While these mechanisms can potently prime $CD8^+$ cytotoxic T cell (CTL) responses without additional adjuvants, the Dectin-1 effector pathways that control CTL induction remain unclear.
Aims: Aims of this study were: To define details of the intracellular signalling pathway responsible for cross-priming of a CTL response after activation of the C-type lectin receptor Dectin-1. To analyze whether identified signalling molecules were indispensable for antitumor immunity. To analyze whether NK cells played a role in antitumor immunity after Dectin-1-mediated CTL induction.
Methods: We used *in vitro* coculture between DCs (wildtype *vs* gene deficient) and CD8 T cells to define signalling components of Dectin-1 induced CTL cross-priming. We used WT and gene-deficient mice to define the signalling pathway of Dectin-1 induced CTL crosspriming *in vivo* and to test the role of this pathway for antitumor immunity by challenging mice with B16-Ova tumor cells intravenously, with or without depletion of CD8 T cells or NK cells, respectively.
Results: Here we demonstrate that Dectin-1-induced CTL cross-priming in mice does not require inflammasome activation but strictly depends on the adapter protein Card9 *in vitro. In vivo,* Dectin-1-mediated Card9 activation after vaccination drives both expansion and activation of antigen-specific CTLs, resulting in long-lasting CTL responses which are sufficient to protect mice from tumor challenge. This Dectin-1-induced antitumor immune response was independent of natural killer (NK) cell function and completely abrogated in Card9-deficient mice. Thus, our results demonstrate that Dectin-1-triggered Card9 signaling but not inflammasome activation can potently cross-prime antigen specific CTLs, suggesting that this pathway would be a candidate for immunotherapy and vaccine development (Figure 1).

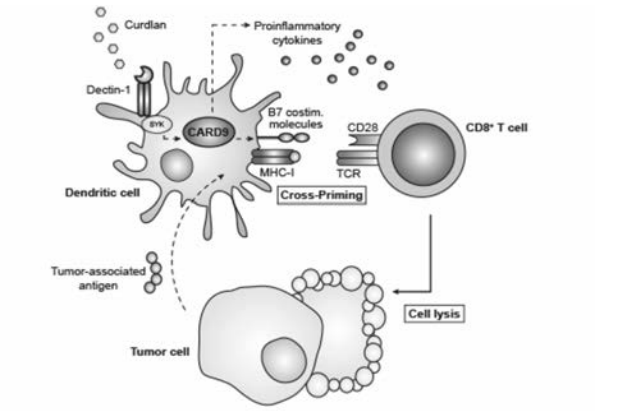

Figure 1.

Summary/Conclusions: We identify Card9 as central regulator of Dectin-1-induced cross-priming of cytotoxic T cells (CTLs) in mice. These antigen specific CTLs mediate potent antitumor immunity independent of inflammasome activity and NK cells. This pathway is a candidate for immunotherapy and vaccine development.

E-POSTERS

Acute lymphoblastic leukemia - Biology

E819

PRECLINICAL COMBINATION OF A NOVEL IRE1 RNASE INHIBITOR MKC-8866 AND TYROSINE KINASE INHIBITION ACTS SYNERGISTIC IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: The role of the Unfolded Protein Response (UPR) in BCR-ABL1$^+$ Acute Lymphoblastic Leukemia (ALL) has been extensively studied, proving the IRE1-XBP1 branch to be required for leukemic cell survival. However, a therapeutic strategy involving UPR inhibition that possesses translational impact is yet to be identified.
Aims: In this study we aim to identify a potential synergistic effect of simultaneous pharmacological inhibition of IRE1 and BCR-ABL1 in BCR-ABL1$^+$ ALL.
Methods: To study the link between IRE1-XBP1 axis of UPR and BCR-ABL1 we utilized both pharmacological and genetic approaches. 1) We tested the effect on proliferation and viability of pharmacological IRE1 inhibition (using MKC-8866) alone and in combination with Tyrosine Kinase Inhibitors (TKI, using Imatinib or Nilotinib) on BCR-ABL1$^+$ human ALL cell lines, SUP-B15 and TOM-1. The cell lines were also co-cultured with immortalized tertMSCs to test the chemo-protective effect of bone marrow stromal cells (BMSCs) on leukemia cells. 2) We tested whether genetic knock-down of XBP1 could sensitize cells towards the effect of Imatinib and Nilotinib. To this end, primary murine pre-B cells from conditional XBP1$^{fl/+}$mice were transduced with BCR-ABL1 construct, and with either inducible cre or empty vector.
Results: IRE1 inhibitor MKC-8866 (MKC) in combination with either Imatinib (IM) or Nilotinib (NL) showed enhanced capacity to arrest proliferation and to induce cell death in BCR-ABL1$^+$ALL cell lines compared to single treatments, after 3 days incubation (Viable SUP-B15: MKC 30μM 94.9%±0.1, IM 10μM 78.4±0.4, Combination 17.0±1.4; MKC 30μM 94.1±0.07, NL 5μM 64.2±0.6, Combination 20.0±0.8. TOM-1: MKC 30μM 85.0±0.9, IM 10μM 89.9±0.4, Combination 17.6±0.07; MKC 30μM 94.6±0.1, NL 5μM 71.0±0.9, Combination 30.6±3.6). Using Bliss independence formula, we confirmed a striking synergistic effect. Successively, to exclude any possible off-target effect at the basis of the observed synergism, we used a genetic approach to block IRE1-XBP1 signaling *in vitro*. B-cell precursors from *Xbp1*$^{fl/+}$ mice, instead of *Xbp1*$^{fl/fl}$, were used in order to warrant a basal signal of XBP1, as present during pharmacological inhibition. After transductions with BCR-ABL1, and either cre or the empty vector, we could observe that heterozygous deletion of Xbp1, induced by 4OHT, significantly increased TKI-induced cell death, after 3 days incubation (4OHT 1μM: 47.5%±13.0, IM 1μM: 70.8±1.7, IM+4OHT: 18.3±2.7, NL 0.5μM: 65.2±0.3, 4OHT+ NL: 6.87±1.2). Finally, we showed whether the tested drugs combinations were still effective in presence of BMSCs. It´s know that BMSCs are a critical component to escape TKI-induced cell death in Ph$^+$ leukemia and that IRE1-XBP1 is responsible for chemoresistance in many different cancer types, although this role has never been confirmed in BCR-ABL1$^+$cells. To shed light on this aspect we co-cultured either SUP-B15 or TOM-1 cells with tertMSCs, and while the stroma was capable to block Nilotinib-induced cell death, after 5 days incubation (in SUP-B15, NL 5μM in standard culture 28.7%±1.9, NL 5μM in co-culture 74.9±0.1; in TOM-1, 29.1±2.8 *vs* 78.7±0.4), this protective activity was partially abrogated upon treatment with IRE1 inhibitor. On the other hand, MSCs were not able to reverse IM effect on cell viability.
Summary/Conclusions: Overall, our data demonstrate that simultaneous inhibition of BCR-ABL1 and IRE1 branch of UPR exerts a potent effect *in vitro*, by acting synergistically on BCR-ABL$^+$ ALL cells. This provides basis for a preclinical application of a combined targeted therapy.

E820

HIGH-THROUGHPUT COPY NUMBER PROFILING IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA USING MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION IN COMBINATION WITH NEXT-GENERATION SEQUENCING

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Background: Development, progression and resistance of pediatric acute lymphoblastic leukemia (pALL) are widely associated with recurrent copy number abnormalities (CNAs). Multiplex ligation-dependent probe amplification (MLPA) is an established technique to screen CNAs, thus providing valuable information for risk assessment in pALL; however, the number of simultaneously analyzable genomic loci is limited to 55-60.
Aims: To introduce and test a high-throughput, high-resolution and comprehensive disease-relevant CNA profiling approach applicable to all subtypes of pALL.
Methods: A new digitalMLPA (dMLPA) technique has been developed which combines the advantages of MLPA and next-generation sequencing (NGS), massively extending the number of genomic targets that can be analyzed for their copy number in a single reaction. Bone marrow samples from 58 patients with pALL were analyzed using this novel assay targeting ~470 genomic loci. dMLPA probes contain sample-specific barcodes as well as Illumina adapters. After sequencing, copy number status of each target sequence was assessed by relative read count quantification. Leukemic cell purity (mean: 81%, range: 60-99%) measured by flow cytometry was considered at the interpretation of copy number changes. Results were compared to conventional MLPA, cytogenetic and FISH data.
Results: CNAs directly indicating structural or whole chromosome aberrations or indirectly referring to gene fusions were detected in 93% of patients, in 44/48 pre-B ALL and 10/10 pre-T ALL cases. Among patients with CNAs, recurrent aberrations specifically affecting putative driver genes varied between 0 and 11 (mean: 3.1, total: 175). *ETV6* and *CDKN2A/B* were the most frequently altered genes in pre-B and pre-T ALL, respectively; followed by *CDKN2A/B*, *PAX5*, *RB1*, *VPREB1*, *MLLT3*, *CD200/BTLA*, *TBL1XR1*, *IKZF1*, *CASP8AP2*, *PTEN*, *RUNX1*, *BTG1*, *TP53*, *IKZF2*, *EZH2*, *NF1*, *NR3C2*, *RAG2* and the PAR region genes in pre-B ALL cases and *PTEN*, *MLLT3*, *PTPN2*, *PHF6*, *LEF1*, *CASP8AP2*, *MYB*, *RB1*, *TP53* in pre-T ALL patients. *STIL-TAL1* and *NUP214-ABL1* gene fusions were also observed in T-ALL cases while in one *BCR-ABL1+* pre-B ALL patient, the copy number profile correctly indicated the presence of an extra Ph-chromosome. dMLPA results showed a congruency of 99.3% with those of MLPA mixes containing probes with different ligation sites for a subset of the genes. The increased resolution of dMLPA (*i*) allowed the detection of subclonal aberrations with an improved efficacy and confidence as compared to conventional MLPA and (*ii*) enabled a more patient-specific characterization of CNAs, *e.g.* by revealing 15 different deletion patterns across 23 samples harboring del(9p). In addition to genomic lesions specifically influencing putative or proven clinically relevant genes, 24 structural and 134 whole chromosome aberrations were detected genome-wide which was strongly facilitated by the inclusion of ~200 digital karyotyping probes covering each chromosome arm.
Summary/Conclusions: A novel NGS-based method has successfully been introduced for high-resolution profiling of CNAs in pALL. dMLPA is a robust, fast and cost-effective technique; its input DNA requirement (≥20ng) is similar to those of other low-input NGS protocols and lower than the requirement for MLPA. Due to its targeted approach, data analysis is computationally less demanding as compared to most NGS methods. The number of genomic sites analyzed in our study was an order of magnitude higher than that achievable with conventional MLPA. Due to its specific probe composition, dMLPA allows both high-resolution analysis of genomic driver regions and a genome-wide detection of aneusomies and large CNAs.

E821

CRITICAL ROLE FOR NOTCH SIGNALLING IN B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA (B-ALL) DRUG RESPONSE

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Background: B-cell precursor acute lymphoblastic leukemia (B-ALL) is the leading cause of cancer-related death in children and young adults. There is still a need of more efficient therapies for the subset of refractory patients. Our group has previously shown that Notch-3 and Notch-4 promote human B-ALL cell survival in presence of stromal cell support. However, the prognosis value of Notch signalling as well as its contribution *in vitro* and *in vivo* to chemotherapy has not yet been investigated.
Aims: In this study we used B-ALL cell lines and samples from new diagnosed B-ALL patients to analyse the contribution of Notch signalling to B-ALL pathogenesis in terms of prognosis, proliferation survival and drug response *in vitro* and in mice xenograft models of B-ALL.
Methods: B-ALL cell lines were obtained from ATCC, while B-ALL primary cells were obtained from bone marrow or peripheral blood of 30 B-ALL patients. Flow cytometry and western immunoblotting were used to study the expression of Notch receptors and ligands. Drugs used were Cytarabine (Ara-C), Dexamethasone (Dexa) and Doxorubicin (Doxo) alone or in combination with Notch modulators including anti-Notch blocking antibodies, gamma secretase inhibitors (GSIs), and Notch transcription factor inhibitor (SAHM1). Mice xenograft model of B-ALL were obtained by injecting the B-ALL line RS4;11 in

NOD/Shi-scid/IL-2Rγnull mice (NOG). Cell viability was evaluated by Annexin-V/PI and MTT assay; proliferation was assessed through CFSE dilution.
Results: Western blot and flow cytometric analysis showed that B-ALL cell lines as well as primary blast cells displayed the same Notch expression pattern consisting in low expression levels of Notch2 and Jagged1, high expression levels of Notch1, Notch3, Notch4, Jagged2, DLL3 and DLL4. Notably, in primary blast cells deriving from patients, the expression of Notch3, Notch4, Jagged2, DLL3 and DLL4 was significantly higher in the cases refractory to treatment as compared to patients achieving complete remission, thus suggesting that Notch signalling could be involved in the response to chemotherapy. In line with this hypothesis, we found that the treatment *in vitro* of B-ALL cell lines with Ara-C or Dexa down regulates the expression of Notch receptors. This down regulation was also observed in human CD19+ blast cells isolated from bone marrow of recipient mice treated with Ara-C compared to cells isolated from not treated mice. In addition, Notch inhibitors significantly improved *in vitro* the cytotoxicity of Ara-C, Dexa and Doxo towards B-ALL cell lines. Finally, we observed that the administration to mice of a pan Notch inhibitor, *i.e.* the GSI XII, significantly lowered the CD19+ leukemic burden in the bone marrow of recipient mice, potentiating anti leukemic effect of ARA-C.
Summary/Conclusions: In this study we used both *in vitro* and *in vivo* assays to highlight the prognostic value of Notch expression in B-ALL as well as its critical role in B-ALL cell survival and response to chemotherapy. We also demonstrated that Notch inhibitors were able to improve Ara-C-mediated reduction of blast cells in bone marrow, revealing that Notch signalling is a possible therapeutic strategy to eradicate minimal residual disease in B-ALL.

E822

REGULATION OF NOTCH AND WNT SIGNALING PATHWAYS BY NRARP IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematological malignancy. Although the outcome of T-ALL patients has improved over recent years, the poor prognosis of patients with resistant or relapsed disease is still a major concern. Even though NOTCH is a known driver in T-ALL, its inhibition cannot be efficiently achieved with the drugs currently available, due to their weak therapeutic effects and severe toxicity. We have shown that loss of *mir-181ab1* blocks Notch-induced T-ALL development partly by de-repressing the expression of NRARP (NOTCH regulated ankyrin repeat protein) a negative regulator of NOTCH signaling. Importantly, NRARP over-expression in murine hematopoietic stem cells impairs T-cell development suggesting that de-regulation of NRARP expression can contribute to the pathogenesis of T-ALL.
Aims: To investigate the role of NRARP in human T-ALL cell growth and survival and its therapeutic potential in T-ALL.
Methods: mRNA and protein expression were determined by real time-PCR and western blot analyses. *in vitro* functional evaluation of NRARP in T-ALL cell lines was performed by flow cytometry analysis of proliferation and viability upon NRARP overexpression using lentiviruses.
Results: We started by characterizing NRARP expression in human T-ALL cells and compared it with the expression of NRARP in human thymocytes. We found that NRARP protein levels are significantly increased in T-ALL cells. This result, although consistent with the fact that NRARP is a transcriptional target of NOTCH, suggests that NRARP is not sufficient to block NOTCH oncogenic signals. To test this hypothesis, we overexpressed NRARP in human T-ALL cell lines. Curiously, NRARP overexpression blocks the expansion of the T-ALL cell lines that display *NOTCH1*-activating mutations but promotes the expansion of the T-ALL cells without *NOTCH1* mutations. Although in both cell types (WT and *NOTCH1*-mutated) NRARP overexpression blocks NOTCH signaling, in *NOTCH1*-WT T-ALL cells we observe an increase in c-Myc expression. Consistent with these results, *NOTCH1*-WT NRARP overexpressing cells are more sensitive to JQ1, a small-molecule bromodomain inhibitor that targets c-Myc. NRARP is known to positively regulate LEF1, a DNA binding transcription factor acting downstream of WNT. Thus we sought to investigate the impact of NRARP in this signaling pathway. Very interestingly, our results show that in *NOTCH1*-mutant cell lines NRARP overexpression results in the down-regulation of the WNT signaling pathway while in *NOTCH1*-WT *T-ALL* cells results in its up-regulation.
Summary/Conclusions: Taken together our results suggest that NRARP may play a dual role in T-ALL pathogenesis, regulating both NOTCH and WNT pathways, with opposite functional effects on leukemia cells depending on NOTCH mutational status and signaling levels. This dual role may have important biological and therapeutic implications.

E823

ETV6/RUNX1-LIKE ACUTE LYMPHOBLASTIC LEUKEMIA: A NOVEL B-CELL PRECURSOR LEUKEMIA SUBTYPE IDENTIFIED BY THE CD27/CD44 IMMUNOPHENOTYPE

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Background: We have shown previously that *ETV6/RUNX1*-positive acute lymphoblastic leukemia (ALL) is distinguishable from other ALL subtypes by CD27^{pos}/CD44$^{low-neg}$immunophenotype. During diagnostic immunophenotyping of 573 childhood B-cell precursor ALL (BCP-ALL), we identified eight cases with this immunophenotype among "B-other ALL" (BCP ALL cases negative for hyper-/hypodiploidy, *ETV6/RUNX1*, *TCF3/PBX1* and *BCR/ABL1* fusion genes and *KMT2A*-rearrangements).
Aims: We aimed to characterize their genetic and biological background, to reveal to what extent they resemble *ETV6/RUNX1*-positive ALL and to elucidate whether they belong to the recently described *ETV6/RUNX1*-like ALL (Lilljebjörn et al., Nature Communications 2016).
Methods: We utilized microarrays to study the gene expression profile (GEP) and biological similarity of the BCP-ALL subtypes. Five *ETV6/RUNX1*-positive and five hyperdiploid ALL cases were analyzed using microarrays in parallel to seven CD27^{pos}/CD44$^{low-neg}$ B-other cases with available material. Microarray data from all 17 BCP-ALL cases were combined with data from an independent Italian cohort of 291 BCP-ALL cases (including *ETV6/RUNX1*-positive, *BCR/ABL1*-positive, *TCF3/PBX1*-positive, *KMT2A*-rearranged, hyperdiploid and B-other ALL cases) whose specimens were analyzed using the same microarray. To study the genomic background, we performed comprehensive profiling using single nucleotide polymorphism (SNP) arrays and whole exome and whole transcriptome sequencing (WES and RNAseq).
Results: In the hierarchical clustering based on GEP all five *ETV6/RUNX1*-positive cases and 5 of 7 CD27^{pos}/CD44$^{low-neg}$ B-other cases clustered within the *ETV6/RUNX1*-positive cluster. These B-other cases were thus classified as *ETV6/RUNX1*-like ALL. We identified multiple regions of acquired copy number aberrations (CNA)/ uniparental disomies (5 to 27 per case) and point mutations (10 to 41 per case) in all 7 cases and 3 in-frame fusion transcripts each in one patient. The most important findings are summarized in Figure 1. All 5 *ETV6/RUNX1*-like cases harbored a deletion of the *ETV6* gene, resulting in an in-frame *ETV6/BORCS5* fusion in one of them. The deletion of *ARPP21* was found in 3 cases, and the deletions of *PAX5*, *ATP10A*, *BTG1* and the gain of *RUNX1* were found in 2 cases each. The *ARPP21* deletions displayed a strikingly uniform character and were highly enriched in *ETV6/RUNX1*-like ALL. Using WES and RNAseq, no recurrently mutated gene and no in-frame fusions were found, respectively, except for the *ETV6/BORCS5.* Integrating data from all platforms, we identified *IKZF1* as another recurrently affected gene; a deletion, a nonsense mutation and an *IKZF1*-involving out-of-frame fusion were each found in one case. The *P2RY8/CRLF2* fusion and the *TCF3/ZNF384* fusion (the former co-occurring with two activating *JAK2* mutations) were found in 2 CD27^{pos}/CD44$^{low-neg}$ B-other cases that were closer to other BCP-ALL subtypes than to *ETV6/RUNX1*-positive ALL as indicated by GEP.

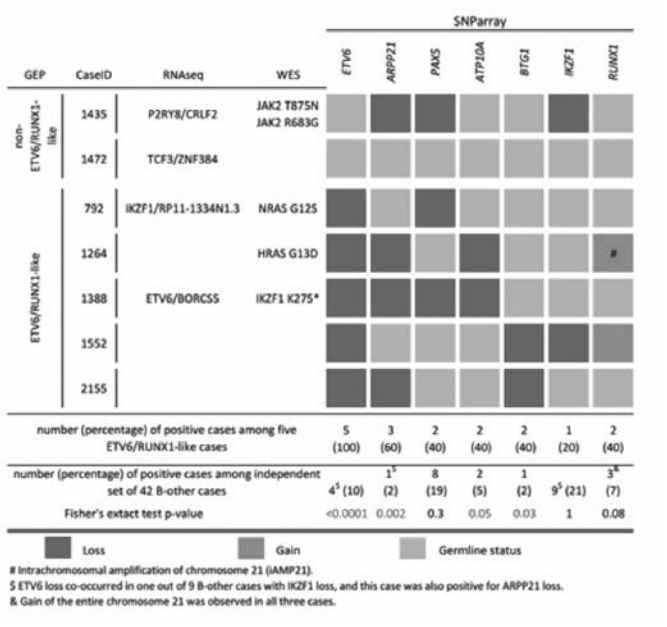

Figure 1.

Summary/Conclusions: We showed that similarly to *ETV6/RUNX1*-positive ALL, *ETV6/RUNX1*-like ALL is also associated with CD27^{pos}/CD44$^{low-neg}$ immunophenotype. We identified deletion of *ARPP21* to contribute to the specific genomic profile of *ETV6/RUNX1*-like ALL in addition to lesions of *ETV6*

and *IKZF1*. In conjunction with the single published study, our study establishes the *ETV6* lesion as the only common genetic aberration and thus the most likely key driver of *ETV6/RUNX1*-like ALL.

Supported by AZV 15-30626A, AZV 15-26588A, UNCE 204012, NPU I nr. LO1604 and the Project for Conceptual Development of Research Organization 00064203.

E824

Abstract withdrawn.

E825

GENETIC ALTERATIONS IN CHILDREN WITH T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA IN TAIWAN

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Background: The leukemogenesis of T-cell acute lymphoblastic leukemia (T-ALL) involves multistep processes of genetic alterations.
Aims: We aimed to determine the genetic alterations including common fusion transcripts, overexpression of T-cell transcription factor oncogenes and deletion or mutations of targeted genes in pediatric T-ALL in Taiwan as well as their impact on outcomes in those treated with TPOG-ALL-2002 protocol.
Methods: Between 1995 and 2015, bone marrow samples from 102 children (<18 years old) consecutively diagnosed with T-ALL were examined. *SIL-TAL1, MLL-ENL*, and *CALM-AF10* transcripts were detected by RT-PCR assays. RQ-PCR with TaqMan assays were used to measure the expression of *HOX11, TAL1,* and *LYL1* oncogenes expressed as normalized copy number (NCN) to *ABL* internal control gene. *TAL1* overexpression was defined as NCN > the lowest level of *SIL-TAL1* positive patients. Overexpression of *HOX11* and *LYL1* was defined as NCN > the upper limits of the 50 normal bone marrow controls. Mutations of *NOTCH1*, *FBXW7*, *PHF6*, *JAK1*, *JAK2*, *RUNX1*, *WT1*, *NRAS*, and *KRAS* genes were analyzed by PCR-based assays followed by direct sequencing. *P16* deletion was determined by RQ-PCR or multiplex ligase probe amplification (MLPA), *PTEN* and *PHF6* deletions, *MYB* duplication and *NUP214-ABL1* were detected by MLPA.
Results: The frequency of *SIL-TAL1* fusion transcript was 16.2%, *MLL*-rearranged 5.1%, *CALM-AF10* 1.0%. and no *NUP214-ABL1*. The frequency of *NOTCH1* mutations was 46.9%, *FBXW7* 13.0%, *RUNX1* 5.2%, *WT1* 6.3%, *NRAS* 6.2%, *KRAS* 2.1%, and no *JAK1* or *JAK2* mutations. *P16* deletion was present in 56.2%, *PTEN* in 11.1%, *PHF6* deletion/mutation in 13.4%, and *MYB* duplication in 4.8%. Overexpression of *TAL1* was present in 46.5%, 22% for *LYL1*, and 9% for *HOX11*. The correlation among the genetic alterations showed that *LYL1* overexpression occurred more frequently in *P16* wild-type compared with *P16*-deleted patients (P=0.0003) and absence of *SIL-TAL1* transcript was significantly associated with *LYL1* overexpression (P=0.018). A comparison of outcomes was made according to the status of each genetic abnormality. *NOTCH1* mutations conferred a favorable overall survival (OS) (P=0.025). *PHF*6 deletion/mutation conferred an inferior OS (P=0.030). *PTEN* deletion was associated with shorter relapse-free survival (RFS) (P<0.0001) and OS (P<0.0001). Status of other gene mutations, deletion or duplication did not influence the RFS or OS. *TAL1* overexpression predicted a higher risk of relapse (37% *vs* 21%, P=0.006), an inferior RFS (P=0.002) and OS (P=0.025) whereas *HOX11* or *LYL1* overexpression had no prognostic impact. By multivariate analysis, *NOTCH1* mutation did not reach statistical significance for an independent predictor of OS (HR 0.167, P=0.112), *PHF6* deletion/mutation was an independent unfavorable predictor for OS (HR 4.596, P=0.006), and *PTEN* deletion was also an independent predictor for both RFS (HR 29.493, P=0.007) and OS (HR 15.830, P=0.003). *TAL1* overexpression was an independent risk factor for both RFS (HR 3.699, P=0.014) and OS (HR 2.701, P=0.047).
Summary/Conclusions: The present study showed that *LYL1* overexpression was negatively associated with *SIL-TAL1* or *P16* deletion. *PHF6* deletion/mutation, *PTEN* deletion, and *TAL1* overexpression were the independent predictors of adverse outcomes. (Grants support: CORPG3C0201, MMH-E-105-09, NSC-101-2314-B-195-004-MY2, and Terry fox Foundation)

E826

COMPUTATIONAL METHODS TO FIND NEW THERAPEUTIC TARGETS IN ALL, SYSTEMATICAL IDENTIFICATION OF ESSENTIAL GENES

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Background: Deletion of chromosomal material is a hallmark of cancer genomes. While these lesions primarily target tumour suppressor genes, neighbouring genes are frequently co-deleted en passant. Loss of one copy(haploinsufficiency) of a neighbouring gene that is essential for the survival of the cancer cells may constitute potential therapeutic targets in that the cancer cells may be selectively sensitive to further suppression of the function of that gene. Identifying such vulnerabilities is one of the current challenges in cancer genomics. We show that vulnerabilities in cancer cells can be identified computationally by applying pattern recognition techniques to a copy-number dataset. This approach will identify genomic regions with potential essential genes. Genes in these regions can be evaluated downstream by genome editing techniques to find novel targets for treatments. Using pattern recognition techniques to find essential genes is a straight-forward, easily applied and non time-consuming method compared to genome wide experimental approaches.
Aims: Develop a computational framework to find regions with potential essential genes from copy-number data, with a primary focus on hematological malignancies and in particular ALL.
Methods: Our computational framework first selected regions of the tumour genome with heterozygous, but not homozygous, deletion. In sections flanking these regions we scanned for linear increases in homozygous deletion frequency. Genes near the start of these increases that have more than one case with homozygous deletion are discarded. Remaining genes were scored by calculating a line of best fit using the least square method towards the nearby peak in homozygous deletion. We sorted the results by settings cut-offs for the slope, amplitude and correlation coefficient of the linear regression line. Genes with the highest scores were then manually evaluated by comparing to known mean copy-number loss dependence score from other data-sets, by graphical visualisation and by investigation of their known function. The data set we analysed contains copy-numbers from tumour samples matched to normal blood samples or normal tissue from the same donor. To validate the essentiality of genes in the discovered regions we used pooled CRISPR/Cas9 editing in ALL cells with and without a deletion of the driving tumour suppressor.
Results: Our framework identified several regions with potential essential genes around well-known tumour suppressors. The strongest signals in the data set were located around the tumour suppressor *CDKN2A*. Downstream analysis with pooled CRISPR/Cas9 editing in ALL cells with and without a *CDKN2A* deletion provided evidence for the essentiality of several genes in this identified region, including one gene that was essential only in *CDKN2A*-deleted cells.
Summary/Conclusions: In conclusion, we explored a computational approach to identify regions with essential genes in copy-number datasets. Application of our approach to real data showed several regions with essential gene candidates around well-known tumour suppressors, indicating the framework works. Downstream genome-editing experiments in model cell-lines provided further evidence for the essentiality of some genes found in such identified regions. While we cannot yet draw conclusions on whether some of these genes are viable therapeutic targets it allows for informed guesses on limited sets of genes for further focused analysis in hematological model cell-lines.

E827

TARGETING ANTIOXIDANT ENZYMES FOR THE TREATMENT OF B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: B-cell acute lymphoblastic leukemia (B-ALL) is a genetically heterogeneous disease characterized by abnormal expansion of B cell precursors and is mainly affecting children and adolescents. The backbone of the treatment is chemotherapy providing high cure rates in pediatric ALL (> 85%) but much worse treatment response is observed in adolescents and adults (< 50%). Patients who relapse develop refractory, chemotherapy resistant disease and remain a clinical challenge. Growing body of evidence suggests that disturbance of redox homeostasis is a promising anticancer approach. Due to high metabolic demands and proliferation rate cancer cells elevate their antioxidative capacity to overcome excessive ROS production and depend on these antioxidants for their survival. One of the oxidative stress protectors are peroxiredoxins (PRDXs) that next to thioredoxins (TXNs) belong to the TXN-family and are the key components of TXN antioxidant system. PRDXs are enzymes involved in scavenging peroxides. TXNs are responsible for cysteine-thiol disulfide exchange in numerous protein substrates.
Aims: To investigate the potential of targeting the TXN antioxidant enzymes as a novel pro-oxidative strategy in B-ALL treatment.
Methods: We have used three different cell lines representing distinct cytogenetic subgroups of B-ALL: BV-173 (BCR-ABL), SEMK-2 (MLL-AF4) and NALM-6 (complex cytogenetics). ROS levels were measured using Cell ROX assay. RNA and protein levels of TXN-family enzymes were measured by quantitative PCR and immunoblotting, respectively. Downregulation of PRDX1 was established by a novel CRISPR/Cas9 gene editing system. We have employed lenti-

CRISPR v2 plasmid to produce lentiviral vectors encoding PRDX1-specific sgRNA and Cas-9 and used them to generate BV-173 cells with PRDX1 genomic deletion. Proliferation rate was evaluated by trypan blue exclusion method. Cytostatic/cytotoxic effects of TXN-family enzymes inhibitors, such as adenanthin (ADE), auranofin (AUR) and SK053 were assessed by MTT viability assay and by detection of propidium iodide-positive cells in flow cytometry.

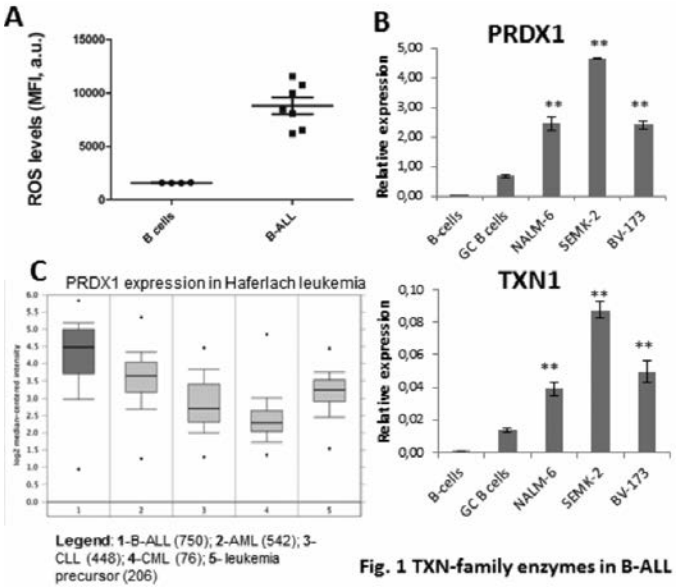

Figure 1.

Results: We have found that B-ALL cell lines exhibit significantly higher levels of ROS as compared to normal B cells isolated from human tonsils (Fig.1A). In accordance with this observation, our analysis of TXN antioxidant enzymes gene expression in B-ALL cell lines showed their upregulation (Fig.1B). Analysis of deposited data revealed that PRDX1 expression level is the highest in B-ALL among the other types of leukemia (Fig.1C). Moreover, we have observed elevated expression of PRDX1 in malignant lymphoblasts derived from pediatric patients at both RNA and protein levels. Genomic deletion of PRDX1 in BV-173 cells leads to suppression of their proliferation rate, comparing to parental cells and cells transduced with mammalian non-targeting sgRNA. These results allow us to suspect that PRDX1 may play growth-supporting role in these cells. Targeting TXN-family enzymes was also performed with the use of various small molecule inhibitors. Both B-ALL cell lines and primary cells are sensitive to PRDX and TXN inhibitors, which reduce cell viability in dose-dependent manner.

Summary/Conclusions: All the above results suggest that targeting TXN antioxidant system may exert desirable anticancer effects in the treatment of B-ALL. Inhibitors of TXN-family enzymes can be considered as putative agents to use in combination with classical drugs and improve existing therapeutic approaches. Further studies are underway.

E828

RNA-BINDING PROTEIN IGF2BP1 PROMOTES SURVIVAL OF ETV6/RUNX1 LEUKEMIA CELLS

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Background: The IGF2 mRNA binding protein 1 (IGF2BP1, other aliases IMP-1 (IMP1), CRD-BP (CRDBP), ZBP-1 (ZBP1), and VICKZ1) belongs to a family of regulatory RNA-binding proteins with an oncofetal expression pattern. IGF2BP1 has also been identified to be exclusively specific for ETV6/RUNX1-positive acute lymphoblastic leukemia (ALL) but biological significance of IGF2BP1 overexpression has not been thoroughly investigated to date (Andersson, Olofsson *et al.* 2005; Stoskus, Gineikiene *et al.* 2011). We have recently contributed by reporting that ETV6/RUNX1 transcript is a target of RNA-binding protein IGF2BP1 in t(12;21)(p13;q22)-positive ALL, suggesting a role of IGF2BP1 in ETV6/RUNX1-mediated leukemogenic events (Stoskus, Vaitkeviciene *et al.* 2016).

Aims: To define the biological significance of IGF2BP1 overexpression in t(12;21)(p13;q22) ETV6/RUNX1-positive ALL.

Methods: In this study we have used stable sublines with downregulated IGF2BP1 from our previously published study (Stoskus, Vaitkeviciene *et al.* 2016). Dynamics of viable cell population was assessed by flow cytometry using 7-AAD staining (BD Biosciences) following 72 hrs culture in complete medium. An EdU flow assay (Thermo Fisher Scientific, TFS) was used to assay DNA replication in proliferating cells. Spontaneous and doxorubicin (Doxo), staurosporine (STS), and STAT3 selective inhibitor S3I-201 (all from Santa Cruz Biotechnology) induced cell death rates were determined by Annexin V (TFS) and 7-AAD staining. All samples were analyzed on Accuri C6 cytometer (Accuri Cytometers) using CFlow Plus and FCS Express software (De Novo Software). IGF2BP1, ETV6/RUNX1, and STAT3 RT-qPCR was performed essentially as reported previously (Stoskus, Gineikiene *et al.* 2011). Statistical analyses performed using GraphPad Prism software (GraphPad Software).

Results: Downregulation of IGF2BP1 by 2-fold have rendered into approximately 2-fold lower population growth rate, increasing levels of spontaneous cell death in dynamics, and modest yet statistically significant attenuation of cell cycle progression (35.13% *vs* 40.40%, p<0.0001). Data from treatment with 50 nM of Doxo, 250 nM of STS suggest that IGF2BP1 downregulation has no effect on pharmacological effectiveness of these drugs. In contrast, IGF2BP1-downregulated cells are more sensitive to pharmacological inhibition of STAT3 even upon treatment with suboptimal 25 μM concentration of S3I-201. Lastly, we have probed if STAT3 transcript levels could be sustained by IGF2BP1 protein as in agreement with previously reported (Stohr, Kohn *et al.* 2012) and our unpublished insights from anti-IGF2BP1 RNA immunoprecipitation datasets. Correlation analysis of RT-qPCR data have confirmed these assumptions as downregulation of IGF2BP1 expression have resulted in a decrease of ETV6/RUNX1 mRNA (r2=0.8253, p<0.001, slope 0.9459) and also STAT3 transcript levels (r2=0.7709, p=0.002, slope 0.6436). These data suggest that STAT3 transcript is also a potentially regulated by RNA-binding protein IGF2BP1 in t(12;21)(p13;q22)-positive ALL model cells (Fig 1).

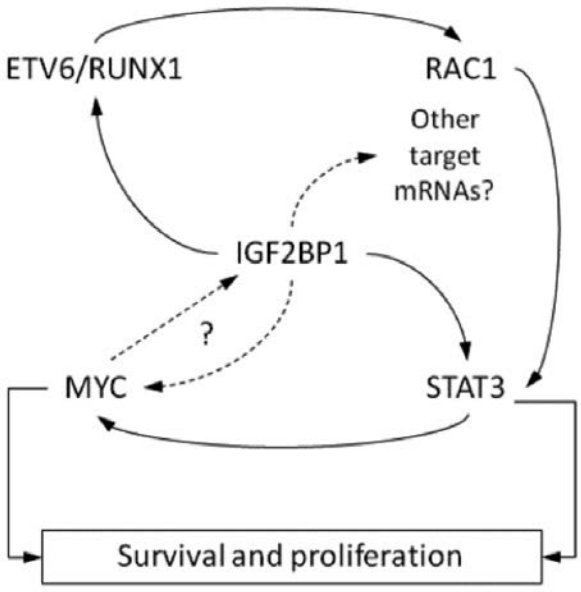

Figure 1.

Summary/Conclusions: We provide evidence that IGF2BP1 promotes survival of t(12;21)(p13;q22)-positive ALL model cells through cell cycle progression and preventing spontaneous cell death. Potentiation of ETV6/RUNX1®STAT3 signaling axis is one of the possible mechanisms responsible for this phenotype as IGF2BP1 maintains appropriate levels of primarily ETV6/RUNX1 and also STAT3 mRNAs. Further studies are clearly warranted to further delineate the role of IGF2BP1 in t(12;21)(p13;q22)-positive ALL (Stoskus, Eidukaite *et al.* 2016).

E829

6-MERCAPTOPURINE PROMOTES ENERGETIC FAILURE IN LEUKEMIC T-CELL LINE JURKAT

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Background: 6-Mercaptopurine (6-MP) is a thiopurine drug with antiproliferative effects by blocking purine synthesis. 6-MP is largely prescribed for the treatment of childhood acute lymphoblastic leukemia (ALL). Recent evidence

suggest that 6-MP inhibits the phosphatidylinositol 3 kinase (PI3K)/ mammalian target of Rapamycin (mTOR) signaling pathway and modulates the transcriptional activity of hypoxia inducible factor 1 α (HIF-1α). As mTOR and HIF-1α are key mediators of metabolic reprogramming in cancer and normal T cells we hypothesized that 6-MP can impact cellular metabolic remodeling through its action on nucleotide synthesis. Metabolic reprogramming fosters glycolysis, glutaminolysis, and biosynthesis of lipids and nucleotides to sustain cell growth and proliferation, a key feature of cancer cells. This metabolic switch is regulated by metabolic checkpoints, including mTOR, AMP-activated protein kinase (AMPK) and the oncogenes Myc and HIF-1α.

Aims: Our objective is to study the impact of the antiproliferative molecule 6-mercaptopurine (6-MP) on proliferating T-leukemia cells metabolic reprogramming.

Methods: *In vitro* experiments were performed in a Jurkat T cell line. Cells were incubated with 6-MP from 6h to 72h. We used RT-PCR, Western Blot, glucose uptake and glycolytic and glutaminolytic flux to evaluate the metabolic effects of 6-MP.

Results: Our results showed that 6-MP reduces ATP content as early as after 2 hours of treatment and this decrease is maintained up to 72 hours. As AMPK is an energetic sensor activated with low ATP content, we studied AMPK activation after 6-MP treatment. We observed that 6-MP treatment activates AMPK after 6 and 48 hours of treatment. Moreover, 6-MP significantly modifies the transcriptional reprogramming of genes implicated in glycolysis, glutaminolysis and nucleotide synthesis after 24, 48 and 72 hours of treatment. In addition, 6-MP inhibits the expression of the metabolic checkpoints mTOR, HIF-1α and Myc after 24, 48 and 72 hours of treatment. 6-MP also decreases glucose and glutamine oxidation after 48 hours of treatment by 60% and 35%, respectively, suggesting that 6-MP inhibited TCA (tricarboxylic acid cycle) and OXPHOS (oxidative phosphorylation). The production of lactate, a marker of aerobic glycolysis, is significantly decreased by 30% after 6-MP treatment for 48 hours, meaning that aerobic glycolysis is also inhibited. However, 6-MP has no effect on glucose uptake or on glucose transporters (Glut1 or Glut3, SLC2A1 or SLC2A) protein expression, suggesting that 6-MP metabolic effects are not linked to glucose uptake.

Summary/Conclusions: In conclusion, our findings offer new insights on the cellular effects of 6-MP treatment by promoting an early energetic stress that influence proliferation and raise apoptosis in leukemia T cells. Interestingly, the inhibition of the metabolic checkpoints (mTOR, HIF-1α, Myc) and the diminution of glycolytic and glutaminolytic fluxes by 6-MP treatment provide an original approach to better understand the cellular effects of 6-MP treatment.

E830

GENETIC ABERRATIONS IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA AND THEIR IMPACT ON CLINICAL OUTCOME

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Background: Genetic alterations have prognostic impact on pediatric patients with B cell acute lymphoblastic leukemia (B-ALL). Genomic landscape and its impact on clinical outcome is less understood in adults with B-ALL.

Aims: To describe the landscape of genomic aberrations and analyze the correlation with clinical characteristics and prognostic impact in adults with B-ALL.

Methods: We assessed bone marrow specimens from 64 consecutive adults with a median age of 51 years (range 18 to 80) with previously untreated B-ALL between 2012 and 2015. The cohort included 23 Philadelphia chromosome (ph)-positive and 41 Ph- negative B-ALL. Sixty patients (94%) were treated with Hyper-CVAD (hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone alternating with high-dose methotrexate and cytarabine)-based regimen; 23 of them received additional tyrosine kinase inhibitor for Ph-positive disease (dasatinib [N=4] or ponatinib [N=19]). Four patients (6%) were treated with augmented BFM (Berline-Frankfurt-Munster) regimen. Genomic DNA extracted from pre-treatment bone marrow specimens were sequenced by targeted capture exome sequencing of 295 genes that are recurrently mutated in hematologic malignancies (median 280x coverage). The panel included more than 1000 cytoSNP position evenly distributed among the genome that allowed estimation of copy number variations (CNVs). Point mutations were called by modified Mutect/Pindel algorithm.

Results: Among the 64 patients, we detected 70 point mutations in 40 genes in 38 patients (54%). Ph-positive ALL had significantly less point mutations than Ph-negative ALL (median number of mutations/patient 0 [range: 0-2] *versus* 1 [range: 0-6], P=0.002). The most frequently mutated genes were *TP53* (17%) followed by *DNMT3A* (9%), *JAK2* (6%), *NRAS* (6%), *NF1* (5%), *PAX5* (5%), *RUNX1* (5%), and *TET2* (5%). *TP53* mutations were strongly associated with Ph-negative B-ALL (P=0.004) and low hypoploidy (P=0.009). Recurrent CNVs involved loss/deletion in genes such as *PAX5* (38%), *TCF3* (38%), *IKZF1* (31%), *CDKN2A/2B* (31%), *BTLA* (25%), *CD200* (22%), *ETV6* (22%), *RB1* (20%), *mir15*-a/*16*-1 (15%), *ERG* (14%), and *MLLT3* (11%), whereas gain/amplification was detected in *NR3C2* (18%), *ERG* (15%), *RUNX1* (15%), and *LEF1* (14%). *MLLT3* loss/deletion was specific to Ph-negative- ALL (0% for Ph-positive *versus* 17% for Ph-negative, P=0.036) and Mir15-a/16-1 loss/deletion had non-statistically significant association with Ph- BALL (4% *versus* 22% respectively, P=0.06). In this cohort, 78% and 100% of the Ph-negative and Ph-positive ALL achieved complete remission, respectively. None of the point mutations or CNVs were associated with differential response to therapy. Survival analysis was stratified by Ph status. Complex karyotype had trend toward worse event-free survival (EFS) (median EFS 3.6 months *versus* 26.3 months, P=0.06) in Ph-negative ALL. None of the point mutations or CNVs were associated with EFS/overall survival (OS) in Ph-negative ALL. Notably, *TP53* mutation nor low hypoploidy did not affect EFS/OS in the current cohort. In Ph-positive BALL, *IKZF1* deletion/loss was associated with a trend toward worse EFS (median EFS 3.6 months *versus* 21.3 months, P=0.07) but it did not affect OS.

Summary/Conclusions: Genetic analysis highlights the molecular heterogeneity of adult B-ALL. Adult B-ALL is frequently associated with CNVs and point mutations are less frequent. Prognostic impact of genetic alteration in adult B-ALL appears to be limited except for *IKZF1* deletion/loss, which may predict worse EFS in Ph-positive BALL.

E831

PROFILING OF RECURRENT COPY NUMBER ALTERATIONS IN RELAPSED ADULT B CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: The survival rate of relapsed adult acute lymphoblastic leukemia (ALL) is around 10%.

Aims: We looked for recurrent Copy Number Alterations (CNA) in relapsed adult B cell progenitor ALL (BCP-ALL) to shed light into the molecular mechanisms of relapse.

Methods: BM or PB samples with at least 30% of blasts from 31 adult BCP-ALL patients at 1st relapse and, of them, 21 paired diagnosis and relapse samples were analysed by MLPA (MRC-Holland, The Netherlands). 19 out of these 21 paired samples were analysed by SNP array with CytoScan HD chips (Affymetrix, Santa Clara, California, USA). True CNA were considered when encompassed a minimum of 25 markers, and 25 markers and ≥20Mb for CN-LOH.

Table 1.

REGION (n=21)	DELETION (n=11)	DUPLICATION (n=10)	RELEVANT GENES
1q24.2-q41		4/15	*KIF14, ABL2, CD34, DHX9, PLA2G4A, PTGS2, ASPM, SMYD2, KCNH1, MAPKAPK2, CENPF, PTPRC, KDM5B, MDM4, CDK18*
1q42.3		4/15	*ERO1LB*
7p12.2	5/15		*IKZF1*
8q21.3-q24.3		4/15	*BAALC, MYC, CCNE2, ENPP2, EBAG9, NBN, CTHRC1, TNFRSF11B, PTK2, PSCA, BOP1, RECQL4, MAPK15*
9p13.2	9/15		*PAX5*
9p21.3	15/15		*CDKN2A/B (11/15, 73% homozygous)*
9p21.3	14/15		*MTAP*
9p21.3	9/15		*MLLT3*
9p23	7/15		*PTPRD*
9q34.12	4/15		*PRDM12*
11q22.3-q23.1	4/15		*CASP12/4/5, NPAT, ATM, DDX10, hsa-miR-34b/c, BTG4*
12p13.2	4/15		*ETV6*
13q14.2	4/15		*ST13P4, DLEU2*
17q12-qter		4/15	*CSNK1D, ABCC3, CDK12, STAT5B, STAT5A, STAT3, AATF, LASP1, PPP1R1B, STARD3, ERBB2, CASC3, hsa-miR-21, GRN, GAST, BIRC5, NMT1, IGF2BP1, DLX4, COL1A1, MLF, MSI2, TRIM37, PPM1D, BCAS3, TBX2, DDX5, GRB2, TIMP2, RNF213, RAC3, SLC16A3, HDAC5, ABCA8, ABCA9, ABCA6, ABCA10, ABCA5, MLLT6, SEPT9, CCL4, BCAS1, CDC6, CCR10, BRCA1, ETV4, WNT3, CDC27, HOXB2, HOXB3, HOXB4, HOXB5, HOXB6, HOXB7, HOXB8, HOXB9, HOXB13, PDK2, CDK3, SRSF2, CBX2, CBX8, CBX4, SPHK1*
21+		4/15	*RUNX1, ETS2, TIAM1, OLIG2, TFF3, TMPRSS3, CSTB, PTTG1IP, S100B, hsa-miR-155, SETD4, ABCG1, ERG, DNMT3L, USP16, CBR1, ITGB2*
22q11.22	4/15		*VPREB1*
X/YPAR1		4/15	*CRLF2*
X/YPAR1		5/15	*IL3RA*
Xp22.11-p11.1		4/15	*USP9X, hsa-miR-221, hsa-miR-222, GATA1, ARAF, ELK1, CDK16, USP11, HDAC6, PIM2, SMC1A, PHF8, USP51, KLF8, FOXR2*
Xp22.33-p22.11		5/15	*TBL1X, CDKL5, EIF1AX, PDK3*
Xq28		4/15	*L1CAM, DKC1, RPL10, IRAK1, TAZ, IKBKG, IL9R, BRCC3*

Results: With a median follow up of 12.43 [2.4;30.3] months, the median OS of the 31 patients at first relapse was 7.9 months, [2,4;13,5]. The OS of patients at first relapse was significantly lower in those having more than 3 CNA by MLPA (median ≤3 CNA 9.7 months [0-20.7] *vs* median >3 CNA 4.2 months (0.6-7.8), p=0.042). CDKN2A/B deletion was the most common CNA observed at relapse (16/31, 52%) and most of these deletions were homozygous (12/16, 75%). Comparing paired CDKN2A/B deletions, homozygous were more frequent at relapse (from 8 heterozygous CDKN2A/B deleted patients at diagnosis, 7 became homozygous at relapse, p=0.070). SNP arrays detected 554 CNA (409 DEL, 125 DUP and 20 LOH) in 34 samples of 19 patients. At diagnosis (n= 16 patients) the mean number of CNA was 12.3 (9.6 DEL, 2.3 DUP and 0.4 LOH) while in first relapse (n=13 patients) was 17.8 CNA (12.6 DEL, 4.2 DUP and 1 LOH) and in second relapse (n=5 patients) was 21 CNA (14.6 DEL, 6.4 DUP and 0 LOH)(p=0.007). All matched diagnosis and first relapse samples (available for 10 patients) showed common CNA. In 6/10 cases some of CNA were retained from diagnosis while others were acquired or lost at relapse (suggesting evolution from an ancestral clone), 3/10 shared all CNA from diagnosis and acquired new CNA at relapse (indicating an evolution from diagnosis clone) and 1/10 showed the same CNA signature at relapse (suggesting a primary resistance of the diagnosis clone). Gene ontology analysis showed a significant enrichment of gene deletions involving B cell differentiation, activation and proliferation, and regulation of cytokine-mediated signaling pathway at relapse (Benjiamini Hochberg test, p<0.01). Table 1 summarizes the frequencies of the most retained or acquired CNA at relapse in at least 4 out of 15 patients. Besides the high genetic heterogeneity observed, some recurrent CNA could be identified such as 9p, 11q, 12p, 22q and 7p deletions and 1q, 8q, 17q, 21+ and Xp duplications. In addition, deletions in important tumor suppressor genes such as *TP53*, *FOXO1*, *FOXO3* or *RB1* were detected in 3 patients.
Summary/Conclusions: BCP-ALL has a high genetic heterogeneity at relapse, with most of the genetic alterations playing important roles for disease progression. This heterogeneity points out the need for search of personalized treatments for patients depending on their molecular alterations. Financial support: Instituto de Salud Carlos III, Ministerio de Economía y Competividad, Spain, Red Temática de Investigación Cooperativa en Cáncer (RTICC, FEDER) (RD12/0036/0044); Sociedad Española Hematología y Hemoterapia; 2014 SGR225 (GRE) Generalitat de Catalunya; Fundació Internacional Josep Carreras, Celgene Spain and "la Caixa" Foundation.

E832

IGF1R/IRS PHARMACOLOGICAL INHIBITION REDUCES CELL PROLIFERATION AND MIGRATION IN ACUTE LYMPHOBLASTIC LEUKEMIA CELLS

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Background: A recurrent clinical complication of the acute lymphoblastic leukemia (ALL) is the infiltration of lymphoblast into central nervous system. The IGF1/IGF1R signaling pathway is initiated through binding of the ligand (IGF1) to its transmembrane receptor (IGF1R), and the subsequent activation of its substrates, IRS1 and IRS2, which transmit mitogenic and antiapoptotic signals, mainly through the modulation of the PI3K/AKT/mTOR and MAPK signaling pathways. These signaling pathways play an important function in cell proliferation, survival and migration of leukemia cells. We have previously noticed that NT157 (IRS1/2 pharmacological inhibitor) significantly decreased cell viability and induced apoptosis in T-ALL (Jurkat and MOLT-4), and in B-ALL (Namalwa and Raji) cell lines and in primary ALL cells (T-ALL [n=2] and B-ALL [n=2]), although did not presented citotoxicity in peripheral blood mononuclear cells (PBMC) from healthy donors. In addition, NT157 was able to induce the p21 (*CDKN1A*) expression, which is a cell cycle arrest-related gene. We also observed that OSI-906 (IGF1R/IR pharmacological inhibitor) significantly reduced cell viability, but did not induce apoptosis in ALL cell lines tested, and did not modulate viability and apoptosis of primary ALL cells and normal PBMC. The molecular mechanism by which leukemia cells break the blood-brain barrier, allowing the infiltration of the central nervous system and causing serious complications remains poorly understood.
Aims: We herein aimed to investigate the impact of the pharmacological IGF1R/IR and IRS1/2 inhibition on cell proliferation and migration in ALL cells.
Methods: T-ALL Jurkat and B-ALL Namalwa were used. Cell lines were treated or not with NT157 at 0.2, 0.4, 0.8, 1.6 and 3.2μM, or with OSI-906 at 1, 5, 10 and 20μM for 24 and 48 hours. After drug exposure, cell lines were evaluated for cell proliferation (Ki-67 assay), migration (Time-Lapse microscopy analysis) and cell adhesion (using human umbilical vein endothelial cells HUVEC monolayer). Statistical analyzes were performed by the ANOVA. *P* value <0.05 was considered statistically significant.
Results: In Jurkat and Nalmawa cells, NT157 strongly reduces cell proliferation in a dose-dependent manner ($p<0.05$) after 24 hours of treatment. OSI-906 was not able to reduce cell proliferation in these cell lines. The 24 hours treatment with 10μM OSI-906 decreased accumulated distance (μm) and velocity (μm/min), while 0.4μM NT157 reduces only the accumulated distance of Jurkat cells under migration assay into fibronectin monolayer, after being filmed by time-lapse microscopy for 3 hours; the images were captured every 1.5 minutes. Although there is a trend for reduction, cell adhesion between Jurkat and Nalmawa leukemia cells and the human endothelial cell monolayer was not significantly modulated by treatment with both inhibitors.
Summary/Conclusions: The reduction on cell proliferation found during IRS/2 pharmacological inhibition reaffirms the important role of these proteins on malignant phenotype of ALL cells. Migration analysis indicated that NT157 and OSI-906 are potential inhibitors of transendothelial migration in ALL cell lines and contribute with new perspectives on the participation of the IGF1R/IRS1 pathway in the break of the blood-brain barrier.

E833

LEUKEMIA-PROPAGATING CELLS DEMONSTRATED DISTINCTIVE GENE EXPRESSION PROFILES COMPARED WITH THE OTHER CELL FRACTIONS IN PATIENTS WITH DE NOVO PHILADELPHIA CHROMOSOME-POSITIVE ALL

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Background: Relapse remains one of the major obstacles in Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph^+ALL) even after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Relapse of Ph^+ALL may result from the persistence of leukemia-propagating cells (LPCs), which are defined by their ability to initiate human leukemia and self-renew in immunocompromised mice. In acute myeloid leukemia, higher LPCs frequencies and a gene expression profile typical of LPCs at diagnosis are predictive of unfavorable clinical outcomes. In Ph^+ALL, we recently reported that LPCs are enriched in the $CD34^+CD38^-CD58^-$ fraction using a xenograft assay. Moreover, our cohort study indicate that the LPCs phenotype at diagnosis is an independent risk factor for relapse in Ph^+ALL. However, little is known about the differential gene expression profiles between LPCs and the other cell fractions in *de novo* Ph^+ALL patients.
Aims: To identify the potential molecular basis of LPCs-mediated relapse, the gene expression profiles of the sorted LPCs and other cell fractions from patients with *de novo* Ph^+ALL were compared.
Methods: Twenty patients with *de novo* Ph^+ALL were enrolled for this study at Peking University Institute of Hematology from 2015 to 2016. The LPCs ($CD34^+CD38^-CD58^-$) and other cell fractions (including $CD34^+CD38^-CD58^+$, $CD34^+CD38^+CD58^-$and $CD34^+CD38^+CD58^+$) were sorted from the bone marrow mononuclear cells of *de novo* Ph^+ALL patients (N=3) using a FACS Aria II. Differential expression analysis between LPCs and the other cell fractions were performed using RNA sequencing (RNA-Seq) and the DESeq R package (1.10.1), Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. RNA-Seq results were partially validated by a TaqMan-based real-time quantitative polymerase chain reaction (qRT-PCR) technique. Moreover, cell cycle status was compared between LPCs and other cell fractions in *de novo* Ph^+ALL patients (N=20) by flow cytometry.
Results: 1021 genes (301 up-regulated and 720 down-regulated), 1245 genes (354 up-regulated and 891 down-regulated) and 1228 genes (248 up-regulated and 980 down-regulated) were differentially expressed between LPC_1 and Other $Cell_1$ (patient No 1), LPC_2 and Other $Cell_2$ (patient No 2), and LPC_3 and Other $Cell_3$ (patient No 3), respectively. Most of differential expression of genes (DEGs) are related to the regulation of cell cycle and metabolism. GO analysis identified enriched terms of biological functions in DEGs including ATP binding process, ribonucleotide binding process, nucleoside binding process, DNA replication process, primary metabolic process, etc. KEGG analysis showed significantly enriched signaling pathways involved in DEGs including cell cycle, DNA replication, nucleotide metabolic pathways, biosynthesis of amino acids, glutathione metabolism, p53 signaling pathway, etc. Consistent with RNA-Seq results, mRNA levels of the cell cycle-related genes, such as *CDK4* and *HMGB1,* were significantly lower in LPCs fraction than those in other cell fractions. Moreover, the frequencies of quiescent cells in LPCs were significantly higher than those in other cell fractions.
Summary/Conclusions: Distinctive gene expression profiles and cluster, which are mostly related to the regulation of cell cycle and metabolism, were demonstrated between LPCs and other cell fractions in patients with de novo Ph^+ALL. Therefore, our data indicate that it would be of value to develop LPCs biomarkers to contribute to personalized leukemia therapy and the need to identify therapeutic targets directed toward LPCs in Ph^+ALL.

E834

T-CELL LEUKEMIA SENSITIVITY TO FARNESYL TRANSFERASE INHIBITION USING TIPIFARNIB

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Background: T-cell leukemia is a collection of aggressive disorders with unfavorable outcome, in which targeted treatments are still at a preliminary phase. The RAS/MAPK pathway is crucial for TCR signaling of T-cells and it is deregulated in T cell acute lymphoblastic leukemia/lymphoma (T-ALL). Farnesyl transferase inhibitors (FTIs) block the localization of some RAS proteins to the intracellular membrane, thereby inhibiting their activation. Tipifarnib is a potent and specific FTI with a prominent anti-proliferative effect in some *RAS* mutated cells.
Aims: This study test tipifarnib in T-cell lines for *in vitro* sensitivity and for biomarker discovery, both genomic and immunohistochemical.
Methods: We selected those cell lines with available genomic data from COSMIC, CCLE or generated by our group. The MAPK, NFAT, NFKB and JAK/STAT pathways were tested by immunohistochemical analysis over FFPE-cell lines at baseline. The range of drug concentrations to perform IC50 analysis was established between 0-10,000 nM (ten points). Cell proliferation analyses were performed using CellTiter-Glo® Luminescent Cell Viability Assay kit from Promega (Madison, WI, USA), following manufacturer´s instructions at 0h, 48h and 96h. All experiments were done in sextuplet and all numerical data were expressed as the average of the values ± the standard error of the mean. IC50 analyses were performed with GraphPad Prism v5. Clinically-relevant drug sensitivity was defined as IC50 <100nM at 96h. Targeted sequencing was performed in 16 genes known to play a potential role in tumorigenesis in T-cell leukemias.
Results: 59.1% (n=13) of cell lines were sensitive to tipifarnib at concentrations which are readily achievable in the clinic (*i.e.* IC_{50} <100nM at 96h). 45.5%, 50% and 27.3% of cell lines harbored mutations in RAS, RAS-guanine nucleotide exchange factors (GEFs) and RAS-GTPase activating proteins (GAPs) genes, respectively. The mutational state of RAS (p=0.38), RAS-GEFs (p=0.192) and RAS-GAPs (p=1.0) genes were not associated with drug sensitivity. Strikingly, the mutational state of *NOTCH1* was associated with tipifarnib sensitivity. The activation of the MAPK pathway biomarker, ERK, was significantly associated (p=0.046) with drug sensitivity. Conversely, RelB (NFKB pathway) was associated with drug resistance (p=0.007). The same findings were observed with the presence of mutations in RAS-GEFs genes and *NOTCH1* and ERK activation (p=0.015 and p=0.023) and the absence of RelB (p=0.02 and p=0.017).
Summary/Conclusions: This study shows tipifarnib as a potential therapeutic option in T-cell leukemias. The mutational state of *NOTCH1* could constitute a predictor of sensitivity in T-cell leukemias. Furthermore, p-ERK and RelB could serve as potential biomarkers of tipifarnib sensitivity and resistance, respectively.

Acute lymphoblastic leukemia - Clinical

E835

HOSPITALIZATION FOR PATIENTS IN THE U.S. AND EU TREATED WITH INOTUZUMAB OZOGAMICIN *VS* STANDARD OF CARE FOR RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA IN A GLOBAL PHASE 3 TRIAL

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Background: Inotuzumab Ozogamicin (InO), an anti-CD22 antibody-calicheamicin conjugate, with its once a week one-hour infusion schedule, has demonstrated lower hospital utilization, in association with a clinically meaningful improvement in overall survival, high rate of complete remission, favorable patient-reported outcomes (PRO), and generally manageable safety profile *versus* standard of care (SOC, intensive chemotherapy) for relapsed/refractory acute lymphoblastic leukemia (R/R ALL) in the phase 3 INO-VATE trial.
Aims: This study aims to determine the regional-specific hospitalization days per patient in the INO-VATE trial.
Methods: Patients receiving study treatment (safety population) and recruited from the US and the EU were included in the analyses. The total number of days hospitalized for each patient was calculated. Hospital days prior to randomization and those after the end of study treatment were excluded. Due to different durations of treatment for InO and SOC (median 1 *vs* 3 cycles), calculations were reported for cycle 1 treatment period (randomization to end of cycle 1) and for the entire treatment period (all cycles - randomization to end of treatment).
Results: A total of 264 patients from the safety population of the phase 3 INO-VATE trial were available for the analyses. 149 were from the US, and 115 from 11 of the EU countries. The percentage of patients requiring hospitalization was lower for InO compared to SOC (Table). The median and mean hospitalization days were shorter for patients in the InO arm compared to the SOC arm across both regions. The difference between the two treatment arms appears to be greater in the US compared to the EU. Hospitalizations in the US appear to be shorter than in the EU, particularly for patients receiving InO.

Table 1. Hospitalizations in R/R ALL patients from the INO-VATE trial.

	Hospitalized N (%)	Mean (Days)	Median (Days) (Min, Max)	Hospitalized N (%)	Mean (Days)	Median (Days) (Min, Max)
	InO (Total N=75)			*SOC (Total N=74)*		
US						
Cycle 1	51 (68%)	11	7 (1, 48)	72 (97%)	23	23 (1, 55)
All cycles	57 (76%)	15	10 (2,53)	72 (97%)	26	25 (4, 81)
EU	*InO (Total N=61)*			*SOC (Total N=54)*		
Cycle 1	46 (75%)	17	15 (3, 56)	48 (89%)	26	27 (3, 43)
All cycles	51 (84%)	25	18 (3, 107)	48 (89%)	33	31 (3, 76)

Summary/Conclusions: InO treatment in R/R ALL is associated with less hospitalization across both the US and EU compared to SOC, consistent with InO's better efficacy, tolerability, PRO and dosing schedule. The finding that US has lower hospitalization than the EU might be explained by different patient care practices in the two regions. Given that hospitalization is the biggest cost driver in cancer care, the data suggest both EU and US could benefit from cost-savings of less hospitalization with InO treatment.

E836

NON-INTENSIVE BUT NON-INTERRUPTIVE TREATMENT WITH FEWER ALLO-HSCT IS EFFECTIVE STRATEGY FOR ADULT PH-NEGATIVE B-CELL PRECURSOR (BCP-) ALL: OUTCOME OF THE RUSSIAN PROSPECTIVE MULTICENTER ALL-2009 STUDY

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Background: As Ph-negative-BCP-ALL in adults remains less favorable in prognosis than T-ALL, and by expert opinion needs intensive protocols with high portion of allo-HSCT, the results of treatment based on the different approach: de-escalated but non-interruptive treatment with low numbers of allo-HSCT- may be of interest and can provide new insights to the common view.
Aims: to evaluate survival data and risk groups in Ph-neg-BCP-ALL pts in the RALL-study.

Methods: The ALL-2009 (NCT01193933) was initiated in Apr2009. The treatment plan was identical for all risk groups with allo-HSCT indicated only for very high-risk BCP-ALL (t(4;11), t(1;19); WBC >100). Since Apr 2009 till Dec 2016, 329 Ph-negative ALL pts (m.age 28 y (15-55), f/m 147/182) were recruited. Phenotype was unknown in 6 pts, biphenotypic AL was diagnosed in–1,2% (n=4), T-ALL/LBL–in 38,7% (n=125), BCP-ALL-in 59,1% (n=194). Among BCP-ALL there were 54 early pre-B ALL (27,8%), 101 common-ALL (52%), 39 pre-B ALL (20,2%). In BCP-ALL pts m.age was 27 y (15-54), f/m 99/95, initial WBC -9,4*10^9/l (0,4-899,0), LDH 901 IU (31-13059), CNS leukemia- in 17 pts (8,7%), mediastinal mass- in 3 (1,5%), splenomegaly– in 111 (57,2%). Standard cytogenetics was done in 124 pts (64%), 11 had no mitosis, so information is available in 58,2% (n=113). 43,4% of BCP-ALL (n=49/113) pts had normal karyotype (NK); 7,9% (n=9) and 1,8% (n=2) - had t(4;11) and t(1;19) respectively; other abnormalities were detected in 53 (46,9%), including p53 (3,2%), +8 (6,3%), complex karyotype (7,9%), high hyperploidy (16%), delp16 (22,2%), etc. 9 BCP-ALL patients (n=4,7%) were not qualified by the risk in the data-base; 68,1% (n=126) were attributed to the high risk (HR) group (WBC ≥30; EGIL BI, LDH>2N; late CR; t(4;11)-pos). The analysis was performed in Feb 2017. 191 pts were available for induction outcomes, DFS and relapse probability (RP), and all pts – for overall survival (OS).
Results: CR rate in 191 pts was 87,4% (n=167), induction death occurred in 8,9% (n=17), resistance was registered in 3,7% (n=9). Late responders constituted 13,6% (n=26). Death in CR on chemotherapy was 6,3% (n=12) and 1 death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (7,4%), 11 of them – in 1CR. Totally 59 pts (34,9%) had relapsed. At 7y OS for the whole cohort constituted – 54,3%, DFS– 56.5%, RP– 35,4%. In a multivariate analysis for BCP-ALL among common risk factors (age >30y, initial risk group, WBC >30, LDH>2N, immunophenotype, late CR >35d, CNS leukemia, cytogenetics) age, WBC, t(4;11) became statistically significant for OS, DFS and RP. We developed a new threshold for the most valuable risk factors. New risk groups stratification demonstrated 7y OS=79%, DFS=71%, RP=23% in the standard risk (SR) group (age <27y,WBC <75*10^9/l, no t(4;11)) and 46%,45%,47%, respectively, in the HR group (age >27y,WBC>75*10^9/l, t(4;11)).

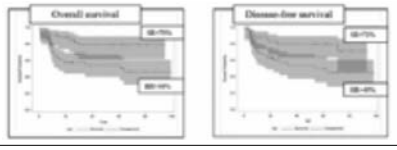

Figure 1.

Summary/Conclusions: Our data demonstrate that non-intensive but non-interruptive treatment with fewer allo-HSCTs is rather effective in adult BCP-ALL producing more than 50% OS at 7 years, though the RP is high. In our study among common risk factors only age, initial WBC and t(4;11) - remained the most valuable markers of poorer prognosis, while immunophenotype, time to CR, CNS involvement, and other cytogenetic markers did not matter. So RALL protocol without intensive highly myelosuppressive consolidation courses and high portion of allogeneic HSCT, may become an alternative and reproducible approach for adult Ph-negative ALL.

E837

POST-INDUCTION MINIMAL RESIDUAL DISEASE RESPONSE DETERMINED BY MULTICOLOR FLOW CYTOMETRY IS A POWERFUL INDICATOR OF EVENT-FREE-SURVIVAL IN THE CHILDHOOD T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Minimal residual disease (MRD) is a powerful predictor of event-free survival in acute leukemia including T-cell acute lymphoblastic leukemia (T-ALL). Due to lower incidence of T-ALL, MRD studies are limited and restricted to a small cohort of patients. Moreover, flowcytometry based MRD (FC-MRD) studies in T-ALL are very few. AIEOP-BFM group showed that late (Day-78) MRD response determines overall risk-of-relapse and event-free-survival (EFS) using RQ-PCR. However, a larger study by COG (Brent Wood *et al.* ASH, 2014) showed that post-induction (Day-29) FC-MRD was more relevant in the prediction of EFS. This indicates that the best time for MRD evaluation for the risk stratification in T-ALL is still not clear and need more studies. We investigated the value of post-induction FC-MRD response in an assessment of EFS in childhood T-ALL. It is a first T-ALL MRD study from India.
Aims: To investigate the value of 10-color flow cytometry based MRD response in the assessment of EFS in childhood T-ALL
Methods: We studied post-induction (Day-35) MRD (PI-MRD) & post-consolidation (Day-78) MRD (PC-MRD) in bone marrow samples from 100 patients of T-ALL treated under modified MCP-841 protocol between 2014 & 2016. In T-ALL with early-thymic-precursor (ETP) immunophenotype, patients received dexamethasone in place of prednisolone. MRD was performed using 10-color FC-MRD assay on Navios flow-cytometer (Beckman Coulter, BC) and MRD analysis was performed with Kaluza software v-1.3 (BC). Any detectable level of MRD (≥20 events) was defined as MRD-positive. Events included relapse & disease related deaths. Statistical analysis was performed using SPSS v.16.
Results: The median age of patients was 11.5 years (range 2–16 y; M:F–4.6). Based on the immunophenotypic criteria, 13 patients were diagnosed as ETPALL & remaining 87 as not-ETPALL type. PI-MRD was positive in 58/100 (58%) with the median level of 0.23% (range, 0.002% to 6%). PC-MRD was not performed in 71.4% (30/42) of PI-MRD-negative & 1.2% (6/58) PI-MRD-positive patients. PC-MRD was available in 64 patients (30/42 of PI-MRD-negative & 6/58 of PI-MRD-positive). PC-MRD was positive in 28% (18/64) (median, 0.2% &range, 0.009% to 4%). PI-MRD positivity was significantly high in ETPALL as compared to non-ETPALL (93% *vs* 53%; *p*=0.01). Median follow-up of all patients was 14 months (3-38 months). Patients were categorized MRD standard-risk (MRD-SR) if PI-MRD was negative and MRD high-risk (MRD-HR) if PI-MRD was positive with any level. Thus, 42% were categorized as MRD-SR & 58% as MRD-HR. Twenty patients relapsed & of them, six died (2 were ETPALL & 18 non-ETPALL; 3 MRDSR & 17 MRD-HR) within 26 months. Median EFS of MRD-HR patients was significantly inferior as compared to MRD-SR (26 months *vs* did not reach; & 70.67% *vs* 92.86%; *p*=0.0017) (Kaplan-Mayer curve shown in Figure 1). Interestingly, there was no difference in EFS for PI-MRD <0.01% *vs* >0.01%, suggesting any level of PI-MRD positive indicated inferior EFS. Furthermore, the PC-MRD response was not found to be significant over PI-MRD (P-value=0.17). ETP *vs* non-ETP status was also not found to be associated with EFS (P-value=0.85).

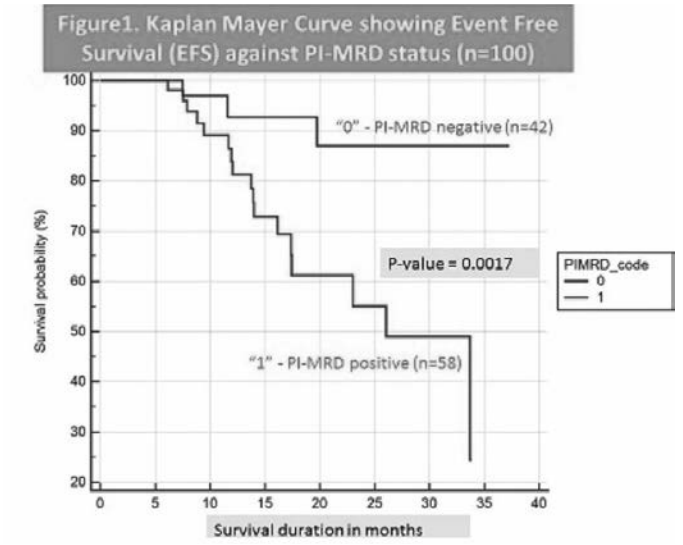

Figure 1.

Summary/Conclusions: We concluded that 10-color FC-based post-induction MRD response is a powerful indicator of EFS in childhood T-ALL. The frequency of PI-MRD positivity was significantly high in ETPALL indicating a lower tumor clearance rate. There was no difference in the EFS based on the level of PI-MRD-positivity indicating even a low level (<0.01%) PI-MRD is important in risk-stratification of childhood-TALL.

E838

SMAC MIMETICS - A NOVEL THERAPEUTIC APPROACH IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Pediatric acute lymphoblastic leukemia (ALL) is one of the most common malignancies in childhood. Survival rates have increased enormously over the past decades, but the prognosis for patients with relapsed ALL or ALL resistant to conventional chemotherapy remains poor. Thus, novel therapeutic options are urgently required. The family of inhibitor of apoptosis proteins (IAPs) has been shown to play an important role in the prevention of cell death, and to mediate gene activation important for cell survival. Many of the cellular processes regulated by IAPs are deregulated in cancer. Thus, IAPs represent a promising target in anticancer therapy. IAP antagonists, also known as Smac Mimetics (SMs), were developed to counteract IAPs' function. SMs have been shown to induce cell death in a number of different cancer entities, amongst them B cell precursor (BCP)-ALL. In BCP-ALL, SM-induced cell death was

shown to depend on autocrine TNF secretion. Of note, only a subset of BCP-ALL cell lines and primografts showed sensitivity to SM treatment. Little is known about the underlying molecular mechanisms conferring resistance to SM treatment.
Aims: Evaluation of the efficacy of different SMs in inducing cell death in BCP-ALL and T-ALL cell lines. Identification of the underlying molecular resistance mechanisms to SM treatment in BCP-ALL and T-ALL cell lines.
Methods: Cell death induced by SMs AT406 (Debiopharm Int.), LCL161 (Novartis), Birinapant (Medivir) and BV6 (Genentech) was evaluated by FSC/SSC in the BCP-ALL cell lines Nalm6, Reh, UoCB6 and RS4;11 and in the T-ALL cell lines ALL-SIL, CEM, Jurkat and Molt4. Expression of cellular inhibitor of apoptosis proteins (cIAPs) 1/2 and X-linked inhibitor of apoptosis protein (XIAP) in presence and absence of different SMs was assessed in the above-named cell lines by Western blot. The mode of cell death was assessed using inhibitors of Caspase activity (zVAD) and receptor-interacting protein 1 kinase (RIP1K) activity (Nec-1). Dependency of SM-induced cell death on TNF secretion was assessed by application of Etanercept, a TNFR2-Fc fusion protein.
Results: BCP-ALL cell lines Reh and UoCB6 and T-ALL cell lines ALL-SIL and CEM were identified to be sensitive to SM-induced cell death with half maximal inhibitory concentration (IC50) values below 1 micromolar. Interestingly, we found that the bivalent SMs Birinapant and BV6 are up to 100x more effective in killing ALL cell lines than the monovalent SMs AT406 and LCL161. SM treatment resulted in efficient and rapid degradation of cIAP1 and 2 in both, sensitive and resistant cell lines. Interestingly, all tested SMs were equally efficient in degrading cIAPs indicating that the resistance mechanisms are likely to be downstream of cIAPs. Next, we assessed the mode of SM-induced cell death in the sensitive cell lines by using zVAD or Nec-1 in order to block activity of Caspases or RIP1K, respectively. These experiments showed that Reh and UoCB6 cells die by apoptosis whilst CEM cells die by necroptosis upon stimulation with SMs. SM-induced cell death in ALL-SIL cells was neither blocked by zVAD nor Nec-1 nor the combination thereof. These results are substantiated by the fact that SM treatment induced typical hallmarks of apoptosis, *i.e.* cleavage of Poly-(ADP-ribose-)Polymerase (PARP), Caspase 8 and 3, in the SM-sensitive BCP-ALL cell lines whereas no Caspase cleavage was detectable in the sensitive T-ALL cell lines following stimulation with different SMs. In addition, the bivalent SMs BV6 and Birinapant more effectively induced cleavage of PARP and Caspases than the monovalent SMs in Reh and UoCB6 cells. In addition, we found that SM-induced cell death in Reh and UoCB6 cells is partially dependent on autocrine TNF-secretion. Interestingly, we identified ALL-SIL cells to die in a TNF-dependent manner, whilst CEM cells die independently of TNF. This strongly suggests that TNF is not the only driver of SM-induced cell death in ALL cells.
Summary/Conclusions: We identified a subset of both, BCP- and T-ALL cell lines to be sensitive to SM-induced cell death with IC50 values below 1 micromolar. Monovalent SMs are less effective than bivalent SMs in killing ALL cell lines. SMs induce differential modes of cell death with a variable dependency on autocrine TNF secretion in the sensitive ALL cell lines. In-depth molecular characterization of resistance mechanisms of ALL cells to SM-induced cell death is required to identify patients that will benefit from a SM-based treatment regimen.

E839

SINGLE-AGENT MOR208 IN PATIENTS WITH RELAPSED/REFRACTORY (R/R) B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (B-ALL): A SINGLE-ARM PHASE II STUDY

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Background: CD19 is a type I transmembrane glycoprotein that is expressed throughout B-cell development until terminal plasma cell differentiation. CD19 is also broadly and homogeneously expressed across different B-cell malignancies, including B-ALL. MOR208 is a CD19 monoclonal antibody with an enhanced Fc region, which leads to a potentiation of antigen-dependent cell-mediated cytotoxicity and antigen-dependent cell-mediated phagocytosis.
Aims: To investigate the efficacy and safety of single-agent MOR208 in the treatment of patients with R/R B-ALL.
Methods: This is a single-arm phase II study of MOR208 in patients aged ≥16 years with histologically confirmed R/R B-ALL with progression after at least one prior therapy. Patients with Philadelphia-chromosome-positive (Ph^+) B-ALL could be included only if they were intolerant or refractory to at least one tyrosine kinase inhibitor. MOR208 was administered at 12mg/kg IV, weekly, over two 28-day cycles, with a loading dose on day 4 of cycle 1. Patients with a partial response (PR) could receive a further 2 cycles of MOR208; patients with a complete response (CR) or CR with incomplete count recovery (CRi) after 2–4 cycles could receive extended MOR208 therapy until progression. The primary endpoint was the overall response rate. The trial was prematurely terminated due to insufficient evidence of single-agent activity leading to slow recruitment.
Results: 22 patients were enrolled; median age was 52 years (range 16–79), 12 (55%) patients were male, 6 (27%) patients had previously received an allogeneic stem cell transplant (SCT), the most common disease subtype was pre-B-ALL (15, 68%) and 2 (9%) patients had Ph^+ B-ALL. 6 (27%) patients received ≥2 cycles of MOR208 and had a subsequent response assessment. Responses were seen in 2 patients; and included a CR and a CRi, giving an overall response rate of 9%. These 2 patients received extended MOR208 treatment. A further 3 (14%) patients did not fulfil the criteria for PR but did not progress; 16 (73%) patients withdrew before completing cycle 2, in most cases due to progressive disease (PD). The patient in CR met the criteria for allogeneic SCT, but declined this at the time; response duration was 8 weeks, with subsequent PD. The patient with the CRi had a response duration of at least 4 weeks, but discontinued due to a treatment-emergent adverse event (TEAE), sclerosing cholangitis. For 12 out of 13 patients with available data, MOR208 treatment led to a rapid reduction in blast/B-cell counts in the peripheral blood; in most cases a reduction of >90% within 1 week of treatment initiation was seen (bone marrow counts were not available). The most common grade ≥3 TEAEs were febrile neutropenia, thrombocytopenia, neutropenia, sepsis and hyperglycemia (each 5 [23%] patients). Infusion-related reactions were reported in 13 (59%) patients; all occurred on day 1 of cycle 1 and were mostly grade 1 or 2, with one grade 3 event; all patients recovered on the same day. Pharmacokinetic data were comparable with previous clinical studies and anti-MOR208 antibodies were not detected.
Summary/Conclusions: MOR208 showed signs of clinical efficacy with rapid reductions in peripheral blood blasts in most patients with R/R B-ALL, but the durability and frequency of achieving CRs was suboptimal, which was not unexpected given the complexity of R/R B-ALL. However, since the safety profile of MOR208 was consistent with previous studies and favorable, further development as a part of a combination treatment in R/R B-ALL remains a promising approach.

E840

UPDATED RESULTS FROM ZUMA-4: A PHASE 1/2 STUDY OF KTE-C19 CHIMERIC ANTIGEN RECEPTOR (CAR) T CELL THERAPY IN PEDIATRIC AND ADOLESCENT PATIENTS WITH RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Acute lymphoblastic leukemia (ALL) exhibits a bimodal age distribution with 60% of cases occurring in children and adolescents (<20 y) and 25% in older adults (>45 y; http://seer.cancer.gov/csr/1975_2013/) and is the most common childhood malignancy (*Hematol Rep* 2014;6:5554; *Front Oncol* 2014;4:63). ALL has an incidence of 1.2 to 1.4 per 100,000 per year in Europe (*BMC Cancer* 2015;15:771). As many as 20% of children relapse after initial therapy, with subsequent poor clinical outcomes (*Front Oncol* 2014;4:63). Promising results were observed with KTE-C19, an anti-CD19 chimeric antigen receptor (CAR) T cell therapy, in B cell malignancies, including refractory, aggressive non-Hodgkin's lymphoma in the ZUMA-1 trial (*Blood* 2016;128:LBA-6). Here, we present updated results from the phase 1 portion of ZUMA-4, a phase 1/2 trial of KTE-C19 in pediatric and adolescent patients with relapsed/refractory (R/R) ALL.
Aims: The aim of the phase 1 study is to evaluate the safety of KTE-C19 in pediatric and adolescent patients with R/R ALL.
Methods: Pediatric and adolescent patients (aged 2-21 y) with high burden R/R ALL (>25% marrow blasts), adequate renal, hepatic, pulmonary and cardiac function received 2×10^6 CAR T cells/kg after low-dose conditioning chemotherapy consisting of cyclophosphamide (900mg/m^2 once) and fludarabine (25mg/m^2/d for 3 days) (CyFlu). The primary endpoint of phase 1 is the incidence of dose-limiting toxicities. Secondary endpoints include efficacy outcomes and biomarker assessments.
Results: As of 19 Jan 2017, 5 patients have enrolled and 4 have been treated with KTE-C19 at 2×10^6 CAR T cells/kg. KTE-C19 was successfully manufactured in a centralized, streamlined 6-8-day process for all patients across a range of baseline absolute lymphocyte counts ($0.21–1.0\times10^9$/L) except in 1 patient who had disease progression with white blood cells 150,000/µL at apheresis and <0.2% T cells in the apheresis collection. All 4 treated patients had high disease burden with a median marrow lymphoblast content of 57%

(range, 41–99%). All 4 patients received bridging chemotherapy during the manufacturing period before conditioning chemotherapy and KTE-C19. No patient experienced a dose-limiting toxicity. One patient had a grade 5 adverse event of disseminated mucormycosis which was not related to KTE-C19. Cytokine release syndrome was reported in all 4 patients (all ≤ grade 3); neurologic events were reported in 1 patient (grade 3). All cytokine release syndrome events resolved with tocilizumab, corticosteroids, and/or siltuximab plus other supportive care with a median duration of 8.5 (range, 4-16) days. Minimal residual disease-negative remission was observed in all 4 patients. One patient received stem cell transplant post-remission, which is allowed per protocol at investigator discretion. Peak expansion of CAR T cells occurred 1-2 weeks post-KTE-C19 infusion. Updated data with additional patients, different dose of KTE-C19, earlier tocilizumb use, and biomarkers will be presented.

Summary/Conclusions: KTE-C19 after low-dose CyFlu has been tolerable and appears safe for further analysis in pediatric and adolescent patients with R/R ALL. No dose-limiting toxicities were observed with KTE-C19 at the 2×10^6 cells/kg dose in patients despite high leukemic burden. All patients receiving KTE-C19 achieved a minimal residual disease-negative remission. Based on these results, ZUMA-4 continues to enroll (NCT02625480).

E841

COMPARISON OF 8-COLOR FLOW CYTOMETRY AND PCR-BASED METHODS IN MEASUREMENT OF MINIMAL RESIDUAL DISEASE IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: The presence of minimal residual disease (MRD) is the most important prognostic factor in adult acute lymphoblastic leukemia (ALL). MRD monitoring is routinely performed by flow cytometry (FCM) and real-time quantitative polymerase chain reaction methods (RQ-PCR).

Aims: We conducted a retrospective analysis comparing these MRD measurement methods in ALL patients treated in three Czech hematology/oncology centers within the CELL group (Czech Leukemia Study Group for Life).

Methods: Adult patients (age 18-55) with both Ph-negative and positive ALL were enrolled in the study, all treated consecutively between 2008 and 2016 according to a pediatric-inspired CELL ALL protocol. Samples for MRD evaluation were acquired from bone marrow on day 26 of induction (D26) and in the 11th week of treatment before the first consolidation (W11). We divided RQ-PCR MRD positive and negative groups using three different cut-off values and analyzed them separately: 1) 1.0×10^{-3}, 2) 1.0×10^{-4}, 3) every RQ-PCR positive result considered MRD positive even below 1.0×10^{-4}. Cut-off value 1.0×10^{-3} was used for FCM MRD. Results were statistically analyzed by the Kaplan-Meier method and log-rank (Cox-Mantel) test.

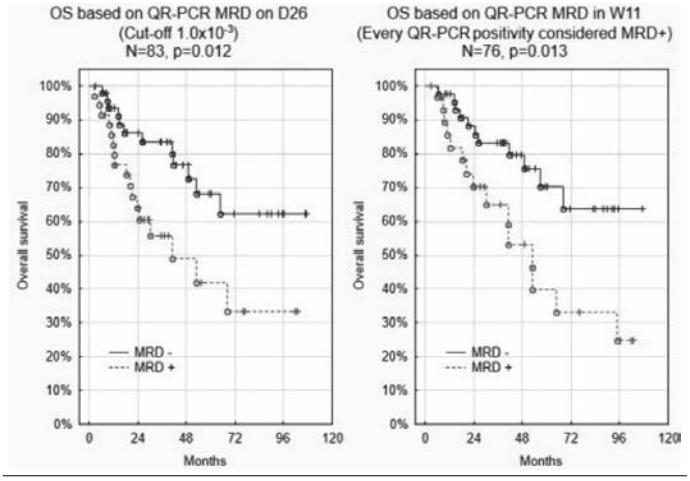

Figure 1.

Results: Total number of 103 patients was evaluated. Nine of them (8.7%) who did not reach a hematological remission on D26 were excluded from the study. The median follow-up of the final cohort was 36.9 months. MRD evaluation was carried out by 8-color FCM (N=73) and RQ-PCR of immunoglobulin heavy chain (IgVH, N=62) or T-cell receptor (TCR, N=3) clonal rearrangements and BCR-ABL (N=24), MLL-AF4 (N=4) and E2A-PBX1 (N=1) fusion genes. Methods with strongest sensitivity for OS prediction on D26 were RQ-PCR with 1.0×10^{-3} cut-off (4-year OS: 76.6% *vs* 48.8%; median OS: not reached *vs* 39.1 months; p=0.012) and FCM (4-year OS: 78.3% *vs* 30.3%; median OS: not reached *vs* 27.4 months; p=0.016). The most sensitive method in W11 was RQ-PCR with every positive result considered MRD positive (4-year OS: 79.6% *vs* 53.1%; median OS: not reached *vs* 46.5 months; p=0.013). Flow cytometry and PCR with other cut-offs were not sufficiently sensitive. The sub-analysis of Ph-negative patients has shown the same results for RQ-PCR (p<0.01).

Summary/Conclusions: Our analysis has shown both RQ-PCR and FCM to be suitable methods for MRD assessment on D26 of induction in adult ALL patients receiving an intensive treatment. Furthermore it seems convenient to take any RQ-PCR positivity (even below 1.0×10^{-4}) into account in W11 and later stages of treatment. FCM can be used for MRD assessment on D26, but it is not sufficiently sensitive in later stages of treatment. We suggest using RQ-PCR as a method of choice for MRD assessment in adult ALL while reserving FCM as a backup method for patients without applicable RQ-PCR target or when faster MRD evaluation is needed.

Supported by MUNI/A/1106/2016 grant of Masaryk University, Czech Republic and the Czech Leukemia Study Group for Life.

E842

QUALITY-ADJUSTED LIFE YEARS (QALY) FOR INOTUZUMAB OZOGAMICIN *VS* STANDARD OF CARE FOR RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA (R/R ALL)

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Background: Inotuzumab Ozogamicin (InO), an anti-CD22 antibody-calicheamicin conjugate, has demonstrated superior clinical activity *versus* standard of care (SOC; intensive chemotherapy), including clinically meaningful improvement in overall survival (OS), high rates of complete remission (CR) and potentially curative hematopoietic stem cell transplantation (HSCT), and favorable patient-reported outcomes for R/R ALL in the phase 3 INO-VATE trial. Quality of life (QoL) is an important consideration for R/R ALL patients in both short- and long-term survival.

Aims: This study aimed to estimate mean overall survival adjusted for QoL (QALY) for patients treated with InO *vs* SOC.

Methods: A Markov model was developed with five health states - No CR, CR, post-HSCT, progression, and death. Lengths and transition probabilities between health states and mortality rates were based on the InO-VATE trial. These rates were extrapolated to a lifetime horizon using parametric survival curves fitted to available OS data, and published literature for survival beyond available data. Utilities (QoL valuations) for each health state were based on the patient-reported EQ-5D scores collected in the InO-VATE trial and a literature review for health states not captured in the trial. Disutilities from adverse events experienced during and after treatments, including adverse events as a result of subsequent HSCT such as veno-occlusive disease (VOD), were taken into account in overall QoL. Outcomes were discounted at 1.5% and half-cycle corrected.

Results: The estimated mean LY and QALY in each health state for InO and SOC and their differences are shown in Table. Most gains in LY and QALY for InO over SOC were from Post-HSCT. These gains are greater in the InO arm as more patients achieved a CR and could undergo a HSCT. Additionally, a "tail-of-the-curve" survival gain Post-HSCT is observed in InO but not SOC.

Table 1.

	LY			QALY		
Health state	**InO**	**SOC**	**InO-SOC**	**InO**	**SOC**	**InO-SOC**
No CR	0.07	0.13	-0.06	0.05	0.08	-0.03
CR	0.25	0.09	0.17	0.19	0.07	0.12
Post HSCT	2.98	0.62	2.36	2.20	0.44	1.76
Progression	0.16	0.28	-0.12	0.05	0.08	-0.04
Total	*3.46*	*1.12*	*2.34*	*2.48*	*0.67*	*1.81*

**Increment values may not always correspond to differences between LYs and QALYs due to rounding*

Summary/Conclusions: This analysis taking into account both quantity and quality of life estimates shows that InO offers an average of nearly 2 more years of QALY compared to SOC in R/R ALL, based on higher CR and HSCT rates, "tail-of-the-curve" survival gains, and better QoL. This can help inform patients, physicians and payers in decision making.

E843

A COST-EFFECTIVE, HIGH SENSITIVITY 10-COLOR SINGLE TUBE FLOW-CYTOMETRY BASED B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA MINIMAL RESIDUAL DISEASE (MRD) ASSAY WITH STUDY OF ARTIFACTS AND MIMICS

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Background: Minimal residual disease (MRD) has been proven to be the most important indicator of relapse in BCPALL. Recently, flow-cytometry based MRD has been shown to achieve a sensitivity of $<10^{-5}$ using a standardised panel with high number of event acquisition. However, high-sensitivity BMRD analysis is based on experience and acquisition of high number of events also includes other rare BM cellular elements and artifacts.We present a study of the cost-effective high-sensitivity 10-color single tube FC-MRD assay in BCPALL along with description of rare BM cellular elements and artifacts causing interference in analysis.
Aims: 1. To study the applicability and sensitivity of a 10-color high event single tube FC-MRD assay for BCPALL; 2. To document the rare BM cellular elements and artifacts causing interference in high-sensitivity FC-MRD assay for BCPALL and describe their prevalence and immunophenotypic features.
Methods: We studied 230 BCPALL MRD samples.FC-immunophenotyping was performed on Navios flow-cytometer using bulk-lysis-and-stain method and data was analyzed with Kaluza-software. MRD was monitored using 10-color single tube FC-MRD assay including CD45, CD10, CD19, CD20, CD34, CD38, CD58, CD86, CD123 and CD25/CD73 with an additional 4-color nuclear-dye (SYTO13) tube. Samples with cluster of ≥20 and ≥2 leukemia associated phenotypes (LAIPs) were called MRD-positive. High number of events were acquired for MRD-assay (1.5 to 6 million). To evaluate the applicability of assay, number of LAIPs were determined in diagnostic and MRD samples. In addition, the frequency and antigen expression pattern of mimics and artifacts were studied.
Results: We studied 230 BCPALL MRD samples. High number of events was acquired for MRD-assay with median-events 3427000 (range, 1678000 to 6052800). We determined the limit of detection (LOD=10 events) and limit of quantitation (LOQ=30 events) by performing dilution assay. MRD was positive in 107 (46.5%) samples with median of 0.135% and range of 0.0003% to 48.3%. We categorized positive MRD results into samples with MRD <0.001%,0.001- <0.01%, 0.01- <0.1%, 0.1- <1.0% and >1% and they were respectively 1.74%, 10.43%, 13.48%, 5.65% and 10.00%. Furthermore, in 24 samples with MRD-positive ≤0.01% and >1.5 million acquired-events, the results were compared between time-gated initial 500000-events, 1000000 events and all events acquired. Sixteen samples among these were found to be negative in initial 500000-events and eight in initial 1000000-events highlighting the importance of acquisition of >1.5 million cells. Further, we categorized different rare cellular events and artifacts in the following way: 1) CD34+ mature B cells; 2) CD10+ mature B cells; 3) CD73+ mesenchymal stromal/stem cells and endothelial cells; 4) CD123+ CD19+ ?PDC precursors; 5) CD86+ CD58+ B cell precursors (BCP); 6) CD19+ NK cells **(Table 1)**. We also described their immunophenotypic features highlighting the differentiating features from MRD and B cell precursors **(Figure 1)**.

Table 1.

	34+ mature B	10+ mature B	73+ mesenchymal cells	123+ 19+ ?PDC precursor	86+ 58+ BCPs	19+ NK cells
Mean	0.192	0.238	0.161	0.159	0.254	7.404
Median	0.100	0.105	0.080	0.100	0.145	2.900
Range	0.008-1.2	0.01-0.8	0.001-1.4	0.0001-.8	0.01-0.9	0.1-32.7

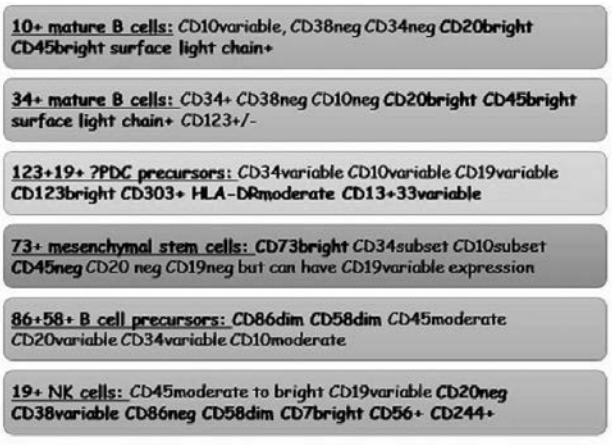

Figure 1.

Summary/Conclusions: We established a cost-effective 10 color single tube FC-MRD assay with high sensitivity ofatleast 1 in 10^5 and applicability in >97% BCPALL MRD samples. We also described the frequency and extent of different cellular events and artifacts that can interfere with high-sensitivity BCPALL FC-MRD analysis. The knowledge regarding presence and antigen expression pattern of these cellular events and artifacts are critical to avoid potential false positive results.

E844

SPECKLE TRACKING ECHOCARDIOGRAPHY IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA: A PRELIMINARY STUDY

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Background: Children with acute lymphoblastic leukemia (ALL) are at risk for late-onset anthracycline-related cardiotoxicity. Left ventricular Global Longitudinal Strain (GLS) by Speckle Tracking Echocardiography has been recently used to identify preclinical late-onset heart failure in both adult and pediatric ALL patients. Nonetheless, efficient strategies for the early diagnosis and management of cardiac toxicity during chemotherapy are still not defined.
Aims: We prospectively studied LV function in ALL patients treated at our Centre, according to AIEOP-BMF ALL 2009 protocol, measuring both GLS and M-Mode Left Ventricular Ejection Fraction (LVEF).
Methods: Out of 42 consecutively examined ALL patiets (pts), 32 (76%) underwent prospective follow-up (*Table*). At basal evaluaton, 19 pts had Hb<9 g/dl, 7 pts had fever, 7 pts had hyperleukocytosis, 2 had BMI>25. The influence of there factors on cardiological parameters could not be evaluated due to low numbers. Echocardiography was performed with Vivid E9 ultrasound system (GE Medical System) and M5Sc probes at the following time points (TP): diagnosis (TP0), after induction phase (anthracycline cumulative dose 120mg/m^2, TP120), at the end of anthracycline exposure (TP240 in standard risk-SR-pts; TP320 in high risk-HR-pts). HR pts underwent intermediate controls before each re-exposure (TP150, TP210, TP270). GLS values>-19% (or GLS drop >15% from basal value) and LVEF values<55% (or LVEF drop >10% from basal value) were considered as abnormal. Statistical analysis was performed using Student's t-test.

Table 1.

A. Main features of SR/HR ALL patients

	SR n^ 26 pts		HR n^ 16 pts
Gender M/F	16/10		12/4
Age	6 (2-16)		13 (4-18)
Phenotype B/T	24/2		12/4

B. Patients with LFVE and/or GLS alteration at different TP

SR	TP0 n^ 26	TP120 n^ 10	TP240 n^ 11	
GLS	3 (11,5%)	3 (30%)	0	
LVEF	0	2 (20%)	0	

HR	TP0 n^ 16	TP120 n^11	TP150 n^ 10	TP210-270 n^ 6	TP320 n^5
GLS	0	7 (63,6%)	6 (60%)	1 (16,7%)	0
LVEF	0	1 (9%)	4 (40%)	1 (16,7%)	0

Results: Basal evaluation was performed on 26 SR and 16 HR pts. Three SR pts with severe anemia (<7g/dl) and/or BMI>25 showed altered GLS at TP0. At the succeeding evaluation, 2 patients recovered, while one obese adolescent confirmed GLS and LVEF alterations. Follow-up after induction therapy (TP120) was performed on 10 SR pts and on 11 HR pts: GLS was altered in 3/10 SR pts (30%) and in 7/11 HR pts (63.6%). Two SR pts showed alterations in both GLS and LVEF values, while one child had isolated GLS impairment. Out of the 7 HR pts, one had both GLS and LVEF impairment and 3 worsened in LVEF at TP150. The 3 remaining HR pts subsequently recovered in GLS value. In 2 additional HR pts both GLS and LVEF were altered at a late evaluation (TP150, TP270). After the induction phase, we found an early 10% increase in mean GLS value (p=0,002), while mean LVEF value was reduced only by 6% (p=0,052), a non-significant difference even by clinical criteria. Thus, according to the recent literature, the combination of GLS and LVEF demonstrated to be able to identify children at risk for LV impairment, as proved later by LVEF fall in 5 cases. Globally, 12 (37%) pts showed GLS impairment at different TP. After the induction therapy, 2/10 SR (20%) and 6/11 HR (54%) pts were considered having significant LV function impairment, even if not clinically evident yet. In 4 patients with GLS impairment, the LVEF never reached pathological values. Eight patients underwent cardiological therapy. We chose to treat them

with b-blockers, as they could limit anthracycline toxicity by their heart rate-lowering activity and antioxidant effect. All the 8 patients subsequently improved in both GLS and LVEF values, despite the occurrence of one episode of mild hypotension in 2 patients.
Summary/Conclusions: ALL children, even if exposed to low doses of anthracycline, show early signs of LV impairment. Overt drop in LVEF, when present, mostly follow GLS alterations. Alterations seem more frequent in HR pts, possibly due to the higher burden of both leukemia itself and HR treatment. Further studies on wider series are needed to confirm the relevance of the early diagnosis of LV preclinical dysfunction in pediatric ALL patients.

E845

NUDT15 VARIANT CAUSING HEMATOPOIETIC TOXICITY WITH LOW 6-TGN LEVEL IN KOREAN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: *NUDT15* polymorphism has been recently identified as a determinant of thiopurine intolerance. 6-thioguanine nucleotides (6-TGN) is monitored to prevent hematopoietic toxicity in acute lymphoblastic leukemia (ALL).
Aims: This study intended to evaluate the impact of *NUDT15* polymorphism on thiopurine intolerance and 6-TGN level in Korean children with ALL.
Methods: Genotyping of *NUDT15* was performed in 258 children with ALL who were registered in Samsung Medical Center. According to *NUDT15* diplotype, patients were classified into low risk (LR, wild-type), intermediate risk (IR, heterozygous variant), or high risk (HR, homozygous or compound heterozygous variant). Total of 182 were fianlly included after 76 patients were excluded for *TPMT* variation or lack of information during maintenance therapy; LR (n=131), IR (n=46), and HR (n=5).
Results: The least 6-mercaptopurine (6-MP) dose (mg/m^2/day) were administered in HR (HR 8.9 vs. IR 25.9 vs. LR 31.8, p<0.01). HR experienced the longest days of therapy interruption (HR 167 *vs* IR 30 *vs* 15, p<0.01) and days of leukopenia (HR 131 *vs* IR 92 *vs* LR 59, p<0.01). The lowest WBC and platelet counts and hemoglobin level were observed in HR. 6-TGN level (pmole/8x10^8 RBC) divided by 6-MP dose (mg/m^2) was the lowest in HR group (HR 4.4 *vs*. IR 13.3 *vs* HR 14.7, p<0.01).
Summary/Conclusions: Patients with *NUDT15* variants encountered significant thiopurine intolerance even with low level of 6-TGN. This concurs with the existing hypothesis that NUDT15 protein may prevent incorporation of thiopurine active metabolites into DNA. Therefore 6-TGN monitoring is not useful to predict hematopoietic toxicity for patients with *NUDT15* variant.

E846

USING NEXT GENERATION SEQUENCING TO DETECT CLONAL TRG AND TRB GENE REARRANGEMENTS

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Background: During early T-cell development, somatic rearrangements occur within T cell receptor beta (*TRB*) locus that bring together, sequentially, the joining of D and J gene segments, followed by joining of a V segment to the DJ pair. This process of random combinatorial selection, coupled with N-region diversity, generates rearrangements that are unique in both length and sequence. Since T cell malignancies arise from transformation and clonal expansion of a single cell, over-represented *TRB* rearrangements can be readily identified, in the majority of alpha-beta T cell malignancies. The T cell receptor gamma (*TRG)* locus rearranges prior to *TRB*, combined use of *TRG* + *TRB* assays identify the vast majority of T-cell, as well as some B-cell malignancies. The current gold standard for identifying these clonal rearrangements is to use PCR assays to amplify all rearrangements, and then identify clonal products on the basis of their uniform size following size separation using capillary electrophoresis (CE). These PCR-CE assays are quite sensitive, but they provide limited information; most notably they don't identify the sequence information required to allow tracking of residual disease during the course of treatment. Recently, next-generation sequencing (NGS) based approaches for immune receptor genes have been developed to both improve the sensitivity of clonal detection and identify the specific V-(D-)J DNA sequences required to track clones in follow-up testing. We have developed such an NGS-based *TRG* + *TRB* clonality assay formatted for the Illumina® MiSeq® platform.
Aims: To develop an NGS-based *TRG* + *TRB* clonality assay for the MiSeq® platform.
Methods: Rearranged products from within the *TRG* and *TRB* locus were generated by PCR using proprietary multiplex master mixes with consensus primers targeting all *TRG* and *TRB* V and J exon families, synthesized with MiSeq specific adapter and individual barcode ID sequences. The PCR products were purified, quantified and pooled into equimolar library. The final library was sequenced on the MiSeq. The sequencing data FASTAQ output file was analyzed using Invivoscribe's LymphoTrack® software. The software generated frequency distributions for the top 200 rearranged sequences, identified the DNA sequences, generated V-J assignments and V-J usage. Cell line DNA known *TRG* and *TRB* V-J rearrangements was tested for the analytical performance. DNA from different clinical sample type (FFPE, PB, and BM) was used to assess the clinical performance.
Results: This NGS assay was able to correctly detect all known *TRB* and *TRG* rearrangements from cell line DNA. The on-target reads per sample were 90% - 100%. Excellent linearity (R^2>0.90), sensitivity of 2.5% for clonality, and reproducibility (<20% CV) were demonstrated with serial dilutions of contrived cell line DNA. The clinical performance of the LymphoTrack® *TRG* + *TRB* NGS assays was evaluated on different clinical samples that have also been tested using the PCR-CE *TRG* and *TRB* assays. Assessment of clonality using the LymphoTrack® MiSeq and PCR-CE assays for *TRG* and *TRB* demonstrated good concordance.
Summary/Conclusions: This combo NGS assay provides a fast, simple, and accurate method to detect clonality. In combination with the LymphoTrack software, the *TRG* +*TRB* MiSeq assay can identify clonal *TRG* and *TRB* V(-D)-J rearrangements and the specific V(-D)-J region DNA sequences required to track clones in follow-up testing. Excellent clinical concordance in detecting clonality with specific rearrangements was demonstrated between LymphoTrack® MiSeq and PCR-CE method.

E847

DETECTION OF CLONALITY IN CLINICAL SPECIMENS FROM SUSPECTED B-CELL MALIGNANCIES USING COMPREHENSIVE IGH LYMPHOTRACK® MISEQ® AND PGM® ASSAYS

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Background: PCR-based capillary electrophoresis (PCR-CE) methods targeting immunoglobulin heavy chain (*IGH*) framework 1, 2, 3 (FR1, FR2, FR3), and joining regions (J) are the current gold standard for clonality testing in suspected B-cell malignancies. Recently, next-generation sequencing (NGS) based approaches for immune receptor genes have been developed that improve sensitivity and identify the specific V-(D-)J DNA sequences required to track clones in follow-up testing. We developed comprehensive LymphoTrack® *IGH* (FR1, FR2, & FR3) Assays for both the Illumina® MiSeq® and ThermoFisher Scientific® Ion PGM™ platforms, which detect the vast majority of rearrangements in a single NGS run. In this pilot study, we compared the performance of both LymphoTrack® *IGH* MiSeq and PGM Assays to the *IGH* PCR-CE assay by testing in 59 anonymized, blinded clinical samples.
Aims: To assess the clinical performance of LymphoTrack® *IGH* MiSeq and PGM Assays
Methods: LymphoTrack® *IGH* Assay has been developed for both the MiSeq and PGM platforms. Proprietary consensus primers targeting the V and J gene segments of *IGH* were designed to include both platform specific adapter sequences and individual barcodes so multiple independent PCR products could be combined and sequenced together on the MiSeq or PGM platforms. MiSeq *IGH* FR master mixes were individually manufactured with 24 indices to allow analysis of 22 samples with 2 controls. PGM *IGH* FR master mixes were manufactured with 12 indices to allow analysis of 10 samples with 2 controls. DNA was extracted from 21 PB, 37 FFPE and 1 BM clinical samples. Single step PCR amplification of 50 ng DNA input was followed by amplicon purification. Equimolar amounts of purified amplicons were pooled and loaded onto the MiSeq or PGM instruments. The sequencing data was analyzed using LymphoTrack® software, which first sorted the sequences by both index and framework region; then generated frequency distributions, V-J usage, identified specific sequences for top sequencing reads, and determined the somatic hypermutation rate of FR1 amplicons.
Results: The analytical performance of the LymphoTrack® *IGH* Assay on both NGS platforms was evaluated using dilutions of contrived samples with known V-J rearrangements. Both NGS assays demonstrated excellent linearity (R^2>0.90), sensitivity to detect 2.5% clonality, and reproducibility (<20% CV). The clinical performance of the LymphoTrack® *IGH* NGS assays was evaluated on 59 clinical samples that have also been tested using the PCR-CE *IGH* assay. Only samples that met the specimen and data acceptance criteria for both methods were evaluated to determine concordance. Assessment of clonality using the LymphoTrack® *IGH* MiSeq and PCR-CE assays demonstrated 95% (42/44) concordance. Concordance was 98% (40/41) comparing results tested using the LymphoTrack *IGH* PGM and PCR-CE assays. Concordance in clonality calls between the LymphoTrack® *IGH* PGM and MiSeq assays was 100% (51/51).

Summary/Conclusions: Comprehensive *IGH* Assays have been developed for both MiSeq and PGM platforms. These assays identify clonal *IGH* V-J rearrangements and provide the clonal DNA sequences of the tumor-specific clonotypes required to perform follow up testing to detect residual disease. Combining FR1, FR2 and FR3 improved the overall clonality detection rate to 96%. Both NGS-based *IGH* assays have demonstrated excellent concordance in detecting clonality regardless of whether clonality was determined using a PCR-CE method or with assays formatted for the MiSeq and PGM platforms.

E848

CORRELATION BETWEEN A 10-COLOR FLOW CYTOMETRIC MINIMAL RESIDUAL DISEASE (MRD) ANALYSIS AND MOLECULAR MRD IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Minimal residual disease (MRD) monitoring in Acute Lymphoblastic Leukemia (ALL) is an accepted standard of care in both adult and pediatric patients as one of the strongest predictive factors for disease outcome and as a stratification tool for treatment intensification and allogeneic stem cell transplant. The currently accepted standard of molecular monitoring with either immunoglobin heavy or kappa chain (IG) or T-cell receptor (TCR) quantitative PCR (qPCR) in Philadelphia negative ALL allows for sensitive monitoring of MRD, but requires a high degree of expertise, and factors such as cost and turnaround time may limit generalized applicability of this technique. Flow cytometric MRD monitoring is utilized in many centers, with increased sensitivity seen with implementation of multi-parameter flow cytometry at 8-colours or more.
Aims: We sought to compare a 10-color flow cytometry assay for detecting MRD in B-ALL with standard molecular monitoring.
Methods: To facilitate rapid identification of MRD in patients with B-ALL, we developed a 10-colour single tube flow cytometry assay utilizing CD19, CD22, CD20, CD38, CD58, CD13/33, CD66c, CD10, CD45 and CD34 as markers. These markers were selected to provide at least two targets for identification of B-lineage cells, and to include the most frequently aberrant markers in precursor B-lineage ALL. Samples were subject to bulk ammonium chloride lysis to maximize cell yields with a target of 1 x 10^6 events. Once normal maturation patterns were established, patient samples were analyzed in parallel to standard of care molecular monitoring with either IG/TCR qPCR in Philadelphia negative (Ph-) disease and *BCR-ABL* qRT-PCR in Philadelphia positive (Ph+) disease. Statistical correlation was performed in Graphpad Prism version 7.0 for linear regression and calculation of correlation co-efficient.

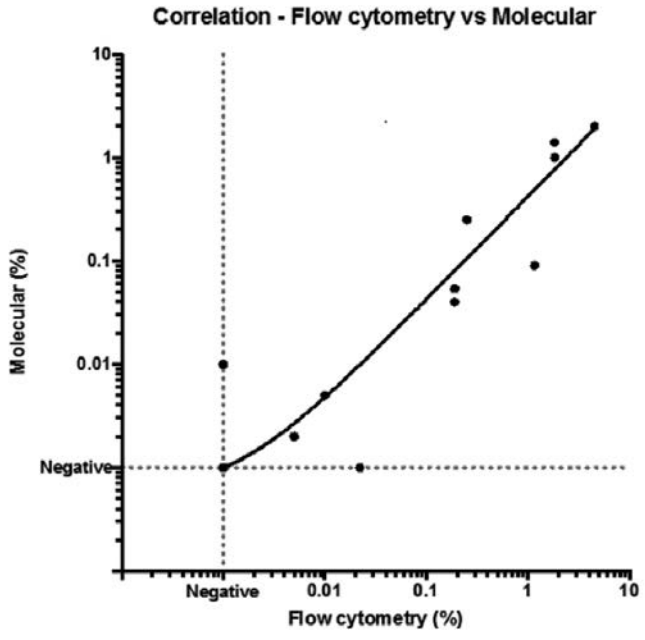

Figure 1.

Results: 33 samples at different time points from 13 patients were analyzed by flow cytometry. 9 samples from 9 patients were taken at diagnosis. Whilst an informative MRD phenotype was identified by flow cytometry in all 9 patients, a molecular assay was not able to be developed in one patient due to lack of an identifiable marker. 24 samples from 13 patients were tested for MRD by flow cytometry. The median lower limit of detection was 0.0078% (range 0.0016% to 0.028%) with a median lower limit of quantification of 0.018% (range 0.002% to 0.07%). A sensitivity of <0.01% was attained in 21 of 24 samples (88%). 20 samples from 11 patients were tested concurrently for MRD by both molecular and flow cytometric methods. 11 samples were in Ph- disease and 9 were in Ph+ disease. MRD was detected by both molecular and flow cytometry in 11 samples and not detected by both methods in 8 samples. In one sample, MRD was detected only by molecular at an unquantifiable level. There was a strong correlation co-efficient between molecular and flow cytometric MRD analysis (R^2=0.905, p<0.001). Correlation was strong with both IG/TCR based molecular analysis (R^2=0.949, p<0.001) and *BCR-ABL* based molecular assays (R^2=0.993, p<0.001).
Summary/Conclusions: 10-color flow cytometric minimal residual disease analysis with bulk lysis attains a high degree of sensitivity in minimal residual disease determination in precursor B-lineage Acute Lymphoblastic Leukemia. There was a strong correlation with molecular MRD monitoring for both quantification of MRD and determination of MRD negative status. Flow cytometric methods may also permit MRD monitoring in patients where a suitable molecular assay cannot be developed.

E849

HYPOGLYCEMIC EVENTS DURING TREATMENT OF PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA: OBSERVATIONS FROM TRIAL AIEOP-BFM ALL 2009

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Background: Hypoglycemia has been reported as a rare side effect in children and adolescents treated for acute lymphoblastic leukemia (ALL). It has been associated to purine nucleoside analogues (PNA), but potential relationship with asparaginase has also been described. Despite these reports, clinicians' awareness of this risk seems to be limited.
Aims: Descriptive evaluation of symptomatic hypoglycemic events during ALL treatment.
Methods: Hypoglycemic events were analyzed among 3293 patients treated in the trial AIEOP-BFM ALL 2009 in four of the participating countries (Germany, Switzerland, Czech Republic, and Australia) between 06/2010 and 08/2016. PNA were administered during induction-consolidation, the second part of the reintensification phase (reinduction-consolidation) and during maintenance (MT). Pegylated asparaginase (PEG-ASP) was given in induction, first part of reintensification, as well as high-risk blocks. Additionally, the benefit of intensified PEG-ASP was tested during induction-consolidation in the high-risk group, and during reinduction-consolidation/MT in the medium-risk group. Adverse events were generally captured in a targeted approach by means of defined events assessed as clinically relevant, not including hypoglycemia. Thus, data collection of these events was based on proactive reporting by the investigators. For analysis, clinical severity of the events was retrospectively graded according to patients' capacity of action and reaction.
Results: In total, 28 hypoglycemic events were reported in 26 of the 3293 patients. 25 events in 23 patients were described as symptomatic, to which further analysis was restricted (22 precursor B- and one T-ALL; 8 standard-risk, 12 medium-risk, and 3 high-risk). Age of patients ranged between 1.7 and 15.5 years at occurrence of symptomatic hypoglycemia. Balanced ratio between both sexes can be observed (13 male, 10 female), median age was essentially similar (male 3.2 y, female 4.1 y). Hypoglycemic events occurred in induction treatment (n=1), induction-consolidation (n=8), reinduction-consolidation (n=4; one in standard reinduction, 3 in reinduction with intensified PEG-ASP treatment), high-risk block (n=1), and in MT (n=11; 4 events during standard MT, 6 events during MT with intensified PEG-ASP treatment, and one event 4 weeks after last PEG-ASP during MT). Seven events were reported with mild symptoms, 6 patients showed moderate symptoms, and in 12 events patients showed severe symptoms (loss of consciousness, seizure-like).
Summary/Conclusions: In accordance with previous reports, hypoglycemic events accumulated in PNA containing treatment phases, but not exclusively. Considering that 324 patients of the total cohort were treated with intensified PEG-ASP in reinduction-consolidation/MT, an additive effect of PEG-ASP and PNA in triggering a hypoglycemic metabolic condition may be assumed although a similar effect was not seen in induction-consolidation with intensified PEG-ASP. However, numbers are small and reporting bias of the present data is probable, as hypoglycemic events were not captured systematically. Investigators' attention to adverse reactions and proactive reporting might be higher

in experimental arms as well as in case of preceding hypoglycemic events in other patients of the respective trial center. Despite these analytical limitations, our data suggest that hypoglycemia during ALL treatment is a relevant and probably underestimated clinical problem. Further investigation including possible identification of predisposing metabolic conditions is required to avoid harm to patients by this preventable complication.

E850

NUDT15 VARIANT IN KOREAN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Acute lymphoblastic leukemia (ALL) is the most prevalent pediatric cancer with cure rates approaching 90% with current therapy. Patient with ALL require long-term maintenance therapy. The combination of weekly methotrexate and daily 6-mercaptopurine (6-MP) consisted with the backbone of ALL maintenance regimens. Genetic polymorphism in thiopurine methyltransferase (TPMP) is well known to affect the 6-MP tolerance. However prevalence of non-function variant of TPMT is rare in Far East. Recently, a study has identified a variant of the *NUDT15* gene associated with intolerance of 6-MP.
Aims: We examined the association between NUDT15 polymorphism and clinical data of Korean pediatric ALL.
Methods: NUDT15 genotyping and collection of clinical data was performed for 74 Korean pediatric ALL patients from two different hospital. For NUDT15 genotyping, DNA was extracted from whole blood/or bone marrow sample and Sanger sequencing was performed for exon 1 and 3 of NUDT15 gene. 6-MP dose intensity, defined as the ration of prescribed 6-MP dose over protocol planned dose.
Results: We found two kinds of variants, c.55_56insGAGTCG(rs869320766) in exon 1 from 8 patients and c.415C>T(rs116855232) in exon 3 from 14 patients. Of them, 7 patients had both variants and all variants were heterozygote. Patients could be divided to four distinct groups according to combinations of genotype (Table 1). 6-MP dose intensity in wild type was higher than three other genotypes during maintenance therapy (p=0.003) (Fig 1). The number of hospitalized days in wild type is small compared to other three genotypes (p=0.017). Frequency of febrile neutropenia, hepatotoxicity, cumulative days of antibiotics use and overall survival did not significantly differ by NUDT15 genotype.

Table 1. Treatement outcome of children with acute lymphoblastic leukemia according to NUDT15 genotypes.

Genotype	Patient no	Relapse	Admission day during maintenance (days)	5-year EFS (%)	5-year OS (%)
Wild type	56	3(5.4%)	152.8 ± 94.7	96.4 ± 2.5	98.2 ± 1.8
w/c.415C>T	8	1(12.5%)	78.3 ± 62.8	87.5 ± 11.7	100.00
c.55_56insGAGTCG/c.415C>T	8	1(12.5%)	190.0 ± 85.2	100	100.00
c.55_56insGAGTCG/w	1	1(100%)	372	0	100.00

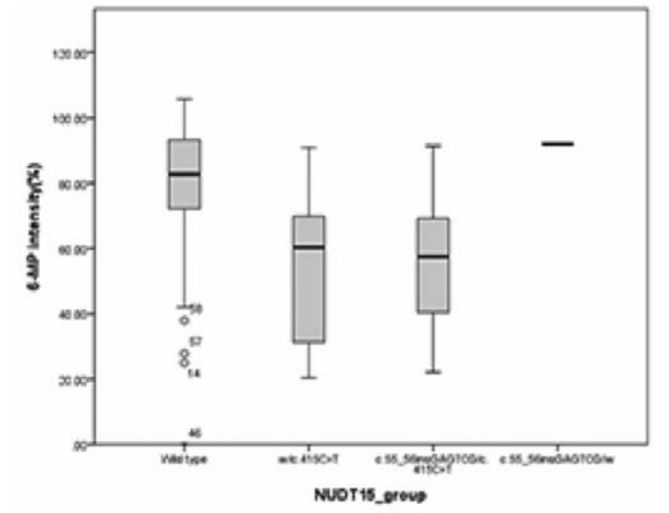

Figure 1.

Summary/Conclusions: Genotyping of *NUDT15* could be beneficial to predict the tolerable dose of 6-MP of pediatric ALL patients.

E851

Abstract withdrawn.

E852

TREATMENT OUTCOME OF ACUTE LYMPHOBLASTIC LEUKEMIA IN KOREAN ADOLESCENTS AND YOUNG ADULTS

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Background: The outcome of acute lymphoblastic leukemia (ALL) has markedly improved for last centuries, but the improvement was mainly observed in children under 10 years old. In contrast, the treatment outcomes of ALL in adolescents and young adults (AYA) still lag beyond those of younger children.
Aims: We conducted this study to investigate the treatment outcome of AYA ALL in Korea, and to define any patterns of care related to the treatment outcome of AYA ALL.
Methods: Clinical data of 10-29 years old ALL patients diagnosed between 2002 and 2010 were extracted from Korean national health insurance service. Data about patients' diagnosis, age, gender, mainly treated department (internal medicine *vs* pediatrics), usage data of medications (L-asparaginase, 6-mercaptopurine, vincristine, prednisolone or dexamethasone), hematopoietic stem cell transplantation (HSCT), radiotherapy, survival, and follow-up duration were collected. Patients who were treated with steroid over 2 weeks, and L-asparaginase at least once in initial 2 months were considered to be treated as pediatric protocol, and who did not fulfill this criteria were considered to be treated as adult protocol.
Results: Total 1,223 ALL AYA patients were diagnosed between the 2002 and 2010, and excluding those who never treated, 1,208 patients underwent ALL treatment. Among them, 665 (55%) patients were treated with pediatric protocol, and the other 543 (45%) patients were treated with adult protocol. Radiotherapy was done in 278 (41.8%) and 186 (34.3%) in each group, and HSCT was done in 205 patients (30.8%) and 216 patients (39.8%) in each group, respectively. Pediatric protocol group showed significantly better overall survival compared to adult protocol group in total age (65% *vs* 43%, $P<0.0001$), 10-13 year old (76% *vs* 57%, $P<0.0001$), and 20-24 year old patients (51% *vs* 31%, $P=0.0116$). In univariable analysis, patient age (younger), treatment protocol (pediatric), L-Asparaginase, 6-mercaptupurine, and steroid over 2weeks in initial 2 months were associated with better overall survival ($P<0.0001$ for each).
Summary/Conclusions: The overall survival rates in Korean AYA ALL were comparable with previous studies done at other countries. Patients treated with pediatric protocol tended to result better overall survival rate when compared to patients treated with adult protocol. Radiotherapy and early HSCT were widely used in the 2000s, and further study is needed to follow up the recent trend of treatment, and outcome as a result.

E853

AUTOLOGOUS TRANSPLANTATION AS TIME-DEPENDENT FACTOR FOR SURVIVAL OF PATIENTS WITH T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA: STUDY DATA AND SIMULATION MODEL

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Background: The role of autologous hematopoietic stem cells transplantation (aHSCT) for patients with T-cell ALL is still being discussed. The resent Russia study of ALL shows the promising effect of aHSCT but there is a skepticism as the study was not randomized. The possible bias was referred to the "time selection" factor.
Aims: It's need to prove that time selection can not explain the magnitude of the effect of aHSCT on patient's survival.
Methods: We have developed SAS macros time-depend graphical and analytic procedures for time dependent factors: Land Mark (LM) methods, Mantel-Bayr test, Cox regression model (CM) and also a base for simulation all end points and study events like remission, transplantation, relapse and death are well approximated by a mixture of exponent distributions. Non-constant (dropping) hazard rate exists in real study data. The consequence of violation of constant hazard assumption as most possible source of biases was tested on our simulation model in different situations. Real data multicenter study of ALL was used to fit simulation model parameters. Russian ALL study group held a prospective multicenter trial RALL-2009 in the treatment of Ph-negative adult ALL patients based on non-intensive but non-interruptive treatment (NCT01193933). The therapy was unified for all Ph-negative ALL pts, but in T-cell ALL/LBL autologous hematopoietic stem cell transplantation (auto-HSCT) after non-myeloablative BEAM conditioning was scheduled as late intensification (+3-4 mo of CR) followed by prolonged 2 years maintenance. From Jan 2009 till Jul 2016, 30 centers enrolled 107 T-ALL/LBL pts. Median age was 28 years (15-54 y), 34 f / 73 m; early T-cell (TI/II) phenotype was verified in 56

(52.3%), mature (T-IV) - in 10 (9.4%), thymic (TIII, CD1a+) ALL – in 41 pts (38.3%). T-lymphoblastic lymphoma (T-LBL= <25% b/m blasts) was diagnosed in 22 pts (20,5%). Autologous HSCT was performed in 35, allogeneic-in 7 pts.
Results: The survival analysis of real data shows 4-fold dropping hazard rate. The effect of aHSCT was confirmed by LM analysis, Mantel-Bayr test - P_{MB}=.0004, Cox model output: 1/HR=15.9, P=0.008. (Fig.1). Simulation model for remission consists of 3 fractions: early (α=10%, τ=0.05 m, δ=0.2 m), normal (α=57%, τ=0.28 m, δ=1 m) and late remission (α=33%, τ=1.31 m, δ=2.2 m), for survival consists of 2 fractions: short life (α=59%, τ=22 m), long life (α=41%, τ=600 m). (Fig.2). The first simulation experiment was performed in preposition that transplantation has no effect (HR=1). To exclude the random effect the sample size was N=4000. Mantel-Bayr and Cox model show no significant (P_{MB}=.50, P_{CM}=.50, HR=.93) but LM plot demonstrates recognizable bias in transplanted patient group (Fig.3). The second experiment supposed that the existed effect of aHSCT (HR=0.5), N=500. Mantel-Bayr and Cox model would show significance, but hazard ratio was underestimated (PMB=.03, PCM=.03, HR=.70 (0.50-0.97)). More experiments were done for repeated simulation, which demonstrated a very good agreement of Mantel-Bayr and Cox methods and their robustness.

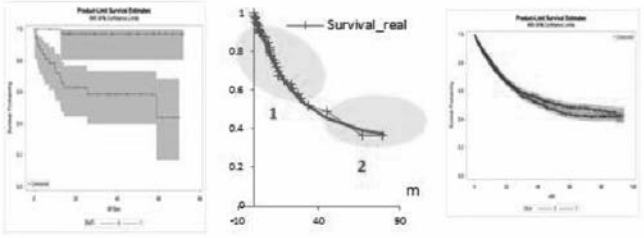

Figure 1. LM plot of RFS in transplanted (red line) and non-transplanted (blue line) groups of T-ALL patients.

Figure 2. Parametric interpolation model for the survival, 1-short life, 2-long life fractions.

Figure 3. LM plot for survival in simulation experiment. Overoptimistic 3% bias in 5-year survival for transplanted patients (red line).

Figure 1.

Summary/Conclusions: The effect of autologous HSCT in T-cell ALL was confirmed by usual analysis and by simulation experiments. It was shown that potential bias caused by no constant hazard rate cannot explain the magnitude of HSCT effect demonstrated on real data. LM plot could express small bias. Mantel-Bayr and Cox Model analytical methods are robust against violation of constant hazard assumption and give very concordant outputs. Cox model underestimates the effect of time-depending factor in case of dropping hazard. Simulations model is a good instrument for testing tests in situations of deviation from theoretical assumptions.

E854

INDUCTION WITH TYROSINE KINASE INHIBITORS, CONSOLIDATION WITH FLUDARABINE, ARA-C AND DAUNOXOME FOLLOWED BY ALLOGENEIC STEM CELL TRANSPLANT IS AN EFFECTIVE AND FEASIBLE STRATEGY FOR PH+ ALL PATIENTS

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Background: The prognosis of Philadelphia positive (Ph+) acute lymphoblastic leukemia (ALL) patients has improved since the introduction of tyrosine kinase inhibitors (TKI). Following TKIs treatment almost all patients rapidly achieve complete hematologic remission (CR). However, only a minority of patients obtain complete molecular response and mot of all will eventually relapse without further treatment. On the other hand, the concomitant combination of TKIs to conventional chemotherapy regimens greatly increases complete molecular responses, but at the price of significant toxicities and high rates of deaths due to toxicity.
Aims: We present here the preliminary results of a sequential therapeutic strategy starting with TKI (Dasatinib) as single agent induction until CR is achieved. Fludarabine (Flu), Cytarabine (Ara-C), Lyposomal Daunorubicine (DNX, FLAD regimen) and Dasatinib were given as consolidation therapy, in order to maximize efficacy and reduce toxicity. Allogeneic stem cell transplantation (HSCT) was planned for all patients in MRD negative CR.
Methods: Dasatinib was given in association with steroids at the dosage of 140mg /die until the achievement of CR. FLAD regimen consisted of a three-days administration of Flu 30mg/sqm followed by Ara-C 2000mg/sqm and DNX 100mg/sqm. Dasatinib was administered again from the end of chemotherapy and G-CSF was given to all patients starting from day 4 until complete hematological recovery. FLAD was administrated for up to two cycles. Minimal residual disease (MRD) was evaluated in all patients after each FLAD either by multicolor flow cytometry (MFC), RQ-PCR for VDJ rearrangements, and RQ-PCR for BCR/Abl.
Results: From January 2008 to December 2016, 8 Ph+ ALL at diagnosis (median age 52 years) have been enrolled in this protocol. The median follow-up was 27 months. All patients received 70 days induction with Dasatinib + Steroids and achieved CR with complete hematological recovery. In all patients but one, however, BCR/Abl was still positive both on day 33 and on day 70. Two patients were MFC MRD positive on day 33 (one on day 70 also), whereas five patients achieved MFC MRD negativity on day 33. After the first FLAD course all patients achieved MFC MRD negativity, with four patients achieving also negativity for VDJ rearrangements and BCR/Abl transcript. FLAD was very well tolerated, with a median ANC and platelet recovery of 7,5 and 4 days, respectively. No patient experienced relapse so far and 5/8 patients proceeded to HSCT. Two patients are currently waiting for transplant. Overall, 6 patients are alive and in MRD negative CR at the time of analysis. One patient died at day +289 after SCT due to non-relapse mortality and one has died after the first FLAD in molecular CR because of an unrelated event.
Summary/Conclusions: This therapeutic strategy proved to be well tolerated and extremely effective for Ph+ ALL patients. Administering FLAD in patients who had already achieved complete hematological response with Dasatinib + steroids allowed us to reduce the period of neutropenia and thrombocytopenia compared to what is reported after combined TKI and chemotherapy treatment given at diagnosis. Most patients underwent HSCT in molecular CR.

E855

BONE MARROW MRD EVALUATION ON DAY 7 OF STEROID TREATMENT OF MODIFIED ST JUDE TOTAL XV THERAPY IN STANDART/LOW RISK PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: In the recent years it was cleary shown that levels of minimal residual disease (MRD) studied by flowcytometry during treatment reflect the overall response to the chemotherapy and give a chance to individualize treatment and improved outcome.
Aims: To determine the clinical significance of MRD on day 7 of initial steroid treatment in patients with childhood ALL we analyzed data from 173 patients treated with modified St Jude Total XV therapy between 1 January 2008 and 31 December 2015.
Methods: According to our previous succesfull results with high dose methl-prednisolone (HDMPX) we add 7 days of HDMPX to the modified St Jude Total XV as an initial treatment and randomized patients at doses of 10mg/kg/d or 20mg/kg/d HDMPX: not exceeding at maximum 1000mg methylprednisolone. After the end of 7th day of steroid concomittant chemotherapy was given and the doses were tapered gradually to 5mg/kg/d and 10mg/kg/d in each group respectively. By the 3rd week of treatment steroid dose was tapered to 2mg/kg/d in both groups and continued with this dose till the end of 3rd week of induction phase. MRD levels were studied at the 15th, 22nd and 42nd days of induction according to the protocol. However we also analyse steroid response rate by the peripheral smear on day 7. Moreover, patients were asked to obtain simultaneously optional bone marrow aspiration after getting informed consents to show whether there will be any concordance with the steroid response and/or whether it can give any idea of the outcome.
Results: Steroid response rate on day 7 by peripheral smear was 91% (n=158) for the whole group. However simultaneously bone marrow MRD measurement was done in 22 of the 173 patients.There were 13 female and 9 male patient with a median initial WBC count of 6400/mm3 (1100-55300/mm^3),all were Calla+ pre B cell ALL (17 low risk ALL, 6 standart risk and 1 high risk ALL),all were in complete remission and all except one is alive at the time of the analysis. There were 10 patients receiving 10mg/kg/d HDMPX and 12 patients were in the group of 20mg/kg/d HDMPX. MRD levels were not statistically different on day 7 between these two groups. Furthermore all patients except 2(one in each group) were steroid responsive by means of peripheral absolute blast count <1000/mm^3. Bone marrow MRD on day 15th and 42nd there were no statistically significant difference in each group(P>0.05).Although some of those patients in each group have high levels of MRD on day 7, interestingly they were all steroid responsive.
Summary/Conclusions: Our preliminary results suggest to think that MRD level on day 7 in a small group of low/standart ALL patients may not predict outcome.

E856

PONATINIB (PON) IN PHILADELPHIA CHROMOSOME (PH)-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (ALL): PRELIMINARY REPORT OF THE OPAL OBSERVATORY.

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Background: PON, a third-generation tyrosine-kinase inhibitor (TKI), displays activity in *de novo* Ph-positive (Ph+) ALL and chronic myeloid leukemia (CML) in lymphoid blastic phase (LyBP), as shown when given as single agent in 42 patients (pts) with resistant disease in the PACE trial (Cortes, NEJM 2013), or combined to first-line chemotherapy in 58 pts (Jabbour, Lancet Oncol 2015; Sasaki, ASH 2016).
Aims: Because data are still limited to few selected pts, we analyzed the outcome of pts treated with PON in the real-life setting (OPAL observatory).
Methods: Pts were recruited if aged ≥ 18 years; with *de novo* Ph+ ALL or CML-LyBP, treated by PON alone or in combination for at least 1 treatment day, for relapsed or refractory disease, between Apr 2012 and Dec 2014 (Expanded Access Program). Twenty-one pts were analyzed (16 men and 5 women; 17 *de novo* ALL and 4 LyBP-CML), with a median age of 60 years (22-73). Time from first ALL or CML-LyBP diagnosis was 6 months (1-123). At PON initiation, 1 pt had primary refractory ALL, 15 pts were in first salvage (1 in second complete remission [CR] after chemotherapy, 3 in molecular relapse only), 2 in second salvage, and 3 in third salvage or beyond. Numbers of patients who had previously received 1, 2, 3, or 4 other TKIs were 4, 15, 1 and 1, respectively (14 imatinib, 17 dasatinib, 5 nilotinib, 1 bosutinib). Six pts had previously undergone hematopoietic stem cell transplantation (HSCT). Of the 18 pts screened for *BCR-ABL* mutations, 5 had none, 3 had T315I, 3 had other PON-sensitive mutations, while 5 had compound mutations (known to be resistant to all TKIs including PON) and 2 had E255V (of intermediate sensitivity to PON). PON was administered alone in 13 pts, combined to low-intensity chemotherapy in 6 pts, intensive chemotherapy or monoclonal antibody in 2 pts. Dose at initiation was 45mg in 17 pts and 30mg in 4 pts.
Results: Median duration of PON therapy was 3 months (5 days-30 months+). Out of the 19 pts who received PON for ≥ 4 weeks, 5 pts failed to reach CR, while 14 (78%) reached or maintained it. Molecular response was not reported uniformly. During induction by PON, 5 grade 3-4 events occurred in 4 pts (1 pulmonary infection; 1 acute renal failure; 1 pancreatitis; 1 hepatitis; 1 venous thrombo-embolic event; no arterial occlusive event). Post-induction therapy consisted in PON-based therapy in most pts. HSCT was performed in 5 pts. Out of 14 pts in CR on PON, 1 pt died in CR from HSCT toxicity, 1 had a CNS relapse at 8 months, and 11 pts ultimately experienced bone marrow relapse, all of them within 6 months after PON initiation, except 2 who relapsed at 13 and 27 months after HSCT. Two patients are alive in CR at 14 and 30 months, 1 after salvage therapy, 1 on PON.
Summary/Conclusions: Our series of resistant pts is comparable to the PACE study population by initial characteristics and high frequency of *BCR-ABL* mutations. CR was achieved in most pts, suggesting the role of PON as a bridge-to-transplant with a favorable risk-benefit ratio. Effective post-induction combinations of PON to chemo/immunotherapy have to be tested in pts not eligible for HSCT. Because global outcome of this very high-risk population remains poor, earlier introduction of PON in the course of the disease is warranted, as underlined by the excellent results of the hyperC-VAD-PON combination in the first-line setting.

E857

JL1 ANTIGEN EXPRESSION OF LEUKEMIC CELLS IN CHILDHOOD ACUTE LEUKEMIA

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Background: JL1 is a novel epitope of CD43, which is known to be specifically expressed depending on the differentiation stages of hematopoietic cells. JL1 antigen is expressed on tumor cells of T, B, and myeloid lineage in >80% of acute leukemia patients, and its expression is limited in normal multipotent hematopoietic cells. The antigen is not expressed on mature peripheral blood cells or other normal tissues. Thus, the clinical phase 1 test of a therapeutic agent for leukemia targeting JL1 is being conducted, and when anti-JL1 antibody was combined with a toxic substance, its therapeutic effect was found earlier in preclinical trials.
Aims: This study aims to examine JL1 expression of leukemic cells in childhood acute leukemia.
Methods: Between December 2014 and January 2016, a total of 71 patients younger than 21 years with acute myeloid leukemia (AML, n=25), and acute lymphoblastic leukemia (ALL, n=46) were enrolled in this study. Expression of JL1 was examined using FACSCanto II (Becton-Dickinson, Sunnyvale, CA, USA) based flow cytometry, and an expression of 20% or above was defined as positive JL1 expression. Pathologic and immunophenotypic characteristics, and clinical outcomes of the patients were analyzed.
Results: Positive JL1 expression was observed in 16 (64.0%) patients with AML, and 27 (58.7%) with ALL. In AML patients, positive JL1 group showed higher expressions than negative JL1 group in CD 14 (P=0.043), CD7 (P=0.026), CD56 (P=0.016) and lower expressions in CD65 (P=0.05). With regard to ALL patients, CD 20 (P=0.002) and CD2 (P=0.005) expressions were significantly higher in JL1 positive group than JL1 negative group. Positivity of JL1 expression did not show significant difference between B-lineage ALL and T-lineage ALL (P=0.671). Positivity of JL1 expression was not significantly associated with overall survival in 71 patients with newly diagnosed childhood acute leukemia (P=0.570).
Summary/Conclusions: 60.5% of childhood acute leukemia displayed positive JL1 expression. This finding is similar to 61.2% of JL1 expression in adult AML and 57.9% of expression in adult ALL reported previously. The JL1 expression was significantly associated with some immunophenotypic features, but was not significantly associated with clinical outcome. These findings demonstrates that anti-JL1 antibody might be used in childhood acute leukemia patient showing JL1 expression

E858

SERUM LEVELS OF CYTOKINES AND ADHESION MOLECULES AND THEIR ASSOCIATION WITH PROGNOSTIC FACTORS IN NEWLY DIAGNOSED ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

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Background: Dysregulated production of cytokines and adhesion molecules has been implicated in the onset and progression of various types of leukemia. Further knowledge gained from multiple cytokine and adhesion molecule evaluation could help to improve treatment outcomes.
Aims: The aim of this study was to evaluate baseline levels of cytokines, cytokine receptors and adhesion molecules in newly diagnosed acute lymphoblastic leukemia (ALL) patients and to assess their correlation with baseline characteristics and prognostic factors.
Methods: A total of 30 newly diagnosed ALL patients (median age 46, range 22–75 years, 20 males) were included in this study. We evaluated serum levels of 31 analytes, specifically 21 cytokines, 4 soluble cytokine receptors, 5 soluble adhesion molecules and Matrix Metalloproteinase-9. From cytokines, we measured Interleukins (IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-23), Epidermal Growth Factor (EGF), Granulocyte Macrophage Colony Stimulating Factor (GM-CSF), Interferon-γ (IFN-γ), Macrophage Inflammatory Protein-1α (MIP-1α), Monocyte Chemotactic Protein-1 (MCP-1), Tumour Necrosis Factor-α (TNF-α), Vascular Endothelial Growth Factor (VEGF) and soluble receptors for IL-2 (sIL-2Rα), IL-6 (sIL-6R), TNF-α type I and II (sTNFR-1,2). From soluble adhesion molecules, we measured E-Selectin (E-SEL), L-Selectin (L-SEL), P-Selectin (P-SEL), Intercellular Adhesion Molecule-1 (ICAM-1) and Vascular Cell Adhesion Molecule-1 (VCAM-1). All analytes were measured by biochip array technology on Evidence Investigator analyzer (Randox). Serum levels of tested analytes were correlated with baseline characteristics and prognostic factors, such as age, sex, risk group according to GMALL (SR 9, HR 9, VHR 12 patients), full blood count parameters (including percentage of blasts), biochemical parameters (LDH, CRP), response to induction therapy (CR rate after induction), progression-free survival (PFS) and overall survival (OS). Statistical evaluation was done by a professional statistician using software R 3.3.2 (R Core Team 2016).
Results: Comparing analytes with baseline characteristics, we found significant negative correlations between IL-7 and leukocyte count (r=-0.633; p=0.032), percentage of blasts in peripheral blood (r=-0.695; p=0.004) and LDH (r=-0.604; p=0.075). Furthermore, we found significant positive correlations between IL-7 and platelet count (r=0.801; p<0.0001), VCAM-1 and LDH (r=0.664; p=0.012). Correlations with baseline risk stratification according to GMALL did not reach statistical significance. In the study population, CR rate after induction was 86% (MRD negative in 29%), 1-year PFS 68% and 1-year OS 73% (2 patients died during induction therapy). Higher levels of EGF were associated with failure to achieve CR after induction therapy (r=0.686; p=0.073). So far, no significant correlations between baseline analyte levels and inferior PFS or OS were found. In newly diagnosed ALL patients, we found statistically significant correlations between sTNFR-1 and sTNFR-2 (r=0.805; p<0.0001), IL-1α and IL-4 (r=0.700; p=0.008), sTNFR-2 and MIP-1α (r=0.657; p=0.037), sTNFR-2 and VCAM-1 (r=0.652; p=0.044). Other correlations between analytes did not reach statistical significance.
Summary/Conclusions: Our findings show that serum levels of IL-7 and VCAM-1 are associated with some baseline characteristics of newly diagnosed ALL patients and EGF with response to induction therapy. Better understanding of leukemia microenvironment is essential for development of new treatment approaches. Further studies in this field are warranted.

The work was supported by a long-term organization development plan 1011 (FMHS) and by MH CZ – DRO (UHHK, 00179906).

E859

IMATINIB VS. DASATINIB FOR OUTCOMES AFTER ALLOGENEIC STEM CELL TRANSPLANTATION FOR PATIENTS WITH PH+ ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: The survival of the patients with Philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ALL) who received allogeneic stem cell transplant (allo-HSCT) has improved over the development of tyrosine kinase inhibitors (TKIs). Currently, Imatinib (IMA) and Dasatinib (DAS) are widely used for the treatment for Ph+ALL. However, there has been no data comparing the outcomes between the patients who received allo-HSCT and the two distinctive TKIs respectively.
Aims: We conducted a retrospective analysis for comparing the two TKIs for the outcome after allo-HSCT.
Methods: Clinical data of patients were retrospectively collected from Hokkaido University Hospital and Sapporo Hokuyu Hospital. The patients' eligibility were as follows: diagnosed as Ph+ALL, aged more than 16 years, and received allo-HSCT between 1990 and 2016 and first time for SCT.
Results: Sixty-six patients were eligible for the study. Fifty-six out of the 66 were administered TKIs (TKI group) and the remaining ten who developed Ph+ALL in the earlier years were treated without TKIs (non-TKI group). Overall survival was not different between the two groups. Of the 56 patients in the TKI group, 39 received IMA (IMA-pts), and the remaining 17 received DAS (DAS-pts). Compared with DAS-pts, IMA-pts received allo-HSCT in relatively older years of age, more frequent myeloablative conditioning regimen, and cyclosporine-containing, not tacrolimus-, regimen for GVHD prophylaxis more frequently. Other characteristics, such as age, disease status at HSCT, or stem cell source were not significantly different between the two groups. Incidences of Neutrophil engraftment and acute GVHD incidence were not statistically different between IMA-pts and DAS-pts. Incidence of chronic GVHD was marginally increased in IMA-pts (IMA; 63%, DAS; 33%, *P*=0.08). At the median follow-up days of 531 after SCT (range: 14-4402 day), overall survival was not different between the two groups by univariate analysis (Logrank, *P*=0.16). However, by multivariate analysis using Cox regression model for adjusting confounding factors, including, overall survival was superior for IMA-pts [Hazard ratio; 0.32 (0.11-0.94), *P*=0.04]. Incidences of transplant-related mortality and relapse were not different between the groups, even though relapse rate tended to be increased in DAS-pts (IMA; 16.1%, DAS; 47.1%, Gray *P*=0.2).
Summary/Conclusions: Our analysis suggests that overall survival may be superior for the Ph+ALL patients treated with allo-SCT and IMA compared with those with DAS. There are some limitations for our analysis due to retrospective fashion and relatively small number of the patients analyzed. Therefore, prospective study comparing survival of the Ph+ALL patients treated with the two distinctive TKIs before HSCT is needed.

E860

IS OLDER AGE AN EXCLUSION CRITERION FOR ALLOGENEIC HEMOPOIETIC STEM-CELL TRANSPLANTATION IN PATIENTS WITH PHILADELPHIA-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA?

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Background: Philadelphia-positive acute lymphoblastic leukemia (Ph+ ALL) is diagnosed more often in older than in younger patients. This type of the acute lymphoblastic leukemia is characterized by very aggressive course of the disease. All clinical recommendations for such conditions indicate allogenic bone marrow transplantation after achieving complete remission. The addition of tyrosine kinase inhibitors (TKI) to chemotherapy has dramatically improved the long-term outcome in Ph+ acute lymphoblastic leukemia patients. Nevertheless whether to administer chemotherapy at all and if yes – how intensive it should be, is still the matter of debate. We have conducted two consecutive trials in Ph+ ALL aiming to evaluate the efficacy of more and less intensive chemotherapy approaches in combination with constant non-stop 600 mg Imatinib. All patients in both protocols with suitable donors underwent hemopoietic stem-cell transplantation (HSCT).
Aims: To analyze the effectiveness of RALL–2009+TKI and RALL–2012+TKI protocols in Ph+ ALL patients with or without HSCT. To analyze the efficacy of treatment with or without transplantation regarding the patient's age.
Methods: From 2010 January to 2017 January, 35 new Ph+ ALL cases were diagnosed in 3 centers of the RALL–group. From 2010 to 2012, 12 Ph+ ALL pts were treated according to RALL–2009 protocol (ClinicalTrials.gov; NCT01193933) with concurrent administration of Imatinib. This protocol includes 8 cytostatic drugs and no intervals between treatment phases. Since 2012 till now 23 pts were included in ongoing RALL–2012 protocol, based mainly on 600 mg Imatinib with prednisolone, VNCR, L-asp, followed by 6-MP and MTX. Both protocols suggested the shift to Dasatinib (100-140mg) after non-achievement of MolCR on day 70 of treatment. MolCR was stated if *bcr/abl* chimeric transcript was <0,01% by PCR with 10^{-4} sensitivity. All patients were considered as candidates for allogeneic HSCT if HLA-identical donor was available. 13 pts (37%) underwent all0-HSCT as the first-line therapy: 1 autologous, 5 matched related and 7 matched unrelated.

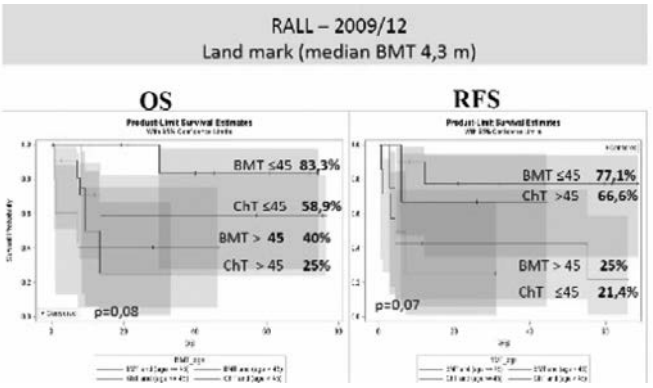

Figure 1.

Results: MolCR on day 70 was achieved in 36% and 59% in RALL–2009 (n=4) and RALL–2012 (n=13) respectively. Death on therapy (within 2 months of induction/consolidation) was registered in 2 cases on less intensive RALL-2012 protocol and 2 cases on RALL–2009. Hematological CR was achieved in 30 (85,7%) of 35 pts (except four early deaths and 2 refractory cases). There was one autologous HSCT in MolCR on the first protocol. Allo–HSCT was carried out in 5 of RALL–2009 protocol pts and in 9 of RALL–2012. The major issue is the non-relapsed mortality after unrelated allo–HSCT in 3 older pts (49, 56 and 59 years old) who were included in RALL–2012 (aGVHD and severe infections, at a median +4 months after HSCT and more than 12 months of CR duration).
The 5y overall survival (OS) and relapse-free survival (RFS) for all 35 pts constituted 54,6% and 40,4% respectively. The long-term outcome on both protocols (RALL–2009 and RALL–2012) was similar: OS – 62,8% *vs* 49,4% (p=0,6), RFS – 55,7% *vs* 45% (p=0,7), respectively. In order to evaluate the impact of allogeneic HSCT we performed a comparison of transplanted and non-transplanted patients by a landmark analysis. The land mark was chosen as the median time from CR to allo-HSCT – 4,3 mo (3-16 mo). So, the 5y OS from land mark was 53,3% for non-transplanted patients and from day of HSCT - 65,6% in transplanted (p=0,18), and RFS was 25% *vs* 62,5 (p=0,19), respectively. OS for older pts (>45 y) was 40% *vs* 25% in transplanted *vs* non-transplanted group of the pts, and RFS was 25% *vs* 66,6%, respectively. OS in younger (£45 y) pts was 83,3% *vs* 58,9% for transplanted *vs* non-transplanted pts, EFS was 77,1 *vs* 21,4%, respectively.
Summary/Conclusions: The results very pessimistic in older (>45 years) patients who received HSCT. The contrary was observed in younger adult patients with very good results after HSCT – OS was 83,3% and EFS 77,1%. We conclude that patients aged>45y should continue chemotherapy without allogeneic HSCT or may be we could apply autologous HSCT for that group of the patients.

E861

TARGETABLE BLINATUMOMAB + TYROSINE KINASE INHIBITORS TREATMENT IN RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS: CLINICAL EFFECTIVENESS AND PERIPHERAL LYMPHOCYTES SUBPOPULATIONS KINETICS

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Background: Blinatumomab is a bispecific monoclonal anti-CD3/CD19 antibody which has clinical activity in relapsed/refractory Ph-positive acute lymphoblastic leukemia (ALL) as monotherapy. Combination of Blinatumomab with

tyrosine kinase inhibitors (TKI) is the promising approach in treating Ph-positive ALL. Some other rearrangements like IKZF1 in Ph-like ALL, FLT3 and JAK2 in Ph-negative ALL are the potential targets to some TKIs.
Aims: To demonstrate effectiveness and toxicity profile of Blinatumomab+TKI treatment. To evaluate peripheral blood lymphocytes subpopulations kinetics during blinatumomab treatment.

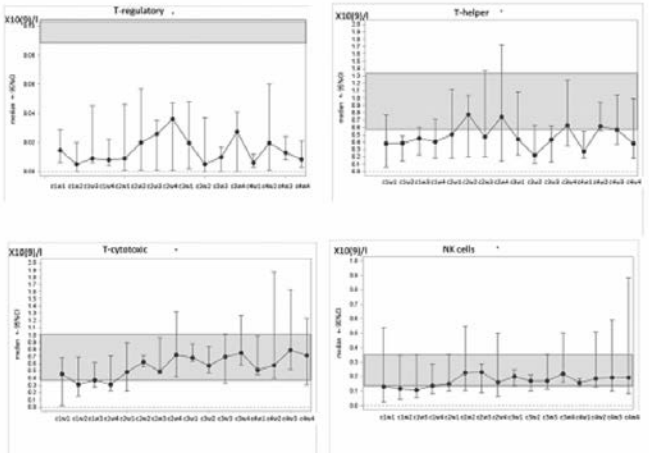

Figure 1.

Methods: From October 2015 to February 2017 10 patients (pts) aged from 24 to 42 (median 31), 7 female and 3 male, with relapsed/refractory ALL were treated in our center. The diagnosis was relapsed ALL in 8 pts (7 – overt hematological, 1 – cytogenetic relapse) and persistent/increasing minimal residual disease of ALL in 2 pts. All pts had strong CD19 positivity. 8 pts was diagnosed as Ph-positive ALL (p190), 1 – Ph-like ALL (IKZF1 rearranged), 1- FLT3+ ALL. Two pts has T315I ABL mutation. In all pts blinatumomab continuous infusion + TKI therapy was started. Blinatumomab dose during 1st week of 1st cycle was 9 mcg/day, 28 mcg – subsequent three weeks. Blinatumomab dose in subsequent 4-weeks cycles was 28 mcg/day. 7 pts were treated with TKI Dasatinib, 1 – Bosutinib (Dasatinib/Nilotinib intolerant), 1 – Ponatinib (T315I), 1 – Sorafenib (FLT3+). ATRA was added to Dasatinib in 1 pt with IKZF1rearranged Ph-like ALL. 1 pt received 1 cycle of 4 weeks blinatumomab, 1 pt – 2 cycles, 6 pts - 4 cycles, 2 pts – 5 cycles. TKIs were administered continuously in all pts. T-helper, T cytotoxic, T-regulatory and NK cells were measured by flow cytometry in every week during all cycles of blinatumomab treatment.
Results: No one pt has neurological toxicity of any grade. All pts has significant decrease of normal IgG level and all of them received intravenous human normal immunoglobulin replacement. Palmar-plantar syndrome in one pt on sorafenib completely resolved after temporarily TKI discontinuation. Diarrhea in 1 pt on dasatinib/nilotinib completely resolved on bosutinib. 8 pts achieved molecular remission (MolCR), one pt – cytogenetic remission and one pt with T315I progressed to overt hematological relapse. T-helper and T-regulatory lymphocytes subpopulations were on or below of lower limit of normal range. T-cytotoxic and NK subpopulations gradually returned into normal range (Fig. 1). AlloBMT was performed in 4 pts. Three pts are awaiting alloBMT and three are continuing Blinatumomab + TKI treatment.
Summary/Conclusions: Lowering toxicity in non-chemotherapy treatment has its significance in such a heavily pretreated patients with relapsed ALL. The treatment has high MolCR rate and low toxicity profile. Treatment effectiveness correlated with T-helper and T-regulatory subpopulations exhausting. T-cytotoxic and NK cells subpopulations restoring also correlated with clinical effectiveness.

E862

VERY VERY LATE RELAPSES OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA, A CASE SERIES

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Background: Recurrence of acute lymphoblastic leukemia (ALL) during childhood usually occurs within the first six years after initial diagnosis.
Aims: The aim of this study is the identification of all relevant characteristics and outcomes in a group of patients with childhood ALL, who relapsed more than six years after initial diagnosis or more than three years after bone marrow transplantation (BMT).
Methods: All children diagnosed with a first relapse of ALL in our Department, from January 1992 till December 2010 were included in this study.
Results: During this period, a total number of 353 patients with childhood ALL were treated in our Department, according to BFM protocols. Recurrence occurred in 86 patients (24.4%, 56 male - 30 female - median age: 4,83 years), within 3 to 184 months from initial diagnosis. Very very late recurrence was noted in 3,1% of our relapses (8 male - 3 female) at 53, 72, 82, 83, 84, 87, 108, 112, 116, 120 and 184 months from initial diagnosis. In 9 patients recurrence involved the bone marrow, in 1 both bone marrow and central nervous system (CNS) and in 1 only the testicles. Two children had received allogeneic BMT from a matched related donor in first complete remission (CR1) and they had a bone marrow relapse 4 and 5 years later, respectively. The mean WBC, Hb, Blasts and PLT values at diagnosis were 29260/mm3, 5.6g/dl, 21360/mm3 and 18000/mm3, respectively. All of them were B-cell ALL except for 1 who had CD33 and CD13 co-expression. Regarding the immunophenotypical profile of the disease at recurrence, it remained almost identical to the initial. Regarding cytogenetic characteristics of the patients at diagnosis, 3 of them had high hyperdiploidy, one del(6)(q12), one BCR-ABL fusion and one 47,XY,+13(9),idem,del(12(p13?); none had MLL rearrangement or ETV6-RUNX1. In 9 cases, the cytogenetic profile remained identical at recurrence, while in 1, trisomia 13 was not detected and another had heterozygous absence of IKZF1, PAX5, EBF1, CDKN2A and CDKN2B genes. On Day 8, nine of 11 patients were Prednisone Good Responders. On Day 15, nine children had bone marrow M1, one M2 and one M3, and on Day 33 only one had M2. Two patients were classified as low risk, 6 as intermediate risk and 3 as high risk. Second remission (CR2) was achieved in 9 children with very very late recurrence. The other 2 died from disease progression. Six of nine patients are still alive and well 6, 8, 10, 10, 11 and 20 years after initial diagnosis. One of 9 patients died from second recurrence and the last two had a second allogeneic BMT and died due to severe infection, 2 and 10 months following that BMT. Interestingly, 3 out of 5 patients who finally died, had the very very late recurrence (10, 10 and 15 years after initial diagnosis) and had been treated with adult type protocols.
Summary/Conclusions: The rate of very very late B-cell ALL recurrence was only 12.8% of all recurrences. The prognosis is worse in patients, older than 18 years, treated with adult type protocols.

E863

NOVEL CRLF2 MUTATIONS AND CLINICAL SIGNIFICANCE IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Cytokine receptor-like factor 2 (*CRLF2*) plays an important role in the development of normal B lymphocytes, which can mediate early B cell proliferation and survival. *CRLF2* overexpression and rearrangement have been observed in acute lymphoblastic leukemia (ALL); and they are reported to contribute to oncogenesis and unfavorable outcome in ALL. We reported that *CRLF2* overexpression in the patients without *CRLF2* rearrangement, indicating the reason other than *CRLF2* rearrangement is responsible to the *CRLF2* overexpression. There is few reported *CRLF2* mutations in adult ALL.
Aims: This study is to investigate the mutations of *CRLF2* and its clinical significance in adult ALL without *CRLF2* rearrangement.
Methods: The 129 patients' BM samples (95 B-ALL, 33 T-ALL and 1T-/B-ALL) were collected between April 2010 and Jan 2015 at the First Affiliated Hospital of Nanjing Medical University. The ALL diagnosis was made according to the cytogenetic, morphologic, Immunophenotypic and molecular criteria of WHO Diagnosis and Classification of ALL (2008). Mutational analysis of *CRLF2* exons 1-6 was performed. Genomic DNA was isolated and DNA fragments spanning the aforementioned *CRLF2* exons were amplified by PCR using AmpliTaq Gold kit (Applied Biosystems, USA) and the exon-specific primers. The resulting PCR products were purified and sequenced by The Beijing Genomics Institute (BGI; Beijing, China).The frequency, positions, types and clinical significance of *CRLF2* mutations were analyzed. For qualitative parameters, overall group differences were analyzed using a $\chi 2$ test. All statistical analyses were performed using SPSS version 17.0 (SPSS, Inc., Chicago, IL, USA). $P<0.05$ was considered to indicate statistical significance.
Results: Six novel *CRLF2* mutants were detected in the 129 patients without *CRLF2* rearrangement, which were L86I (0.8%), R186S (7.8%), P224L (8.5%), W255C (0.8%), and two silent mutations F232F (0.8%) and A11A (12.4%). The overall rate of *CRLF2* mutation was 26.6%. Exon6 is a hot spot exon, detected three types of mutants. The incidence of A11A in B-ALL was significantly higher than that in T-ALL (14.7% *vs* 2.4%, *P*=0.037), whereas R186S was only detected in B-ALL. Exon1, exon5 and exon6 mutations were detected in B- and/or T-ALL patients; but no mutations were detected in exon2 and exon4. None of these mutations were reported in the COSMIC and SNP databases. The patents with R186S, P224L mutations showed significant differences with that of non-mutant patients in sex, age, white blood cell count, hemoglobin level, and platelet count. The median neutrophil count in the patients with P224L mutation

was lower than that of non-mutation (8.53x10^9/L *vs* 28.9x10^9/L, *P*=0.032). The positive rate of Ph chromosome in patients with R186S was lower than that without the mutant (10.0% *vs* 31.8%, *P*=0.018). In addition, the incidence of splenomegaly in patients with R186S and P224 L mutants was lower than that in non-mutant patients (0.0% *vs* 29.5%, *P*=0.026; 0.0% *vs* 29.7%, *P*=0.034, respectively). The B-ALL patients with L86I mutant had myeloid antigen expression, high white blood cell count (248.4×10^9/L) and low platelet count (10×10^9/L), and relapsed in two months after the first induction chemotherapy; and the overall survival was only 2 months. The patient with W255C mutation did not achieve complete remission (CR) with the first induction chemotherapy. Interestingly, the patient with silent mutation, A11A showed higher age (46 *vs* 30 years, *P*=0.033), higher HLA-DR (100% *vs* 75.3% *P*=0.035), CD22 (83.3% *vs* 47.4% *P*=0.020) than those without the mutation; and the patient with F232F mutation relapsed in 6 months.

Summary/Conclusions: Six novel *CRLF2* genetic mutations were identified in adult ALL patients and may associate with clinical outcome, such as *CRLF2* R186S indicating favorable, while L86I and W255C indicating poor outcome. Our data indicated that the *CRLF2* mutations may be new prognostic markers and play an important role on oncogenesis in ALL.

Acute myeloid leukemia - Biology

E864

THE MUTATIONAL SPECTRUM OF T(8;21)(Q22;Q22) POSITIVE ACUTE MYELOID LEUKEMIA DETERMINED BY HIGH-THROUGHPUT TARGETED SEQUENCING

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Background: Recently, comprehensive genetic profiling of pediatric and adult core-binding factor (CBF) AML revealed a variety of cooperating events in a cohort of 85 t(8;21) AML patients (Faber *et al. Nat Genet* 2016). These mutations comprised alterations in genes encoding for proteins in tyrosine kinase (TK) signaling, epigenetic regulation (ER), and in the cohesin complex (CC).
Aims: To validate and to further extend our recent findings by comprehensive characterization of the mutational landscape of t(8;21) positive AML using a high-throughput targeted sequencing (HTS) approach.
Methods: The HTS panel comprised the entire coding region of 244 genes that are involved in hematological malignancies. Pretreatment blood (n=23) or bone marrow specimens (n=72) of 95 additional adult t(8;21) positive AML patients (pts) (median age: 51 yrs, range 18-72 yrs) were analyzed. 92/95 pts were enrolled in one of seven prospective AMLSG treatment trials. Libraries (total probes size: 1.359 Mbp) were prepared using SureSelectXT custom solutions (Agilent). Paired-end sequencing was carried out on a HiSeq 2000 (Illumina). The variant allele frequency (VAF) cutoff for reporting mutations was set at ≥0.05.
Results: The median coverage per pt was 900x. Mutations were detected with an average of 5.1 (SD: ±2.6) per pt with 99% of all pts harboring at least 1 mutation and 87% ≥ 3 mutations. Consistent with previous studies, mutations in TK signaling pathways were common events: *KIT* mutations were found in 22/95 pts (23%) followed by mutations affecting *NRAS* (16/95; 17%), *FLT3* (11/95; 12%; point mutations only), and *KRAS* (4/95; 4%). A significant enrichment of mutations was also observed in genes involved in epigenetic regulation, *ASXL1* (15/95; 16%), *ASXL2* (12/95; 13%), *KDM6A* (11/95; 12%), *CREBBP* (8/95; 8%), *SRCAP* (8/95; 8%), *EZH2* (7/95; 7%), *SETD2* (5/95; 5%), *TET2* (12/95; 13%) and *DNMT3A* (5/95; 5%), highlighting their contribution in altering the epigenetic state of this leukemia subtype. Moreover, mutations affecting members of the CC were found with a high frequency: *RAD21* (13/95; 14%), *SMC1A* (5/95; 5%), *STAG2* (3/95; 3%), and *SMC3* (2/95; 2%). Of note, mutations in CC genes were almost mutually exclusive. We also identified additional mutations in previously detected cooperating genes such as mutations clustering in exon 2 of the *ZBTB7A* gene (15/95; 16%), encoding for a transcription factor involved in hematopoietic lineage fate. Recurrent mutations were also observed in *CCND2* (9/95; 9%), that plays an important role in regulation of hematopoietic cell proliferation, as well as *DHX15* (6/95; 6%) being involved in spliceosome function and ribosome biogenesis. With respect to the clonal architecture we found that the median VAF in genes belonging to ER and CC (0.30; range 0.03-0.91; 0.31, range 0.05-0.73; respectively) was higher than in genes associated with TK signaling (0.19, range 0.05-0.53). These data suggest that alterations affecting the epigenetic state and differentiation occur earlier than those in signaling during t(8;21) leukemogenesis.
Summary/Conclusions: Using a comprehensive, deep sequencing approach we could further characterize the mutational landscape of t(8;21) positive AML. Here, mutation clusters in genes involved in TK signaling, ER and CC were confirmed as well as novel CBF-associated gene mutations that play an essential role in regulation of hematopoietic cell proliferation and differentiation. Further analyses in terms of sample size extension as well as correlation of findings with clinical parameters are ongoing.

E865

NFKB PATHWAY PROMOTES TUMOR PROGRESSION THROUGH BRUTON'S TYROSINE KINASE IN MLL+ ACUTE MYELOID LEUKEMIA

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Background: Mixed Lineage Leukemia's (MLL's) are characterised cytogenetically by reciprocal translocations of the MLL gene and clinically by unfavourable outcomes. Evidence indicating that MLL leukemia's are resistant to apoptosis encourages the identification of novel drug targets.
Aims: Using cord blood (CB) CD34+ cells (control) and CB CD34+ cells expressing MLL-AF9, we sought to determine the potential role of BTK in the development and progression of MLL+ leukemia. We further aimed to uncover possible downstream target/s of BTK, improving the therapeutic efficacy of the drugs used.
Methods: Experiments were performed using control and MA9.3 cells and leukemic blasts from 3 AML (MLL+) patients. Signalling events were evaluated by immunoblotting. p65 mediated BTK expression was determined by promoter assays. Cells were treated with specific inhibitors of BTK (Ibrutinib (IBR): 0.25, 0.5, 1.0 and 2μM) in combination with Daunorubicin (DAU 5nM) or RAC (NSC 23766 (NSC): 5, 10, 15 and 20μM) for 48 hrs and cell viability was assessed using Annexin V/ Sytox-Blue based flow cytometric analysis.
Results: To determine the relevance of BTK as a therapeutic target in MLL+AML, we examined the whole cell lysates (WCL) from control cells, two clones of CB expressing MA9 (MA9.3 and .6) and leukemic blasts from the 3 AML patients. Activated BTK (pY223) was detectable in both the clones of MA9 and MLL+AML samples. Interestingly, the cells demonstrated activation of p65 (pS536) but not in control cells. To address if activated p65 could potentially drive BTK expression, we performed BTK promoter assays with reporter construct and empty vector. MA9.3 cells electroporated with test construct demonstrated significantly higher transcriptional activity. At the protein level, p65 inhibitor treatment (MG132 or Bay 11-7082) reduced total BTK expression, indicating the involvement of activated p65 in the expression of BTK. Treatment of control and MA9.3 cells with various concentrations of IBR for 48 hrs induced a dose-dependent reduction of cell viability (Annexin V and Sytox blue negative). We further sought to determine if the use of IBR in combination with Daunorubicin would further sensitize MA9.3 cells. The apoptotic rate of the cells with combination treatment was significantly higher than that of cells treated with IBR or DAU alone. The coefficient of drug interaction (CDI) values indicated that IBR-DAU combination synergistically reduced cell viability (CDI >1.0-antagonistic; <1.0 synergistic and =1 additive effect). Recent studies suggested RAC-GTPase signaling may also represent a target in AML, particularly in the context of *MLL* gene rearrangements (Wei J et al., Cancer Cell 2008 and B Mizukawa et al., Blood 2011). This intrigued us to test if BTK is possibly upstream of RAC. We measured activation of the GTPase RAC via active RAC pull down assay. Interestingly, treatment with IBRU significantly reduced RAC activation, positioning BTK upstream of RAC. In line with observations reported earlier; we also observed that MA9.3 cells are responsive to RAC inhibitor, NSC. This effect of NSC on cell viability was further potentiated in combination with IBR (0.5μM). CDI values once again indicated that the drugs together have a synergistic effect on reducing the cell viability.
Summary/Conclusions: Taken together, our data support a biological link between NFκB, BTK and RAC pathways in the modulation of cell survival in MLL-rearranged AML cells. Aberrantly active p65 drives the expression of BTK and contributes to the progression of the AML. Combination treatment using IBR-DAU and IBR-NSC might be a promising therapeutic strategy, minimizing high drug dose-related side effects but increasing the therapeutic efficacy.

E866

A PRECISION MEDICINE PLATFORM FOR ACUTE MYELOID LEUKEMIA TO HELP UNRAVELING THE MOLECULAR ADDICTIONS OF FLT3-ITD-DRIVEN AML

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Background: Acute myeloid leukemia (AML) is an aggressive disease with poor prognosis (Tzelepis *et al.* 2016). No single driver mutation is present in all cases of AML, making its treatment a challenge (The Cancer Genome Atlas Research 2013). Current standard of care for AML is an aggressive cytostatics-based treatment that has remained unchanged for the past 30 years (Longo *et al.* 2015). Weak or elderly patients might not be eligible for intensive treatment, leading to poor survival rates. Many such patients are labeled as "untreatable", although a portion of them could benefit from specific, individualized treatment. A precision medicine strategy can help to find the specific treatment for these 'untreatable' AML patients.
Aims: Drug-driven personalized medicine aims to directly test the sensitivity of primary cancer cells taken from individual AML patients to a selection of targeted cancer drugs, compare these results with drug sensitivities of healthy donor samples and select the most effective drug for each patient. This approach considers any combination of mutations or epigenetic changes that might not be found in the standard sequencing panels, an advantage when dealing with such a heterogeneous disease. Proof of principle of this strategy was recently demonstrated by FIMM (Helsinki, Finland) (Pemovska *et al.* 2013), not only providing immediate clinical benefit to leukemia patients, but also identifying drugs that can potentially be repurposed for future treatment of patients.
Methods: We have established a drug-driven personalized medicine platform for AML where we check the *ex-vivo* drug sensitivity and resistance of bone marrow primary cells to a panel of around 400 drugs and drug combinations covering the standard of care treatments, cancer chemotherapeutics as well as many clinically available and emerging molecularly targeted compounds. We calculate the IC_{50} values for all the drugs for each individual donor or patient, and then the differential drug sensitivity scores, selecting the drugs that affect preferentially the cancer cells when compared with healthy cells. To date we have successfully processed 6 healthy donors and 66 AML samples identifying subgroups of patients who respond with a similar dynamic to certain classes of drugs, as the subgroup of cells carrying internal tandem duplications in the receptor tyrosine kinase FLT3 (FLT3-ITD).
Results: FLT3 activating mutations, particularly FLT3-ITD, have been observed in approximately 30% of AMLs (The Cancer Genome Atlas Research 2013), and are associated with increased risk of relapse and poor clinical outcome (Abu-Duhier *et al.* 2008). The cellular pathways that support FLT3-ITD-driven cell proliferation and survival remain poorly understood. We discovered that FLT3-ITD-expressing AML blasts show an enhanced sensitivity to HSP90 inhibitors such as Ganetespib compared to healthy donors and any other subgroups of leukemia. In addition, HSP90 inhibitors specifically sensitize FLT3-ITD-expressing bone marrow-derived cells to TKIs, whereas cells derived from healthy donors are unaffected. HSP90 inhibitors also preferentially eradicate a population of patient-derived FLT3-ITD+ AML cells expressing leukemic stem cell markers.
Summary/Conclusions: In summary, our study reveals a molecular basis for HSP90 addiction of FLT3-ITD-driven AML and provides a rationale for treatment of this form of AML with HSP90 inhibitors.

E867

SECRETION OF SOLUBLE FACTORS BY AML CELLS INFLUENCE CD33/CD3 BITE® ANTIBODY MEDIATED CYTOTOXICITY AND T-CELL PROLIFERATION

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Background: In our previous work, we showed that the CD33/CD3 BiTE® antibody construct (AMG 330) is able to recruit autologous, residual T cells and induce cytotoxicity against primary AML cells *ex vivo*. However, as described previously (Mussai et al, Blood 2013) primary AML cells are able to secrete soluble factors, which might not only influence T-cell proliferation but also negatively impact AMG 330 mediated cytotoxicity.
Aims: In this study we characterized the influence of soluble factors secreted by primary AML cells on AMG 330 mediated cytotoxicity.
Methods: We used plasma samples (from heparinized serum tubes or after density gradient centrifugation) from newly diagnosed and relapsed AML patients in AMG 330 cocultures of healthy donor (HD) T cells and AML cell lines. In flow cytometry based experiments we determined the influence of AML plasma in comparison to fetal calf serum (FCS, heat inactivated) on AMG 330 mediated cytotoxicity and T-cell proliferation. In transwell experiments using primary AML cells physically separated from AMG 330 cocultures, we evaluated if AML cells are the source of soluble factor secretion.
Results: The influence of AML plasma from bone marrow (BM) of AML patients on AMG 330 mediated cytotoxicity and T-cell proliferation was heterogeneous: In 15/30 samples, AMG 330 mediated cytotoxicity was significantly reduced compared to cultures containing FCS (mean% specific lysis FCS *vs* BM: 72.8 *vs* 25.1). This was accompanied by a reduction in T-cell proliferation (mean% proliferation FCS *vs* BM: 27.7% *vs* 9.5%). The degree of immunosuppression could not be correlated to percentage of bone marrow blasts. Interestingly, the effect was not observed in AML plasma samples from peripheral blood (PB) (mean% specific lysis FCS *vs* PB: 84.7 *vs* 83.5;% proliferation FCS *vs* PB 32.2 *vs* 36.5, n=19). In the remaining 15 plasma samples from AML BM no influence on AMG 330 mediated T-cell function was observed (mean% specific lysis FCS *vs* BM: 82.1 *vs* 78.3;% proliferation FCS *vs* BM: 25.7 *vs* 26.8). In control cultures plasma from AML patients in complete remission (CR) or from HD BM was used which did not negatively impact AMG 330 mediated cytotoxicity (mean% specific lysis FCS *vs* CR: 95.6 *vs* 94.4, n=5; FCS *vs* HD: 89.6 *vs* 90.5, n=6), or T-cell proliferation (FCS *vs* CR: 76.6 *vs* 68.2; FCS vs HD: 58.9 *vs* 65.4). To further explore the influence of soluble factors from primary AML cells, we performed transwell experiments. Primary AML cells were cultured in the previously described long term culture system (Krupka et.al, Leukemia 2016) and HD T cells and MOLM 13 cells were placed in transwell devices (3μm). In analogy to our findings with AML BM plasma, we observed a strong reduction in AMG 330 mediated cytotoxicity and T-cell proliferation in 7/14 experiments (mean% specific lysis control *vs* AML: 95.0 *vs* 70.8;% proliferation control *vs* AML 78.6 *vs*

41.8, n=7). In the remaining 7 primary AML samples, no immunosuppressive effect was observed (mean% specific lysis control *vs* AML 98.9 *vs* 98.2;% proliferation control *vs* AML 82.8 *vs* 77.7, n=7).
Summary/Conclusions: In summary we demonstrated that BM derived plasma from AML patients at primary diagnosis or relapse reduced T-cell proliferation and AMG 330 mediated cytotoxicity in half of the samples tested. The immunosuppressive factors were secreted by primary AML cells as demonstrated by transwell experiments. Unraveling mechanisms of resistance to BiTE^{O} antibody mediated cytotoxicity will allow the exploitation and usage of enhanced strategies to increase response rates.

E868

CLONAL EVOLUTION OF FLT3-ITD POSITIVE AML AT DIAGNOSIS AND RELAPSE IN PATIENTS TREATED WITHIN THE CALGB 10603 (RATIFY) AND AMLSG 16-10 TRIALS

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Background: Internal tandem duplications (ITD) in the receptor tyrosine kinase *FLT3* occur in about 22% of patients (pts) with acute myeloid leukemia (AML) and confer a poor prognosis depending on the mutational load. The multi-targeted tyrosine kinase inhibitor (TKI) midostaurin has been shown to improve outcome in *FLT3*-ITD positive (*FLT3*-ITD^{pos}) and *FLT3*-TKD mutated (*FLT3*-TKD^{mut}) pts in combination with intensive chemotherapy [CALGB 10603 (RATIFY) trial], thus representing a promising targeted treatment approach. However, a significant number of pts relapse after initial response due to yet unknown mechanisms.
Aims: To study the clonal evolution in *FLT3*-ITD^{pos} pts treated in the AMLSG16-10 (NCT01477606) or CALGB 10603 (RATIFY, NCT00651261) trial in paired samples obtained at diagnosis (Dx), complete remission (CR) and relapse (Rel) by whole exome sequencing (WES).
Methods: WES was performed in 17 *FLT3*-ITD^{pos}pts using the Nextera Rapid Capture Exome kit (Illumina) for library preparation followed by sequencing on a Illumina HiSeq2000. 6 pts were treated in the RATIFY trial receiving either midostaurin or placebo combined with intensive chemotherapy during induction and consolidation; 11 pts were treated in the AMLSG16-10 trial, which includes treatment with midostaurin combined with intensive chemotherapy during induction and consolidation followed by a one-year maintenance therapy with midostaurin; 4 pts in the AMLSG16-10 trial received allogeneic hematopoietic cell transplantation. The presence of *FLT3* and *NPM1* mutations (mut) and the allelic ratio (AR) of *FLT3*-ITD were analyzed according to standard protocols.
Results: The median AR of *FLT3*-ITD was 0.51 (0.10-18.94) and 0.54 (0.07-26.31) at Dx and Rel, respectively. Loss of *FLT3*-ITD was observed in 5 pts; changes of the ITD clone at Rel occurred in 7 pts. Of those, 5 pts had a change of the insertion site and 1 pt gained an additional ITD clone at Rel. 3 pts had a D835Y *FLT3*-TKD^{mut} that was lost at Rel. 6 pts had a *NPM1*^{mut} that persisted at Rel in all pts. Using WES, 301 mut (226 missense, 24 nonsense, 41 indels, 6 splice sites, 4 unknown) were identified. The average coverage was 125 (186-67) among all samples. 131 (43%) mut were present at both time points (Dx and Rel). 83 (28%) mut were found only at Dx or only subclonal at Rel. 73 (24%) mut were detected only at Rel and 14 mut with only 1 read at Dx. Besides *FLT3*-ITD, the average number of mut per sample (Dx or Rel) was 13. Mut were most frequently observed in genes related to signaling (23%), transcription (20%), DNA methylation (5%), chromatin-cohesin (4%), components of the membrane (4%) and ubiquitin-proteasome (4%). Pre-leukemic mut (*DNMT3A*, *TET2*, *IDH1/2*) were detectable in 10 pts at both time points and persisted at CR in 7 pts. Recurrent mut in transcription related genes occurred in 8 pts at Dx and Rel, with *WT1* mut being most frequent (n=7). Mut in signaling related genes present at both time points included *NRAS* (G12V/D) and *NF1* mut. At the time of Rel, gene mut frequently referred to signaling (34%) including a *KRAS* (G13D) and a *KIT* (D816V) mut, both in pts with loss of *FLT3*-ITD at Rel.
Summary/Conclusions: Analyzing the clonal evolution of *FLT3*-ITD^{pos} AML, known pre-leukemic mut were stably detectable at Dx and Rel in most pts, whereas known signaling related gene mut were also acquired at Rel. To further investigate more comprehensively pathways underlying therapy resistance with a focus on TKI treatment, larger cohorts of pts are currently analyzed for the detection of recurrent mutational patterns.

E869

MICROENVIRONMENT SECRETED PROTEINS MEDIATE RESISTANCE TO TARGETED THERAPY IN PRIMARY AML CELLS

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Background: The bone marrow stromal microenvironment (BMSM) plays an important role in the pathophysiology of acute myeloid leukemia (AML). This is demonstrated by primary AML blasts dependence on stromal conditioned media to survive long-term in culture. Although some of the components of the stromal secretome (the totality of secreted proteins by biological cells) that augment AML survival are known, the precise molecular mechanisms of the stromal-blast interactions are not fully defined.
Aims: i) Identify proteins secreted by bone marrow stromal cells that mediate AML survival; ii) Investigate global changes in signalling pathway activity induced by stromal factors in primary AML; iii) Validate the functional significance of these interactions through targeted inhibition of BMSM activated signalling pathways.
Methods: We used primary AML cells and established cells lines. Four different human AML lines were grown individually or in co-culture with a mouse bone marrow stromal line (MS-5). The resulting conditioned medium from these experiments (4 AML lines alone, 4 AML lines + MS-5, MS-5 alone) was purified to obtain the secretome (in triplicate). Proteins in these secretomes were quantified using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS). Peptide sequence searches against both mouse and human proteomes allowed for discrimination between the mouse stromal and human AML proteins. Guava EasyCyte Flow Cytometry was used to measure the viability and proliferation of these cell populations, assessing the capabilities of the identified factors above on primary AML cells (n=6) as well as the effects of kinase inhibitors Torin-1, Trametinib, Tofacitinib and Midostaurin over 72hrs with and without stromal conditioned media (n=10). Label-free LC-MS/MS based phosphoproteomics quantified >5,000 phosphorylation sites in primary AML patient cells treated with identified stromal factors individually and combined in triplicate. Commercial (MASCOT) and in-house (PESCAL, KSEA) software were utilised to identify and quantify proteins, determine kinase activities and interpret intracellular signalling.
Results: Initially by comparing secretomes of the four AML lines (on their own or in MS-5 co-culture) we identified 520 bone marrow stromal proteins and 293 AML blast proteins. From these, six stromal proteins were selected (including S100-A11, connective tissue growth factor [CTGF] and bone morphogenic protein-1 [BMP-1]) based on their ability to effect growth and likely signalling capacity in AML cells. These six proteins were used in varying combinations to determine their effect on growth of primary AML cells from patients (n=6). We also analysed the phosphoproteomes of primary AML cells displaying the maximal effects on growth in response to the six factors above to determine the underlying biological mechanisms. These studies have shown that several different pathways are activated as a result of secretome treatment including mTOR and MAPK. Primary AML cells were sensitive to targeted inihibition of these pathways. However, the inclusion of stromal secreted factors to the same AML cells would induce sensitivity towards another kinase inhibitor and insensitvity towards the previously effective inhibitor.
Summary/Conclusions: This proteomic approach has allowed identification of a panel of key proteins (including S100-A11, CTGF, BMP-1) secreted by the stromal cells that modulate cell signalling and cell fate in AML blasts. Using a proteomic approach to study growth factor effects we were able to dissect the factor specific effects on AML signalling. Subsequent survival assays and targeted inhibition studies demonstrate that despite heterogeneity in patient response to these factors, activity in key signalling pathways such as MAPK and mTOR switch under stromal influence. These observations suggest that resistance to targeted therapies *in vivo* in part may arise from changes that AML cells induce in the microenvironment.

E870

CHARACTERIZATION OF FLT3 MUTATIONS AT DIAGNOSIS, REFRACTORY DISEASE OR RELAPSE IN AML PATIENTS TREATED WITH MIDOSTAURIN WITHIN THE CALGB 10603 (RATIFY) AND AMLSG 16-10 TRIALS

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Background: Internal tandem duplications (ITD) and mutations (mut) in the tyrosine kinase domain (TKD) of the receptor tyrosine kinase *FLT3* occur in about 25% of acute myeloid leukemia (AML) patients (pts). *FLT3*-ITD is associated with an unfavorable prognosis in particular in pts with a high allelic mutant to wildtype ratio (AR;>0.5) as well as localization of the ITD in the beta1-sheet of the receptor. FLT3is targetable by tyrosine kinase inhibitors (TKI) and the combination of chemotherapy with the TKI midostaurin has been recently investigated within the CALGB 10603 RATIFY trial and is still under investigation within the AMLSG 16-10 trial.
Aims: To study the *FLT3*^{mut} status at the time of diagnosis (Dx), refractory disease (RD) and relapse (Rel) in AML pts treated within the CALGB 10603 (RATIFY, NCT00651261) and AMLSG 16-10 (NCT01477606) trial with regard to AR of *FLT3*-ITD and *FLT3*-TKD^{mut}, loss of *FLT3*-ITD and *FLT3*-TKD^{mut} and change of ITD clones (ITD insertion site, length, number of clones).
Methods: *FLT3*-ITD and *FLT3*-TKD^{mut} were detected using Genescan-based fragment-length analysis according to standard protocols. In the randomized phase-III RATIFY study, *FLT3*^{mut} pts were treated with induction (daunorubicin/cytarabine) and consolidation (high-dose cytarabine) plus midostaurin or placebo, followed by maintenance therapy with midostaurin or placebo for 1 year. The phase-II AMLSG 16-10 trial evaluates midostaurin in induction, consolidation (allogeneic hematopoietic cell transplantation or high-dose cytarabine) and maintenance therapy in *FLT3*-ITD positive pts.
Results: In total, 83 pts were analyzed, of which 33 were treated in the RATIFY and 50 within the AMLSG 16-10 trial. 36 pts had RD and 47 pts had relapsed. *FLT3*-ITDwas present at diagnosis in all pts treated in the AMLSG 16-10 trial; one pt had an additional *FLT3*-TKD^{mut}. Pts entered in the RATIFY trial had either a *FLT3*-ITD (n=22), a *FLT3*-TKD^{mut} (n=9), or both (n=2). The median AR of *FLT3*-TKD^{mut} at Dx was 0.82 (0.07-2.66) and the majority of pts showed loss of *FLT3*-TKD^{mut} at RD or Rel (n=9/12; 75%). In relapsed pts, loss of *FLT3*-ITD occurred in 14 (36%) pts. There was no significant difference between the median *FLT3*-ITD AR at Dx [0.62 (0.10-18.94)] and Rel [0.65 (0.07-38.75); p=.98]. A shift of the ITD clone was found in 14 (36%) pts at Rel, with switch of the ITD insertion site or length in 8 (21%) pts. 8/14 pts with change of the ITD clone at Rel had multiple ITD clones at Dx. For 35 *FLT3*-ITDpositive pts with refractory AML, *FLT3*-ITD loss was observed in 17 (49%) pts. The median AR of *FLT3*-ITD was significantly lower at the time of RD [0.29 (0.05-2.37)] compared to Dx [0.58 (0.05-8.91); p=.002]. The ITD clone changed in 5 (14%) pts with RD. In pts with shift of the ITD clone at RD (n=5) or Rel (n=14), no significant difference of the median ITD length was observed (p=.84).
Summary/Conclusions: Comparing the *FLT3*-ITD status at Dx, at the time of RD or Rel, we found a lower median AR of *FLT3*-ITD in pts at RD compared to Dx, whereas no significant change of AR was observed at Rel. In addition, loss of *FLT3*-ITD was observed in 49% of pts at RD and in 36% of pts at Rel. These findings suggest that the *FLT3*-ITD clone can be targeted in a significant number of pts and other clones might mediate resistance to treatment. We also observed a switch of the ITD clone in about 20% of pts with Rel, indicating the presence of ITD subclones at the time of Dx that might confer resistance to treatment. Despite the small number of TKD mutations in our study, it was remarkable that most of the TKDs (75%) were lost at the time of RD or Rel.

E871

A NOVEL PML-RARG FUSION IN ACUTE PROMYELOCYTIC LEUKEMIA

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Background: Acute promyelocytic leukemia (APL) is a unique subtype of acute myeloid leukemia (AML) characterized by specific translocation involving retinoic acid receptor alpha (*RARA*) locus. Retinoic acid receptor (RAR) is a member of nuclear receptor family, and has three types of isoforms such as RARA, reinoic acid receptor beta (RARB) and retinoic acid receptor gamma (RARG). These RARs share high structural homology (90%). Although the oncogenic properties of the artificial *PML-RARG* fusion gene was observed in an *in vitro* study, there has been no report on the *PML-RARG* fusion in human APL patients.
Aims: We report here a novel *PML-RARG* rearrangement in a patient with AML displaying suitable morphologic and immunophenotypic features of the classic hypergranular APL.
Methods: Whole genome sequencing (WGS) and further analysis of mRNA and gDNA were performed to clarify the atypical gene rearrangement observed by karyotyping and FISH.
Results: Laboratory, morphology and immunophenotypic analysis results suggested the classic APL with hypergranular type. A clonal translocation t(12;15)(q13;q22) was identified by karyotyping. No evidence of fusion of *PML-RARA* was detected by RT-PCR and *PML*-split was found on FISH analysis using *PML-RARA* dual color dual fusion probes. WGS analysis performed to clarifying the partner gene of *PML* located on chromosome 12q13 strongly suggested a *PML-RARG* fusion. RT-PCR following sanger sequencing were performed to verify the presence of *PML-RARG* fusion transcript, then two kind of transcripts was detected, one with the fusion of *PML* exon 3 and the middle part of exon 1 of *RARG* and the other with the fusion of *PML* exon 3 and exon 2 of *RARG*. The breakpoint of DNA was clarified on intron 3 of *PML* and 5' region of *RARG*. Despite of ATRA treatment for 9 days, cell count did not show any response. Then induction chemotherapy composed of idarubicin and cytarabine was combined on ATRA. ATRA was finally stopped after 18 days, then cytogenetic remission was acquired day 36 after induction therapy.
Summary/Conclusions: We first report the presence of *PML-RARG* fusion in a human APL patient. This report supports the possibility of a new molecular mechanism involving *RARG* not *RARA* in APL and suggests the need of different therapeutic approach for this variant case showing the potential ATRA resistance.

E872

COOPERATION OF MLL-PTD WITH DNMT3A OR RUNX1 MUTATIONS IN AML LEUKEMOGENESIS

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Background: Our previous study showed that *DNMT3A* or *RUNX1* mutations were frequently coexisted in the *MLL*-PTD AML patients (Oncotarget 2015).
Aims: We aimed to investigate the role of coexisted *DNMT3A* or *RUNX1* mutations in leukemogenesis of *MLL*-PTD AML.
Methods: After lentiviral-mediated over-expression of *RUNX1* or *DNMT3A* mutants in *MLL*-PTD mouse bone marrow (BM) cells or human *MLL*-PTD^{+} AML cell lines, colony formation, cell proliferation, differentiation and apoptosis assays were carried out. Interaction of RUNX1, HIF-1α, and MLL-PTD were evaluated by co-immunoprecipitation assay. Differential genes and protein expression, histone modifying protein expression, and enrichment of histone-4 acetylation (H4Ac) were assessed by RT-qPCR, Western blot, and ChIP-qPCR, respectively. For BM transplantation assays in mice, *MLL*-PTD^{+} BM cells over-expressing *DNMT3A*-wild type (Wt)/mutants and empty vector (EV) control were injected into C57BL/6 mice via tail vein.
Results: We observed that *MLL*-PTD mouse BM cells with *RUNX1* mutants lacking C-terminal VWRPY sequence (H377fsX and V425fsX576) had increased self-renewal, proliferation, increased HIF-1α and its downstream gene expression. In addition, the interaction of HIF-1α and MLL-PTD was disrupted in the cells transduced with C-terminal truncated RUNX1 mutants or *RUNX1* shRNA. Compared with those expressing *DNMT3A* mutants, over-expression of *DNMT3A*-Wt reduced cell growth, colony formation and self-renewal activities of EOL-1 and *MLL*-PTD^{+} BM cells. All *DNMT3A* mutants impaired Na-butyrate-induced differentiation, but only R882H mutant impaired ATRA-induced differentiation *in vitro*. *DNMT3A* mutations were associated mostly with up-regulation of *homeobox B* (*HOXB)* genes. The expressions of *BCL2A1*, *AREG, PRKCA, HLX, hBEGF* and *MCL1* were increased in the *DNMT3A*-mutant cells compared to those of *DNMT3A*-Wt. Cells with *DNMT3A* mutants showed a reduction of H4Ac enrichment at the *HOXB* and *HOXC12* promoter regions compared to the control cells or the cells with *DNMT3A*-Wt. DNA methylation microarray analysis identified both hypo- and hypermethylation features in different regions throughout the whole genome of *DNMT3A* mutants-transduced EOL-1 cells. Up-regulated genes including *HLX* and *hBEGF* were hypomethylated in the EOL-I cells transduced with *DNMT3A* mutants. *In vivo* study showed that white blood cells including neutrophils, lymphocytes and monocytes increased significantly (P<0.03) in the *DNMT3A*-mutants mice compared to EV or Wt- mice at 10 months post-BM transplantation.
Summary/Conclusions: The present study showed that both *RUNX1* and *DNMT3A* mutants dysregulated self-renewal, proliferation and apoptosis in the mouse *MLL*-PTD BM cells. Disruption of MLL-PTD-RUNX1-HIF-1α complex in the *RUNX1*-mutant and aberrant methylation in the *DNMT3A*-mutant cells might play an important role in AML pathogenesis. Our results showed that cooperative *RUNX1* or *DNMT3A* mutations had impact on leukemogenesis of *MLL*-PTD AML.

E873

AML BLASTS INDUCE A SENESCENT PHENOTYPE IN THE BM-MSC THROUGH THE UPREGULATION OF P21

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Background: Acute myeloid leukaemia (AML) is a heterogeneous clonal disorder that arises from the haematopoietic myeloid progenitor cells within the

bone marrow microenvironment (BMM). Survival of patients with AML is presently poor; two-thirds of younger adults and 90% of older adults die of their disease. Even in patients who achieve remission with chemotherapy, relapse is common and occurs from minimal residual disease sequestered in protective niches in the BMM. Reciprocal interactions between that of the AML and bone marrow mesenchymal stromal cells (BM-MSC) are central to the survival and progression of the leukaemic blasts through micro-environmental promotion of quiescence in malignant cells as well as the activation of anti-apoptotic and pro-survival pathways.
Aims: To investigate how BM-MSC are programmed by AML to generate a pro-tumoural environment.
Methods: Primary AML and BM-MSC were isolated from the pelvis of AML patients following informed consent and under approval from the UK National Research Ethics Service (LRCEref07/H0310/146). Low input RNASeq of 10 AML BM-MSC and 10 healthy BM-MSC (taken from the pelvis of patients undergoing elective hip replacement surgery) was performed following CD271 MicroBead selection. Primary AML blasts $1x10^6$ were co-cultured on confluent primary BM-MSC for 48 hours (h), 72h and 168h. Real-time PCR was used to verify the RNA sequencing data and Western Blot analysis to confirm protein expression. Lentivirus mediated knockdown was used to target gene expression in the BM-MSC. Senescence was assayed by β-Galactosidase staining.
Results: Results from the RNA sequencing carried out to compare 10 healthy and 10 AML BM-MSC show that 1125 genes were differentially expressed, with 924 down-regulated in AML derived BM-MSC and 201 up-regulated. From this analysis, we found that CDKN1A (p21) is up-regulated in BM-MSC from AML patients (7.406 logFC) compared to BM-MSC from patients with normal bone marrow. Further analysis of p21 mRNA expression confirmed an increase in AML BM-MSC compared to normal BM-MSC. In-vitro experimentation showed that p21 mRNA and protein expression is increased in BM-MSC when co-cultured with primary AML. Furthermore, we show that AML increased senescence β-Galactosidase staining in BM-MSC and that p21 knockdown in BM-MSC reversed the senescent phenotype. Finally, primary AML cultured on p21 knockdown BM-MSC had reduced survival compared to control BM-MSC.
Summary/Conclusions: We have identified that AML induces a senescent BM-MSC niche via the p21 mediated pathway which in turn promotes survival and proliferation of AML. Silencing of p21 within the BM-MSC reduces AML survival. In identifying this novel microenvironment feedback loop in AML we highlight a potential new target for future AML therapies.

E874

HYPOXIA DRIVES AML PROLIFERATION IN THE TUMOR MICROENVIRONMENT THROUGH HIF1A/MIF SIGNALLING

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Background: The bone marrow microenvironment is hypoxic and furthermore hypoxia contributes to the development and maintenance of acute myeloid leukemia (AML) cells within the bone marrow microenvironment. The hypoxic state is principally maintained by members of the hypoxia-inducible factor (HIF), in particular HIF1a and its target genes, including MIF. We have previously shown that AML cells express constitutively high macrophage migration inhibitory factor (MIF) which drives IL-8 expression by the BM-MSC which in turn supports AML cell survival and proliferation (Abdul-Aziz et al, 2017).
Aims: The aim of the present study is to determine the role of hypoxia in regulating MIF signalling in AML.
Methods: Primary AML were isolated from the bone marrow and peripheral blood of AML patients following informed consent and under approval from the UK National Research Ethics Service (LRCEref07/H0310/146). Differential expression analysis of RNA sequencing data (GEO ID: GSE49642) was used to compare hypoxia-associated gene expression of BM AML blasts with AML blasts obtained from the peripheral blood (PB). AML cell lines and primary AML blasts were cultured under normoxic (20% oxygen) or hypoxic conditions (1% oxygen). MIF mRNA expression was determined by RT-PCR and protein was determined using target specific ELISA. HIF1a protein expression was determined by western blotting. Lentiviral-knockdown of MIF and HIF1a were performed on AML blasts prior to in-vivo xenograft mouse model injection. Finally, in-vivo analysis of the MIF inhibitor, ISO-1, was performed in the AML xenograft model.
Results: Our results show that MIF gene expression was significantly higher in AML samples from the BM compared to those from PB. To determine If MIF is regulated by HIF1a in AML cells, we mimicked the hypoxic conditions of the BM using $CoCl_2$, DFO and a hypoxic chamber. We found that 1% O_2, $CoCl_2$ and DFO upregulate MIF transcription and protein expression in OCI-AML3 cell lines and in primary AML blasts. Lentiviral mediated KD of HIF1a decreased MIF expression in human AML cells and significantly reduced leukaemic proliferative capacity. Moreover, KD of HIF1a in OCI-AML3 significantly increased survival of NSG mice compared to control-KD. Finally, in-vivo lentiviral mediated knockdown of MIF and pharmacological targeting of MIF using ISO-1 significantly increased survival of AML xenografts.
Summary/Conclusions: The results reported here suggest that hypoxia significantly affects the expression of the pro-tumoural cytokine MIF in AML blasts and that this hypoxia regulated HIF1a/MIF axis supports AML blast survival in the bone marrow niche.

E875

BONE MARROW ECOLOGICAL COLLAPSE IN ACUTE MYELOID LEUKEMIA IS MEDIATED BY REMODELING OF ENDOSTEAL VESSELS

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Background: Bone marrow vascular niches have been proposed to support acute myeloid leukemia (AML) growth. However, anti-angiogenic therapies do not improve patient outcome suggesting that a complex relationship between AML cells and the microenvironment influences the disease process.
Aims: We aim to study the complex vascular remodelling occuring during AML progression.
Methods: Using a murine model of AML we performed intravital microscopy to investigate leukemia behavior in the bone marrow.
Results: We show AML is an invasive species causing highly localized disruption of the endosteal stroma and outcompeting non-malignant cells. Particularly affected are endosteal microenvironments containing osteoblastic cells and type H endothelium, typically associated with hematopoietic stem cells (HSCs). In contrast, splenic endothelial cells expand, suggesting *de novo* niches in the spleen could potentially support extramedullary hematopoiesis in leukemia. Intravital microscopy further revealed that the endothelium in AML is more adhesive and permissive to transendothelial migration of hematopoietic cells. Pharmacological intervention known to induce type H endothelium preserved HSCs in endosteal areas of diseased mice.
Summary/Conclusions: Together, these data suggest that AML-induced vascular damage contributes to cell egress from the bone marrow, and that new therapeutic approaches aiming to normalize bone marrow vasculature may support normal hematopoiesis.

E876

CLONAL HETEROGENEITY IN PATIENT-DERIVED XENOGRAFT OF ADULT ACUTE MYELOID LEUKAEMIA

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Background: Acute myeloid leukaemia (AML) is the most common leukaemia in adults. Currently, despite intensive chemotherapy and bone marrow transplantation, outcome is still dismal. In particular, therapeutic stratification remains suboptimal, which is largely attributed to the clinical and molecular heterogeneity of AML.
Aims: To better characterize and study this heterogeneity, we developed an *in vivo* model of AML using patient derived xenografts (PDX).
Methods: Blasts cells obtained from bone marrow of 45 *de novo* AML were intravenously injected into NOD-scid gamma (NSG) mice. Engraftment was surveyed by chimerism of CD45 (human *versus* murine) by flow cytometry. At sacrifice (peripheral blast count greater than 70% or clinical sign of illness), cells collected from bone marrow and spleen were used to perform targeted sequencing (AmpliSeq, Thermo Fisher Scientific®) and gene expression analyses (HG-U133 Plus 2.0 microarray, Affymetrix®). Bone marrow cells were serially transplanted into secondary and tertiary animals. We then compared mutational and gene expression profiles of patient samples at diagnosis and corresponding PDX samples.
Results: Eighteen out of 45 injected samples (40%) successfully engrafted into mice with a median delay of 2.5 months (range : 26-154 days). Leukaemia infiltration into bone marrow was concordant with peripheral blood and spleen infiltration. Successful xeno-engraftment was linked to younger age (50 *vs* 61 years, p=0.04) and elevated white blood cell counts at diagnosis (132 *vs* 35 G/L, p=0.001). No association was found between engraftment and karyotype or ELN classification. Relapse free survival (RFS) was worse for patients with successful PDX (0.3 *vs* 0.9 years, p=0.017). Despite previous reports suggesting better engraftment of AML harbouring FLT3-ITD mutations, we did not find

a preferential engraftment in the presence of FLT3-ITD mutation (9 of 18). Furthermore, we found that the mutational fraction of 3 out of 4 patients harbouring a FLT3-ITD mutation enriched for this mutation in the primary PDX and then remained stable in subsequent xenotransplantations. Similarly, eight PDX with respective primary AML were analysed by next-generation sequencing (NGS) of 27 AML relevant genes. We found stable variant allele fractions (VAF) among the primary and serial PDX bone marrows and spleens for 50 mutations (6 mutations on average per patient); except for DNMT3A and NRAS mutations, which were lost each for one AML sample at the secondary PDX. Concordantly, gene expression profiles were stable between primary patient sample and serial PDX samples up to the tertiary xenograft.
Summary/Conclusions: NSG mice xenotransplantation may be a clinical relevant model for *in vivo* studies of clonal heterogeneity in AML and may be used as preclinical model to test novel therapies.

E877

COSTIMULATION INCREASES INTRACELLULAR SIGNALLING IN BITE® ANTIBODY CONSTRUCT MEDIATED T-CELL ACTIVATION

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Background: The CD19/CD3 BiTE® antibody construct, blinatumomab, has been approved in Ph-, relapsed/refractory B-cell precursor Acute Lymphoblastic Leukemia (ALL). Despite the promising response rate of 43% in heavily pre-treated ALL patients, reasons for resistance have not been determined. In contrast to classical T-cell activation, BiTE® antibody construct mediated T-cell activation relies solely on binding to the CD3ε chain of the T-cell receptor (TCR) complex. Resolving the exact mechanism of BiTE® antibody construct mediated T-cell activation is a prerequisite for our understanding of mechanisms of resistance.
Aims: In the present study we characterized the role of costimulation on intracellular signalling in CD33/CD3 BiTE® antibody construct (AMG 330)-mediated T-cell activation.
Methods: We generated a murine cell line stably expressing human CD33 and devoid of human costimulatory molecules (B33). In *in vitro* cocultures, cytotoxicity against B33 cells and the AML cell line MOLM-13 was evaluated by flow cytometry. Activation of downstream signalling pathways was assessed by a phospho-flow cytometry protocol for T-cell recruiting antibodies.
Results: Coculture of B33 cells with CD3+ healthy donor T cells (n=4) resulted in AMG 330 mediated mean cytotoxicity of 58.3%. In contrast, MOLM-13 cells were completely lysed (% specific lysis relative to control B33 *vs* MOLM-13: 58.3±32.9 *vs* 99.9±0.1, n=4), despite comparable CD33 expression levels (CD33 median fluorescence intensity (MFI) ratio: B33 116.1 *vs* MOLM-13 67.6). However through the addition of an anti-CD28 antibody or recombinant human IL-2, cytotoxicity against B33 cells could be restored (% specific lysis AMG 330 *vs* AMG 330 + anti-CD28 *vs* AMG 330 + IL-2: 58.3±32.9 *vs* 82.6±11.1 *vs* 91.3±9.0). At lower E:T ratios (1:4) the additional costimulatory signal also increased AMG 330 mediated cytotoxicity against MOLM-13 cells (mean MFI Ratio of CD80 and CD86 on MOLM-13 cells: 1.4±1.2 and 3.0±1.0 respectively, n=3) (% specific lysis AMG 330 *vs* AMG 330 + anti CD28: 65.1±19.7 *vs* 80.7±16.1, n=3). We next analysed intracellular Akt and Erk phosphorylation levels of T cells after stimulation with AMG 330 or a control BiTE® antibody construct (cBiTE®) and MOLM-13 cells. Crosslinked anti-CD3/anti-CD28 antibodies served as positive control. In the presence of target cells, AMG 330 induced significantly lower Akt and Erk phosphorylation (mean% phosphorylated (p)Akt and pErk 7.9 and 7.6, n=3-5) compared to crosslinked CD3/CD28 antibodies (mean% pAkt and pErk 43.0 and 34.6). However, the combination of AMG 330 and CD28 increased the amount of phosphorylated proteins (mean% pAkt and pErk 11.6 and 11.1), but not to the level achieved by CD3/CD28 stimulation. In the absence of target cells, no Akt phosphorylation was observed upon incubation with AMG 330, suggesting a highly target cell dependent T-cell activation (mean% pAkt with *vs* without target cells: 0.8 *vs* 7.9).
Summary/Conclusions: Our data support the hypothesis that costimulation influences the susceptibility of target cells to lysis by T-cell recruiting antibody constructs. Currently, we are validating our results in a larger cohort using T cells from healthy donors and patients with AML. Furthermore, we will analyse the phosphorylation pattern within different T cell subsets and upon knock out of B7 molecules in MOLM-13 cells. Our results will contribute to the understanding of BiTE® mediated activation of T cells, which is a prerequisite for clinical responses.

E878

ESTABLISHING SINGLE CELL WHOLE EXOME SEQUENCING ANALYSIS AS A DISCOVERY TOOL IN NPM1/FLT3 POSITIVE PEDIATRIC ACUTE MYELOID LEUKEMIA

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Background: AML is a rare hematological disorder in children and adolescents caused by distinct genetic aberrations, which are relevant for leukemogenesis, prognosis and therapy. Although major players in the molecular landscape and clonal evolution of AML have been identified in adults, it remains a major technical challenge to genetically characterize the few leukemic stem cells (LSCs) cells against a noisy background of AML blasts and normal hematopoietic cells.
Aims: The aim of this study was to establish a simple workflow for reliable genetic analysis of single LSCs in pediatric patients with AML, where often limited research material is available.
Methods: For three pediatric AML patients with mutations in the genes NPM1 and/or FLT3, we performed single cell sorting for CD34+ CD38- AML blasts by FACS and subsequently whole genome amplification (WGA) using the REPLIG single cell kit (Qiagen). The amplified single cell DNA and additionally the DNA of the corresponding bulk bone marrow was analysed by exome sequencing (WES). Bulk DNA was additionally evaluated by an amplicon-based sequencing approach targeting 54 genes (TruSight Myeloid Panel, Illumina) associated with myeloid malignancies.
Results: The analysis revealed that the median read coverage obtained in the WES of the five DNAs amplified from the single CD34+ CD38- cells and in the corresponding bulk DNAs from the bone marrow of all three patients was comparable for three out of the five single cells. For three amplified single cell genomes, between 92 and 98% of all reads could be mapped to the human genome, however the median coverage for the two failed single cells was 0. For validation of the WGA quality from single LSC DNA, data from 50 out of 54 genes genes analyzed by both sequencing approaches, WES and TSM Panel, were available for all three patients. The majority of variants detected in the WES bulk data could consistently be found at a comparable variant frequency in the TSM panel data. The variant frequencies in the single cell data from WES were more variable and more variants could not be detected in the TSM panel data derived from bulk DNA. We were able to detect n=79 (66%) out of n=121 somatic variants (SNVs, InDels) present in the patients' AML blasts with all three sequencing approaches. WES readily identified n=103 (85.1%) and n=93 (76.9%) of all 121 variants in the bulk and single cell DNA, respectively. Only n=4 (3.3%) variants were not detected by WES at all. We were able to retrace the NPM1 and FLT3 mutations for each of the three patients in the targeted sequencing approach. However the NPM1 mutations and one FLT3 ITD could not reliably be called in the WES approach due to insufficient coverage.
Summary/Conclusions: In summary, WES of amplified single cell DNA is an excellent discovery tool also in pediatric AML for detecting unique changes in potential LSCs that should be validated by targeted sequencing approach with sufficient read counts for finding of rare events.

E879

RAF KINASE INHIBITOR PROTEIN IS INVOLVED IN THE DEVELOPMENT OF MYELOID SARCOMA

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Background: Myeloid sarcoma (MS) is a subgroup of acute myeloid leukemia (AML), where leukemic cells invade non-hematopoietic tissues and form solid tumor masses. It may occur as isolated event or simultaneously with leukemic infiltration of the bone marrow (BM). Loss of RAF kinase inhibitor protein (RKIP), a negative regulator of RAS signaling, has recently been described as a frequent event in AML and to be functionally involved in leukemogenesis. Although RKIP has been shown to inhibit the formation of metastases in solid tumors previously, its role in the development of MS is currently unknown.
Aims: In this study, we aimed to delineate the role of the metastasis-suppressor RKIP in the development of MS.
Methods: RKIP protein and mRNA expression was evaluated in formalin-fixed paraffin-embedded biopsies of MS and BM by immunohistochemistry and quantitative real-time PCR (qPCR). Sequence analysis of MS biopsies defined as RKIP loss was carried out from 40ng of total DNA employing Ion Torrent Next Generation Sequencing (NGS). For functional assays, both RKIP overexpression and knockdown was performed in THP-1 AML cells by lentiviral transduction of a FLAG-tagged RKIP expression construct and by RKIP shRNA, respectively. Subsequently, these cells were tested in migration and invasion assays using the Transwell-methodology.
Results: This study comprised 14 patients with MS (MS-group) and 14 patients with AML without any evidence of extramedullary involvement (BM-AML group). Of the 14 cases within the MS-group, MS occurred as isolated event in three cases and concomitantly with systemic AML in eleven cases. Both groups were age- and sex-matched and clinical as well as laboratory values were comparable between them. Most importantly, however, when we measured the protein expression of RKIP in leukemic tissues of these patients (MS biopsies in the MS-group and leukemic BM biopsies in the BM-AML group), we observed a

significant increase of specimens exhibiting loss of RKIP expression in the MS-group (7/14 *vs* 1/14; P=0.0329). Interestingly, RKIP loss in MS specimens of cases with concomitant systemic AML was also present in the corresponding leukemic BM samples, thereby excluding a geographical clonal heterogeneity during MS formation in respect to RKIP expression. We then analyzed RKIP mRNA levels by qPCR and observed that RKIP loss correlated with decreased expression of its mRNA (P=0.041). To gain more insight into the molecular landscape of MS patients with and without RKIP loss, we performed NGS of 39 genes that are recurrently mutated in AML. Interestingly, five out of six (83%) MS patients with RKIP loss demonstrated mutation(s) affecting the RAS-pathway, suggesting a potential functional synergism between these events. Consequently, we performed stable overexpression and knockdown of RKIP in the RAS-mutated THP-1 AML cell line and subsequently studied these cells in functional migration and invasion assays. Importantly, RKIP knockdown increased both migration (relative ratio to control cells [rrtcc] of 193%, P=0.0097) and invasion (rrtcc of 171%, P=0.0197), whereas RKIP overexpression had the opposite effects (rrtcc of 56%, P=0.0036 for migration; rrtcc of 35%, P=0.0037 for invasion).
Summary/Conclusions: Loss of the metastasis-suppressor RKIP is associated to MS and to mutations affecting the RAS signaling cascade. In functional assays employing RAS-mutated AML cells, RKIP knockdown increased both, migration and invasion, thereby indicating a role of RKIP in the development of this condition.

E880

INHIBITING MIR-10A OVERCOMES CYTARABINE-RESISTANCE IN ACUTE MYELOID LEUKAEMIA

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Background: Chemoresistance is the principle cause of treatment failure in acute myeloid leukaemia (AML) despite a promising response to induction chemotherapy. Emerging evidence suggest the roles of autophagy, a self-eating process contributing to chemoresistance of leukaemia cells. We previously demonstrated that miR-10a, highly expressed in a subgroup of AML harbouring Nucleophosmin1 mutations, promotes cell survival by inhibiting non-canonical cell death pathway, suggesting its function in autophagy and thus chemoresistance in AML.
Aims: We aim to demonstrate evidence that miR-10a, a regulator of autophagy, plays important roles in chemoresistance in acute myeloid leukaemia.
Methods: Apoptosis and proliferation in miR-10a inhibited and overexpressed leukaemia cells after cytarabine treatment was measured by Annexin V binding and MTT assay. Autophagy was measured by monitoring the levels of LC3I/LC3II proteins, autophagy-related proteins via Western Blotting and monodansyl-cavaradine (MDC) staining (flow cytometry).
Results: First, we observed a decreased expression of miR-10a in the leukaemia cells after the exposure to stress induced by serum starvation. Overexpressing miR-10a in miR-10a low MV4-11 cells decreased apoptosis induced by nutrient starvation and resulted in the resistance to cytarabine. In contrast, its inhibition in OCI-AML3 cells, which express high miR-10a constitutively, resulted in the induction of apoptosis and increased chemosensitivity towards cytarabine. miR-10a was shown to directly downregulate key members of the p53-mediated tumour suppressor gene network, including the CDKN1A (p21) inhibitor Transcription Factor AP2-gamma (TFAP2C). The inhibition of either miR-10a itself or CDKN1A by siRNA treatment inhibited autophagy induced by serum starvation, treatment with autophagy inducer,mg132 or p53 stabiliser, Nutlin3a.
Summary/Conclusions: The data suggests miR-10a as an important regulator of autophagy via modulation of the p53-p21 tumour suppressor signalling axis in subtypes of AML. It also emphasizes the significance of autophagy in chemoresistance in AML, supporting the targeting of the autophagy pathway as a potential therapeutic approach for AML.

E881

BY AN MCL-1-DEPENDENT MECHANISM, ALVOCIDIB POTENTIATES THE ACTIVITY OF CYTARABINE AND MITOXANTRONE WHEN ADMINISTERED IN A TIME SEQUENTIAL REGIMEN IN AML

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Background: Treatment with alvocidib has shown significant improvements in the complete remission rates in newly diagnosed acute myeloid leukemia (AML) patients when administered before cytarabine and mitoxantrone (ACM regimen) in a randomized Phase 2 study compared to 7+3. Although the mechanism of alvocidib action as a single agent is documented, the mechanism underlying synergy found in the ACM regimen is not fully understood. The ACM regimen was originally developed based on the perceived benefit of a time-sequential regimen starting with cell-cycle arrest (alvocidib), followed by release of the cells from arrest and inhibition of DNA replication (cytarabine/mitoxantrone) during S-phase. However, recent reports suggest that the transcriptional repression of key anti-apoptotic proteins (eg., MCL-1) mediated by alvocidib's CDK9 inhibition, may contribute to the activity in the ACM regimen.
Aims: We hypothesized that MCL-1 transcriptional repression constitutes the primary mechanism for the synergism observed with the ACM treatment regimen.
Methods: Following treatment, cell viability and caspase activation, an indicator of apoptosis, were assessed using CellTiter-Glo and Caspase-Glo assays, according to manufacturer protocol. mRNA levels were assessed using RT-PCR. Protein levels were assessed using standard immunoblotting technique.
Results: In this study, we demonstrate that treatment with alvocidib, followed by treatment with cytarabine and mitoxantrone, synergized *in vitro* and correlated with the downregulation of MCL-1 protein and mRNA expression. Indeed, the ACM regimen resulted in a 2.4 or 3.4-fold increase in caspase activity relative to any single agent within the combination in MV4-11 or OCI-AML3 cells, respectively. As has been previously reported, we also observed that increased activity of cytarabine in alvocidib-treated cells corresponded with progression into the S-phase of the cell cycle, following the washout of alvocidib. However, this observation accounted for only a small portion of the inhibition of cell proliferation. This was further confirmed by the observation that CDK4/6 (cell cycle) specific inhibitors, such as palbociclib, did not show synergistic increases in caspase activity following treatment in the same setting. In various AML cell lines treated with MCL-1 siRNA, followed by cytarabine and mitoxantrone treatment, we also observed a synergistic increase in the inhibition of cell proliferation.
Summary/Conclusions: Considering our earlier work showing that MCL-1 dependence predicts AML patient response to the ACM regimen, we propose that MCL-1 repression is the primary mechanism of alvocidib's clinical activity. As MCL-1 also confers resistance to cytarabine, the current study provides additional rationale for the inclusion of alvocidib in the treatment of AML, and in the ACM regimen specifically. Taken together, this data suggests that the ACM regimen may be an effective regimen in treating patients with high-risk AML, because of alvocidib's inhibition MCL-1.

E882

DYSREGULATION IN KEY REGULATOR GENES OF AUTOPHAGY AS A MECHANISM OF THERAPY RESISTANCE AND POOR PROGNOSIS IN ACUTE MYELOID LEUKEMIA (AML): RESULTS FROM MICROARRAY ANALYSIS ON 148 PATIENTS

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Background: To date, there are no clear evidences if autophagy can lead to therapy resistance or favor apoptosis in cancer. Autophagy can function as a pro-apoptotic mechanism, or can improve stresses survival clearing damaged mitochondria and proteins accumulation. Levels and activity of pro-apoptotic and anti-apoptotic proteins, particularly BCL-2 and p53, high levels of cAMP, and a complex made by PINK/PARK could play as fulcrum of this yin and yang effect of autophagy.
Aims: Our study aims to define the role of PI3P pathways in AML, and to establish if autophagy could reduce the patients' chance to respond to induction, and to worsen OS.

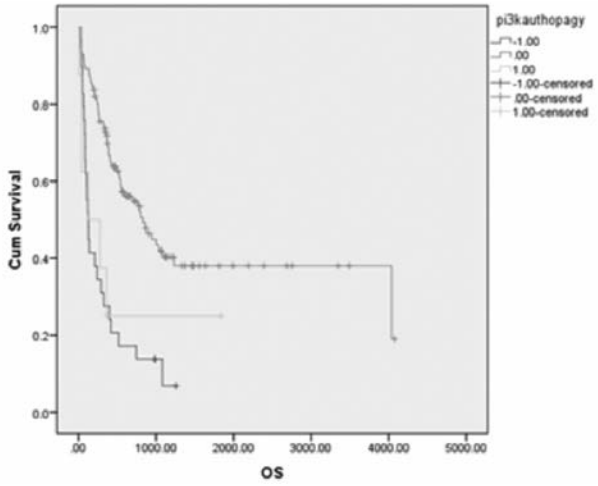

Figure 1.

Methods: We analyzed 148 consecutive newly diagnosed non M3 AML patients treated with induction chemotherapy regimens containing at least one dose of anthracycline. We screened all patients for TP53, FLT3, NPM1 mutations. In all

patients, we perform Microarray-based High-Throughput Technology with Affymetrix SNP array 6.0 or Cytoscan HD. Survival data were collected prospectively from the time of diagnosis, with a median follow-up of 18 months. Survival analysis was performed with Kaplan Meyer method using log rank test. Univariate and multivariable regression and Cox Hazard Ratio (HR) model was performed. Correlation between variables was assessed with Fisher's exact test.
Results: Autophagy alteration (gene group 1: *ULK1 CHR11; ULK1 CHR17; BECN1; ATG14; AMBRA1; UVRAG; ATG9A; ATG9B; PIK3C3; PIK3R4*) were related to lower Complete Remission rate (CR%) after induction in univariate (p<.001) and multivariable regression with age, karyotype, secondary AML, *TP53* mutation (p=.014). Autophagy alteration showed to confer worst OS (p<.001) and was significantly associated with complex karyotype and *TP53* mutation (p<.001). We detected significant differences in term of survival independently both in Copy Number (CN) Gain and CN Loss in group 1 genes (p<.001). Furthermore, we investigated genes in **AMPK pathway** (group 2: *SESN1; PRKAA1 CHR 3; PRKAB1: PRKAA1 CHR 1: PRKAG1 CHR11; PRKAG1 CHR 7; PRKAG3; PRKAB1*) and other genes that could be related to a **switch from a physiological role of autophagy to a resiliency mechanism** (group 3: *CCND1; BCL2; PINK1; PARK2; TP53; MDM1; MDM4*): alterations in those genes were shown to confer worst OS (p<.001 in both groups). Alteration in group 2 and group 3 were related to lower CR% after induction (p<.001 in both groups). Whole Exome Sequencing on 56 patients in our set did not found any significant mutation in genes we analyzed with the exception of TP53.
Summary/Conclusions: Our work investigates for the first time with a genomic approach the role of autophagy in AML. We found that both CN gain and CN loss in autophagy key regulator genes are associated with poor prognosis and therapy resistance. A CN loss in autophagy could enhance proliferation and block apoptosis, a CN gain could give cell resiliency, favoring cytoplasm turnover, damaged mitochondria elimination, and neutralizing oxidative damages. Further fucntional studies will be necessary in order to confirm these results.

Acknowledgements: ELN, AIL, AIRC, PRIN, Progetto Regione-Università 2010-12, FP7 NGS-PTL project, HARMONY.

E883

NO EVIDENCE FOR MICROSATELLITE INSTABILITY (MSI) IN 1,394 PATIENTS (PTS) WITH ACUTE MYELOID LEUKEMIA (AML)

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Background: MSI is the addition or loss of bases within repetitive DNA sequences called microsatellites (MSs), caused by defects in DNA mismatch repair. MSI is most often observed in endometrial and colorectal carcinomas, and pts with MSI-positive (MSI$^+$) solid tumors have shown promising responses to therapy with immune checkpoint inhibitors. MSI's existence in AML has been examined in several studies, but the results are equivocal, with some reporting a total absence of MSI and others finding as many as 20% of *de novo* AML pts are MSI$^+$. These studies tested for MSI using polymerase chain reaction (PCR) of small numbers of MSs (usually 5-7), and typically examined <100 pts. To our knowledge, the largest published AML cohort studied for MSI contained 132 pts.
Aims: To screen 1,394 AML pts for MSI.
Methods: Diagnostic DNA samples from 1,371 pts (1,364 with *de novo* AML and 7 with therapy-related AML [t-AML]) and 86 paired germline DNAs were sequenced for 80 genes using amplicon-based next-generation sequencing (NGS). Variants were detected by MuTect and Varscan with variant allele fraction cut-offs of 0.30, and considered mutations if not reported in 1000 Genomes or dbSNP142. NGS-based MSI detection was performed similar to mSINGS (Salipante *et al*., *Clin Chem*. 2014;60:1192) combined with a MS variant caller we previously developed (Walker *et al*., *Hum Mutat*. 2016;37:1004). Briefly, 18 MSs with >50 reads in all samples and that were not polymorphic in germline DNAs were selected from the target panel; then the number of different length MSs (alleles) each AML sample had was calculated for each of the 18 MS loci. Outlier samples with elevated allele counts were flagged for each locus. Based on previously established mSINGS crtieria, samples flagged in ≥20% of MSs (4 out of 18) were considered MSI$^+$. Paired DNAs were separately screened for MSI by comparing the alleles in each AML sample to its paired germline DNA, using the 2xn version of Fisher's exact test to determine significance. The MS PCR analysis system (Promega) was used on any pairs with significant *P*-values (*P*<0.05*)*. 23 additional t-AML cases were screened using PCR-based detection of 4 MSs (BAT25, BAT26, D2S123 and D17S250).
Results: The NGS-based MSI screen showed that 1,303 samples did not have elevated allele counts for any of the MSs, 67 samples had elevated counts for one MS and one sample had elevated counts at 2 MSs. Because none of the samples met the criterion of ≥4 MSs with elevated allele counts, these data indicate that none of the samples are MSI$^+$. Comparison of alleles between the 86 paired AML and germline samples showed no differences in 78 pairs, and small but statistically significant differences in 8 pairs. However, subsequent assessment of these 8 pairs with the MS PCR analysis system proved that none of them were MSI$^+$. Because MSI$^+$ tumors have 10-100 times as many mutations as MS stable (MSI-negative) tumors, we examined the mutation counts in the 1,371 AML samples for the 80 genes on the target panel. Most samples (64%) had 2-4 mutations and the 2 most highly mutated samples had only 9 mutations each. Since any putative MSI$^+$sample would harbor tens if not hundreds of mutations, these data support the absence of MSI in all samples. Finally, because it has been proposed that t-AML might be more prone to MSI than *de novo* AML,we performed PCR-based MSI detection on an additional 23 t-AML cases, and found all were MS stable.
Summary/Conclusions: The absence of even a single MSI$^+$ case within this large cohort provides strong evidence that MSI is non-existent in AML.

E884

SY-1425, A POTENT AND SELECTIVE RARA AGONIST, REPROGRAMS AML CELLS FOR DIFFERENTIATION ALONG DISTINCT LINEAGES, UNCOVERING PD MARKERS FOR CLINICAL STUDIES

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Background: SY-1425 (tamibarotene) is a potent and selective agonist of the retinoic acid receptor alpha (RARα) transcription factor (TF), currently in a biomarker directed Ph2 clinical study in AML and MDS patients (NCT02807558). A subset of AML and MDS has been found to have RARα pathway activation characterized by a large enhancer at the *RARA* locus (RARA-high) and/or upregulation of IRF8, a TF associated with RARα signaling, forming the basis of SY-1425 sensitive tumor identification.
Aims: We sought to understand how SY-1425 agonism of RARα acts to promote maturation and halt proliferation of AML blasts locked into an immature cell state by the cancer circuitry. This characterization could further inform clinical pharmacodynamics markers.
Methods: We analyzed the epigenomic and transcriptional landscape of 66 non-APL AML patients and normal primary myeloid cells by RNA-seq and ChIP-seq for the enhancer mark H3K27ac. AML cell lines were profiled by RNA-seq, ChIP-seq for H3K27ac and RARα, and ATAC-seq with or without SY-1425 treatment. Cell surface marker changes were assessed by flow cytometry.
Results: A subgroup of the patient samples was defined by an SE driving *RARA*, which co-occurred with SEs driving *FOS* and *JUNB,* or *IRF8*. FOS and JUNB form the AP-1 heterodimeric TF known to promote an immature cell state and the interferon regulatory factor 8 (IRF8) pathway has been implicated in AML pathogenesis. Previously reported crosstalk between INF and retinoic acid signaling was supported by the strong induction of interferon gene sets by SY-1425 in IRF8-high AML models. We found that each AML cell line had distinct compositions of lineage factors consistent with cancer initiation from different stages of myeloid development. SY-1425 induced maturation features associated with monocytic, macrophage, dendritic, and granulocytic cell types. While APL has well characterized and specific granulocytic differentiation, we found that RARA/IRF8-high AML could follow multiple differentiation paths depending on the initial state of the AML model, necessitating different marker panels to capture full cell typing. Functional validation confirmed surface marker changes consistent with the observed epigenomic alterations including CD11b, CD11c, CD66b, and CD38 upregulation. We integrated epigenomic data, DNA accessibility, and SY-1425 response to understand RARα agonist perturbation to cell circuitry. Enhancer elements directly bound by RARα were associated with greater response to SY-1425 as were enhancers bound by other TFs involved in myeloid differentiation. The accessibility of RAR elements and IRF motifs were increased and their associated TFs were upregulated. The target genes of known immature/proliferative state drivers, such as RUNX1 and CEBP, were downregulated. Importantly, the FOS/JUN circuit, identified as a component of the oncogenic RARα circuit in patient samples, was found to be suppressed.
Summary/Conclusions: As in normal myelopoiesis, RARα and associated cell state TFs play a critical role in the differentiation of AML. SY-1425 perturbation of this circuitry leads to differentiation toward multiple potential lineage paths depending on the initial state of the cancer. These pharmacodynamic changes can be assessed clinically and combined with common AML/MDS assays, such as blood and marrow aspirate smears or white blood cell differentials, to support the differentiation mechanism of action and offering the potential for early biologically relevant data to inform current and future clinical studies.

E885

GENETIC CHARACTERIZATION OF A LARGE GROUP OF CEBPA MUTATED AML PATIENTS AND THE EFFECT OF TET2 AND GATA2 MUTATIONS ON OUTCOME

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Background: Mutations in the *CEBPA* gene are detected in about 10% of patients (pts) with cytogenetically normal (CN) acute myeloid leukemia (AML). *CEBPA* mutations can either be biallelic (bi) or monoallelic (mo). Only pts with bi*CEBPA* mutations have favorable outcomes when compared to other CN-AML pts. bi*CEBPA* mutations are rarely associated with other prognostic mutations like internal tandem duplications (ITD) or mutations in the tyrosine kinase domain (TKD) of the *FLT3* gene or mutations in *NPM1*. There is a specific association of bi*CEBPA* mutations with mutations in the transcription factor *GATA2*.
Aims: In this study we aimed to characterize the mutational spectrum of CN-AML pts with mo- and bi*CEBPA* mutations. We further analyzed the effect of *TET2* and *GATA2* mutations on outcome in pts with bi*CEBPA* mutations.
Methods: Targeted amplicon resequencing (Agilent Haloplex, target region: 63kbp) was used to analyze 42 target genes or hotspots known to be mutated in AML or other hematologic neoplasms.
Results: In 48 bi*CEBPA* and 32 mo*CEBPA* we found mutations in 20 and 26 different genes respectively. Mo*CEBPA* pts had significantly more additional mutations compared to bi*CEBPA* pts (mean: 3.9±1.7 *vs* 2.2±1.5; p<.001). We also compared the mutational profile of mo*CEBPA* and bi*CEBPA* pts with a cohort of 287 wildtype (wt) *CEBPA* pts. Eight genes with a mutation frequency of ≥5% were significantly associated with one or more groups. We confirmed the mutual exclusiveness of bi*CEBPA* and *NPM1* and the association between *GATA2* and bi*CEBPA* (35.4%). *TET2* was frequently mutated in both mo- (43.8%) and bi*CEBPA* pts (41.7%), but not in wt*CEBPA* (16.3%; mo- *vs* wt*CEBPA* p<.001; bi- *vs* wt*CEBPA* p<.001). Mutations in TKD1 or TKD2 of *FLT3* were frequently identified in mo*CEBPA* (25%) and wt*CEBPA* pts (17.8%) but not in bi*CEBPA* pts (2.1%). The *FLT3*-TKD1/2 mutation frequency in bi*CEBPA* significantly differs from mo*CEBPA* (p=0.002) and wt*CEBPA* (p=0.004). There was a significant difference in the frequency of *FLT3*-ITD in bi*CEBPA* (20.8%) *vs* wt*CEBPA* pts (40.8%; p=.009) but not in comparison to mo*CEBPA* pts (43.8%). *IDH2* was found mutated only in wt*CEBPA* (21.6%) and mo*CEBPA* (18.8%). In 48.8% of wt*CEBPA* pts *DNMT3A* was mutated, this significantly differs from bi*CEBPA* pts (14.3%; p<.001) but not from mo*CEBPA* patients (28.1%). *CSF3R* was frequently mutated only in bi*CEBPA* (10.4%) but not in wt*CEBPA* (0.35%; p<.001) or mo*CEBPA* (3.1%; ns). *STAG2* was associated with mo*CEBPA* (25%), while *STAG2* mutations were significantly less frequent in bi*CEBPA* (6.3%; p<.001) and wt*CEBPA* pts (6.27%; p=.002). *TET2* mutations had a negative prognostic impact on overall survival (OS) in biCEBPA pts, but not in mo*CEBPA* pts. OS (HR: 3.1; 95% CI: 1.2-8.2; p=.023) was significantly worse in bi*CEBPA* pts with a *TET2* mutation, but relapse free survival (RFS) and cumulative incidence of relapse (CIR) was not different depending on *TET2* mutational status. In bi*CEBPA* pts we also evaluated the clinical impact of *GATA2* mutations. For 30 of 48 bi*CEBPA* pts survival data was available, 15 of these pts had a *GATA2* mutation. We found no significant difference with respect to RFS (p=0.216), OS (p=0.479) and CIR (p=0.059). In a combined analysis, the *GATA2*mut and *TET2*wt genotype was associated with a lower relapse risk and a trend towards a higher RFS compared to the *GATA2*wt and *TET2*mut genotype.
Summary/Conclusions: bi*CEBPA*, mo*CEBPA* or wt*CEBPA* pts show a distinct profile of co-occurring mutations that might explain the biological differences between these groups. *TET2* mutations were found in 40% of all *CEBPA* mutated pts and might have a prognostic impact in bi*CEBPA* pts.

E886

MECHANISMS OF SYK-MEDIATED SUPPRESSION OF DIFFERENTIATION AND APOPTOSIS IN ACUTE MYELOID LEUKEMIA (AML)

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Background: Spleen tyrosine kinase (SYK) is a critical mediator of integrin b3 signaling in AML, leading to leukemia cell growth and disease initiation upon transplantation into healthy recipients. Genetic or pharmacologic inhibition of SYK leads to increased differentiation, reduced proliferation and apoptosis. However, the comprehensive description of mediators and effectors of SYK signaling in AML is lacking.
Aims: To identify key downstream mediators of SYK signaling in AML responsible for differentiation block, proliferation and leukemic stem cell (LSC) maintenance.
Methods: AML cell lines (KG1, MOLM14) or bone marrow primary AML blasts, were incubated 24h with R406 (1uM, 4 uM) or vehicle. Activity of SYK, ERK, STAT5 was assessed by western blot and/or intracellular phospho-flow. Proliferation, apoptosis and clonogenic potential were assessed by MTS, PI staining and methylcellulose colony-forming assay. Differentiation was assessed by Giemsa-Wright staining, quantitative NBT, CD14/CD15 staining and qPCR. Constitutively active form of MEKDD and STAT5A1*6 were retrovirally transduced to KG1. Activity toward LSC was assessed using leukemia cell line with stem cell properties, TEX. ROS level, mitochondrial mass were assessed by DCF, MitoTracker Green FM staining. Expression of MYC, TFAM, NRF1, NRF2, EF-Tu were assessed by western blot and/or q-PCR.
Results: To identify downstream mediators of SYK in AML, we assessed the activity of key signal molecules in KG1 and MOLM14. AML cells exhibited basal level of pSYK, pERK and pSTAT5. R406 decreased phosphorylation of these proteins, reduced proliferation, clonogenic potential, induced myeloid differentiation and apoptosis. Since ERK can block maturation, we assessed impact of this pathway on differentiation arrest downstream of SYK in KG1 transduced with MEKDD. R406 induced morphological evidence of differentiation and increased expression of myeloid differentiation genes in control cells, whereas in MEKDD transduced cells, R406 had no effect. Given the role of STAT5 in self-renewal of HSC we next assessed impact of this factor on clonogenic potential. R406 reduced clonogenic potential of control cells, while in cells expressing STAT5A1*6, clonogenic potential was maintained. Moreover, STAT5A1*6 cells had significantly higher cell surface expression of an LSC marker, CD25, and R406 reduced CD25 level only in control cells. Since activity of SYK is required for LSC survival, and R406 induced differentiation and reduced CD25 expression, we decided to explore the mechanism of LSC depletion following R406. R406 reduced proliferation, clonogenic potential and induced dose-dependent apoptosis in LSC-enriched TEX cells. Since high pSYK is associated with overexpression of MYC and increased expression of MYC and its targets that drive mitochondrial biogenesis is a characteristic feature of LSC, we hypothesized that R406 deplete LSC by reducing mitochondrial biogenesis/oxidative phosphorylation (OXPHOS). In TEX and primary CD34+ AML blasts R406 reduced expression of MYC, transcription factors associated with mitochondrial biogenesis (NRF1, TFAM, EF-Tu, NRF2), and lowered cellular mitochondrial mass.
Summary/Conclusions: Taken together, we found that SYK inhibition obviates differentiation arrest imposed by ERK activity, and reduces clonogenic potential via decreased STAT5 activity. Moreover, we show that pSYK is required to sustain MYC expression and mitochondrial biogenesis/OXPHOS, a key features of LSC in AML.

This work was supported by NCN grant#2013/11/N/NZ5/03704.

E887

MUTATIONAL PROFILE OF RELAPSE-RISK GROUPS IN ACUTE PROMYELOCYTIC LEUKEMIA PATIENTS

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Background: Although the fusion oncogene PML-RARA is known to initiate acute promyelocytic leukemia (APL), other cooperating mutations have also been implicated in the disease pathogenesis. However, the spectrum of mutations of APL patients within the relapse-risk groups, based on patient leukocyte and platelet counts at diagnosis, are not yet known
Aims: 1)To identify genetic alterations that might cooperate with PML-RARA in the leukemogenic process within the three APL relapse-risk groups. 2)To find mutations at diagnosis responsible for poor outcome by comparing patients who experiment relapse *vs.* patients who do not relapse in each group.
Methods: We performed multi-amplicon targeted deep sequencing on bone marrow samples of 91 patients diagnosed with APL (PETHEMA LPA99/2005/2012) with a median follow-up of 2.8 years (range 0.2-10) (Table 1). APL patients were classified into relapse-risk groups according to initial leukocyte (WBC) and platelet counts (Score Sanz *et al*. 2000). Libraries were prepared by using the TruSight Myeloid Sequencing Panel (Illumina) that assesses 54 myeloid related genes. Paired-end sequencing runs were performed on a MiSeq (Illumina) genome sequencer. Minimum depth for reliable analysis was fixed in 100x and minimum variant allele frequency (VAF) in 5%. FLT3-ITD mutations were analyzed by fluorescent PCR and capillary electrophoresis. Sequences obtained were analyzed with the Variant Studio v2.1 software (Illumina) and the Integrative Genome Viewer (Genome Browser).
Results: Distribution of 91 patients in the 3 relapse-risk groups was: 28 in low-risk group (31%), 48 in intermediate-risk (53%) and 15 in high-risk (16%). We

found 150 mutations in 31 genes, in 73 out of the 91 patients included (a median of 1 mutation per patient (range: 0-5) with a mean read depth of 10360x. Eighteen patients remained wild-type for all analyzed genes (Figure 1).Only one of this patients suffered relapse (5%). In the global series, no single mutation or functional category showed an association with clinical variables or prognostic impact in terms of overall survival or relapse free survival (RFS). There were no differences in the mean number of mutations per patient in each risk APL group (p>0.05). Patients who lack mutations belonged to the intermediate (13/48, 27%) and low risk (4/28,14%) groups, except for only one patient (1/15, 6%) in high-risk group. FLT3 was the most frequently affected gene in high risk APL subgroup (10 out of 15): 8 patients carried an FLT3-ITD mutation and 2 patients had *amino acid* substitutions at codon *835.* Seven patients assigned to intermediate-risk relapsed (7/38, 18%). All but one carried mutations that have been reported as unfavorable in AML (FLT3, PTEN, ASXL1, CUX1 and WT1). By contrast, patients who remain in complete remission in this group, lack mutations with a greater frequency (12/31, 39%). Finally, within the low-risk group 3 patients suffered relapse (3/27; 11.5%) and all of them presented missense mutations in the Ras domain of *NRAS* at diagnosis (p.Ser65Arg & p.Gln61Arg). Therefore, we could identify a small subgroup of patients at a very high risk of relapse (RFS at 5 years, 25% *vs* 100%, p<0.001).

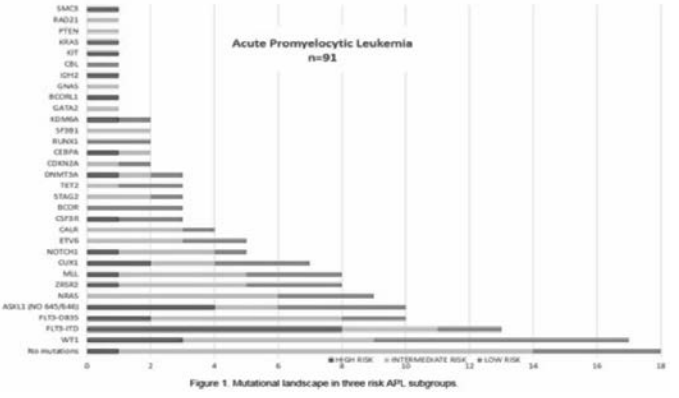

Figure 1. Mutational landscape in three risk APL subgroups.

Figure 1.

Table 1.

	Global serie N=91	Low risk n=28	Intermediate risk n=48	High risk n=15
Age (years)	49,6±19,4	52,7±23,4	50,7±16,8	47,4±21,5
Male Sex, N (%)	48/91(53)	17/28(61)	24/48(50)	7/15(47)
BM blasts (%)	75±18	70±19	75±17	83±15
WBC count (x10^3/L)	8,7±18,3	2±1,5	2,6±2,6	37,4±24,8
Platelets (x10^3/L)	38,9±32,4	73,3±33	19,6±9,4	27,8±19,6
Hemoglobin (g/dL)	9,9±2,3	10,3±2,4	9,4±2,2	10,8±2,4
FLT3 mutations, N (%) ITD D835	14(15) 10	2(7) 2(7)	4(9) 6(14)	8(53) 2(2)
CR rate, N (%)	78/86(90)	27/28(96)	38/43(88)	13/15(86)
Relapse rate, N (%)	12/78(15)	3/27(11)	7/38(18)	2/13(15)
PML-RARa isoform bcr1 bcr2 bcr3	60 6 25	20 2 6	31 4 13	9 0 6

Table 1. Clinical and biological characteristics of 91 APL patients.

Summary/Conclusions: In summary, the present study shows that the mutational status of NRAS and FLT3 genes could be used as genetic markers for prognosis in APL, especially in the intermediate and low-risk groups, allowing a more accurate patient risk classification. Our data suggests the need to search for new mutations required for progression in APL, in order to benefit from a change in post-remission therapy.

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ANALYSIS OF THE PD-1/PD-L1 AXIS POINTS TO ASSOCIATION OF UNFAVORABLE RECURRENT MUTATIONS WITH PD-L1 EXPRESSION IN AML

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Background: Programmed death ligand-1 (PD-L1) is regulated through miR-34a molecules in AML patients. Moreover, Cortez *et al.* for the first time identified novel, complete mechanism of PD-L1 regulation by p53 via miR-34a in non-small cell lung cancer (NSCLC).
Aims: In this study, our comprehensive analyses of *PDCD1* (PD-1), *CD274* (PD-L1), *TP53* and *miR-34a* expression in AML patients shed new light on the complex regulation of PD-1/PD-L1 axis during development of this disease.
Methods: We performed analysis of *TP53*, *CD274* and *miR-34a* expression in 197 AML patients available from The Cancer Genome Atlas (TCGA) database. Moreover, we assessed mRNA expression of *PDCD1* in independent cohort of 54 AML, 62 MDS and 8 s-AML patients samples using qRT-PCR method. For miRNA analysis, CD33+ cells from 29 AML patients were isolated and *miR-34a* expression was analysed. We also characterized several SNP for *PDCD1* that demonstrate relevant associations with a higher risk of developing autoimmune diseases: PD-1.1 (rs36084323), PD-1.3 (rs11568821), PD-1.5 (rs2227981), PD-1.6 (rs10204525), PD -1.7 (rs41386349), PD-1.9 (rs2227982) in 54 AML, 62 MDS and 100 HVs samples.
Results: We observed significant differences in *PDCD1* expression in groups of 54 AML, 62 MDS, 8 s-AML patients compared to HVs. TCGA data analysis showed that *CD274* expression was elevated in group with *TP53* mutations compared to unmutated *TP53* group (p<0.001). We also found negative correlation of *TP53* and *miR-34a* expression with *CD274* expression (p=0.02 and p=0.005, respectively). The expression of *miR-34a* tended to be elevated in group with high expression of *TP53* compared to group with low *TP53* expression (p=0.17). We have not found any differences in *CD274* expression between groups with or without following mutations: *IDH1*, *TET2*, *RUNX1*, *NRAS*, *CEBPA*, *PTPN11*, *KIT*, *KRAS*, *FLT3*, *DNMT3*, *NPM1* and *IDH2.* Patients with more than 4 recurrent mutations were characterized with higher expression of *CD274* compared to group of patients with 0-3 recurrent mutations. We also found that patients with >14 of all mutations had elevated expression of *CD274* compared to group 0-13 mutations (p=0.06). We observed significant differences in *PDCD1* expression level regarding to PD-1.1.5 polymorphism. Moreover, analysis of a PD-1.1.3 polymorphism in HVs and MDS groups revealed that genotype GG was associated with nearly fivefold lower risk of disease (OR=4.93, p=0.009). We observed significant differences in OS in AML patients in case of presence of certain genotypes of PD-1.1.6. Genotype AA was significant associated with higher risk of shorter OS compared to the rest of the genotypes (58 *vs* 333 days, HR=35; p=0.0188).
Summary/Conclusions: Our analyses indicate that p53 might specifically modulate the tumor immune response by regulating PD-L1 via miR-34a which directly binds to the PD-L1 3' UTR and blocks its expression. Moreover, we found that high *CD274* expression is associated with the higher numbers of recurrent and all mutations as well as poor cytogenetic and molecular risk groups of AML patients. We found significant differences in *PDCD1* expression in AML patients compared to HVs that might indicate deregulation of a signal transduction through the PD-1/PD-1L axis. While our SNP analysis in AML patients suggested a prognostic impact of PD-1. 6 polymorphism, further studies are warranted to evaluate the impact of the PD-1/PD-L1 Axis in AML.

This work was supported by National Centre for Science Grant HARMONIA (UMO-2013/10/M/NZ5/00313).

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DISSECTING THE DYNAMICS OF SINGLE-TUMOR-CELL-LINEAGES THAT UNDERPIN RELAPSE OF AML

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Background: Cancers kill primarily via disease recurrences after transient treatment responses. The emergence of therapy-resistant tumor escape variants is fueled by intra-tumor heterogeneity, underpinned by interference and Darwinian evolution across continuously developing sub-clones in the residual tumor. Several non-genetic factors add significant variation, on top of the diversity provided by the often complex intra-tumor genetic landscape, resulting in an extremely heterogenous and dynamic tumor cell population that can drive disease under many conditions. The relapse of human acute myeloid leukemia (AML) is a prime clinical example of how evolving sub-clonal dynamics can frequently drive treatment-resistant cancer recurrence after initially potent therapeutic responses.
Aims: We aimed to understand how sub-lineage interference is regulated in AML in response to standard and emerging treatments - and clarify how this impacts the development of therapy resistance. Specifically, we aimed to dissect if relapse from each drug regimen was driven by predetermined or stochastically selected sub-lineages and determine the functional impact of such differences.
Methods: We dissected the intra-tumor population dynamics of relapsing AML, beyond the genetic level, by performing single-cell lineage-tracing through cellular barcoding technology (lentivirus-integrated non-coding DNA-tags). We

specifically evaluated the impact of *in vitro* exposure of a barcoded AML cell line (HEL) to chemotherapy regimens (doxorubicin (DOX) and/or cytarabine (CYT)) and/or the hypomethylating agent decitabine (DCT) by comparing the barcode composition of the tumor population recurring after each therapy, *versus* non-treated (NT) controls. By comparing the barcode architectures between parallel replicate cultures for each therapy, we could further delineate whether AML relapse was driven by predetermined (if recurrent barcodes found in multiple replicates) or stochastically selected (if mainly diverse barcodes in each replicate) cells in response to each treatment regimen.
Results: Only treatment regimens containing DOX caused marked decreases in HEL cell numbers and barcode architectures diverging strongly from the non-treated control cultures. Replicate AML cultures regrowing after treatment with DOX all converged to a very similar barcode architecture, reflecting that relapse following this mono-therapy was driven by predetermined single-cell lineages. Combination of DOX with CYT increased the degree of overall cell elimination by ~10-fold, while addition of DCT to either chemotherapy regimen had minimal quantitative impact (*i.e.* yielded similar cell number reductions and re-growth kinetics). Interestingly, DCT additions nevertheless qualitatively changed which sub-lineages that regrew - specifically making replicates more divergent from each other, indicating a more stochastic selection of the cells emerging when DCT had been added to the respective chemotherapy regimens. Importantly, this effect was accompanied by the reversion of resistance to chemotherapy re-treatment, which the DOX-containing treatment regimens potently induced in the absence of DCT.
Summary/Conclusions: The development of curative treatment combinations requires deep understanding of how non-genetic factors synergize with cancer genetics to drive intra-tumor heterogeneity, which is key for tumor escape/ disease recurrence. Our detailed analyses of the heterogenous dynamics among single-cell lineages in AML, following different treatment regimens with apparently similar global impact, represent an important step in dissecting kinship-dependent aspects that go beyond the genetic level. Critically, these studies directly provide the rationale for combining standard chemotherapy with administration of hypomethylating drugs to target AML. The mechanism is prevention of the development of chemotherapy resistance (mediated by selective relapse of a specific set of predetermined sub-lineages) - by partially randomizing which sub-lineages that emerge to drive relapse when DCT is added to the chemotherapies. Maintaining the chemosensitivity of relapsing AML would represent a paradigm shift, turning the currently often lethal recurrences into survivable/ repeatedly clinically manageable episodes of a type of chronic leukemic disease.

E890

Abstract withdrawn.

E891

MRD ANALYSIS BY NEXT-GENERATION SEQUENCING APPROACH FOR ACUTE MYELOID LEUKEMIA FOLLOW-UP

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Background: Sensitive detection of molecular marker of minimal residual disease (MRD) in acute myeloid leukemia (AML), could improve prognostication of a possible relapse during the remission. Traditional methods for measuring minimal residual disease (MRD) in AML, such as real time PCR and multiparametric flow cytometry (MFC) are associated with high technical complexity, low applicability and laborious standardization. However, some patients who achieve a negative MRD become to relapse and several MRD- patients have a long survival, which indicates that the sensitivity and specificity of traditional techniques for MRD assessment can be improved.
Aims: To detect minimal residual disease in AML follow-up sample using high-throughput sequencing as a standard and accurate technique.
Methods: We studied 54 *gDNA* bone marrow follow-up samples (27 after induction, 10 after first consolidation, 17 after second consolidation) from 30 AML patients treated according *PETHEMA AML* clinical protocols and with DNA sample at diagnosis. All patients had achieve CR at the moment of MRD assessment. We developed a custom-targeted sequencing panel of 32 genes *(Ion Torrent Proton System-Thermo Fisher*) for mutation (SNV and/or InDels) detection at diagnosis sample; From the 32 genes, we use specific primers to amplify the specific region of the four most frequent alterations at diagnosis (Samples at follow-up: *FLT3no-ITD n=2, NPM1n=46, IDH2 n=9* or *IDH1 n=7)*. We analysed and detected at diagnosis and at follow-up (after induction, first consolidation or second consolidation), and sequenced with high-throughput approach. We achieve a technical sensibility around $10^{-4}$for point mutations and $10^{-5}$for Indels mutations according our specificity and sensitivity calibration curves.
Results: We analyse the results of assessing MRD by NGS, and the presence or absence of MRD was established at a cut-off level of 0.0017 (between $10^{-4}$and 10^{-5}technical sensibility) by ROC curve with a sensibility of 0.5 for DFS and 0.571 for OS, and a specificity of 0.92 for DFS and 0.897 for OS; thereby a result above this level was considered as MRD positive. DFS (Disease Free Survival) and OS (Overall Survival) rates in this group were 29.9% and 24.1%, respectively; positive MRD sample was independent marker associated with shorter DFS (p=0.002, HR=0.33, 95% CI:1.60-33.51) and OS (p=0.002, HR= 8.33, 95% CI:1.87-37.15) (see figure 1). These results support the usefulness of MRD evaluation in patients with AML by NGS in the context of molecular biology studies.

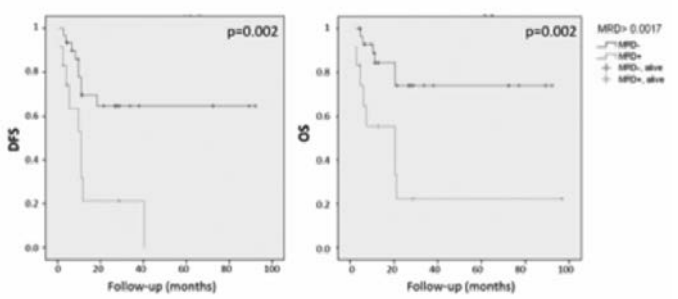

Figure 1. Kaplan-Meier curves for DFS (p=0.002; HR=7.33 95%, CI:1.60-33.51) and OS (p=0..002, HR=8.33 95%, CI:1.87-37.15) of patients with MRD positive (MDR> 0.0017)

Figure 1.

Summary/Conclusions: High-throughput NGS is a technique with the capacity to measure, identify and classified MRD levels. In fact, NGS MRD evaluation has a better DFS and OS prediction than other traditional methods. Implementation of NGS technique on MRD detection could help to anticipate to disease progression.

This study was funded by Instituto Carlos III (PI13/02387).

E892

THE ROLE OF MYELOID-DERIVED SUPPRESSOR CELLS-LIKE BLASTS WHICH SUPPRESS T CELL PROLIFERATION IN LEUKEMIC CELL GROWTH

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Background: Myeloid-derived suppressor cells have an ability to suppress T-cell function and have been known to facilitate tumor growth. We elucidated the immune suppressive function of leukemic blasts which resembled MDSC phenotype and their role in the growth of leukemic cells.
Aims: We elucidated the immune suppressive function of leukemic blasts which resembled MDSC phenotype and their role in the growth of leukemic cells.
Methods: $CD11b^+CD33^+HLA\text{-}DR^-$blast (MDSC like blast) were isolated using flow-cytometry from bone marrow mononuclear cells of primary acute myeloid leukemia (AML) patient samples. CD14, CD15, Arg1 and iNOS expression were checked by flow-cytometry to identify the phenotype of MDSC like blast. To evaluate the ability of MDSC like blasts to suppress T cell proliferation, $CD8^+$ T cells from healthy donor and MDSC like blasts were co-cultured with a ratio of 1:1 with/without phytohemagglutinin A 10ug/ml. T-cell proliferation was measured by carboxyfluorescein diacetate succinimidyl ester dilution assay after 3 days of culture. Then, various leukemic cell lines were co-cultured with jurkat T cells and/or MDSC like blasts at a leukemic cell line:jurkat T cell: MDSC like blast ratio of 4:4:1. The effect of jurkat T cells and MDSC like blasts on the proliferation of leukemic cells was assessed by the CCK-8 assay after 1 and 3 days of culture.
Results: MDSCs like blast can be divided into two subtypes, monocytic subgroup expressing CD14 and granulocytic subgroup expressing CD15, and CD14 expression was more frequent than CD15 (67.5% *vs* 39.3%). MDSC-like blasts showed higher expression of Arg1 (77.1% *vs* 38.5%, P<0.001) and iNOS (33.0% *vs* 1.1%, *P*<0.0001) compared to non-MDSC-like blasts. $CD8^+$T cell proliferation induced by PHA was significantly suppressed when co-cultured with MDSC-like blasts compared to without them. Among the various leukemic cell lines, the proliferation of NB4 cells were significantly suppressed when co-cultured with jurkat T cells on day 3 (NB4 23.49±6.26% of control, NB4+jurkat 12.62±3.92%, P<0.01). The decreased proliferation of NB4 cells was partially recovered after 3 days of co-culture with MDSC-like blasts (NB4+jurkat 12.62±3.92%, NB4+jurkat+MDSC like blast 18.71±6.19, P=0.022).

Summary/Conclusions: CD11b^{+}CD33^{+}HLA^{-}DR^{-} MDSC-like blasts subpopulation which expressed the iNOS and Arg1 existed in AML, and showed ability to suppress the T cell proliferation. MDSC-like blasts partially restored the suppressed leukemic cell growth of NB4 cells by jurkat cells. MDSC-like blasts might play a certain role in immune-escape mechanism of AML.

E893

GENERATION OF NEW CELLULAR MODELS FOR THE STUDY OF PEDIATRIC NON DOWN SYNDROME ACUTE MEGAKARYOBLASTIC LEUKEMIA BASED ON HUMAN PLURIPOTENT STEM CELLS

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Background: Acute megakaryoblastic leukaemia (AMKL) is a rare and complex type of Acute Myeloid Leukaemia (AML), more frequent in children than in adults, characterized by the accumulation of immature megakaryoblasts and thrombocytopenia. Paediatric AMKLs are classified in Down Syndrome AMKL (DS-AMKL) with a good prognosis; and AMKL non-related to Down Syndrome (non-DS-AMKL), a more aggressive disease with a mortality rate close to 80%. There is a limited amount of research done on infant non-DS AMKL due to its low frequency and the heterogeneity of the gene mutations linked to it. Among the genetic alterations found in non-DS-AMKL, approximately half of the patients carry the chromosomal translocations t(1;22) and t(11;12), that generate the fusion proteins RBM15-MKL1 and NUP98-JARID1 respectively, and the inversion of chromosome 16, that originates the fusion protein CBFA2T3-GLIS2.
Aims: It is essential to establish new human models to provide enough biological material for functional and molecular studies. As the genetic alterations that drive infant leukaemia occur in the developing fetus, we propose that human pluripotent stem cells (hPSCs) are ideal models to study non-DS AMKL, as these cells allow us to mimic human embryonic hematopoietic development. In this project, we aim to use human hPSCs expressing non-DS AMKL-associated fusion oncogenes as cellular models for this leukaemia, to study the molecular and cellular pathways involved in the development of pediatric non-DS-AMKL.
Methods: Generation of human models of non-DS AMKL using hPSCs: 1. Generation of hPSCs with the oncogenic fusion proteins RBM15-MKL1, CBFA2T3-GLIS2 and NUP98-JARID1 using transduction with lentiviral vectors. 2. Generation of hPSCs with the chromosomal translocations t(1;22)(p13;q13) RBM15-MKL1 and t(11;12)(p15;p13) NUP98-JARID1 using the CRISPR/Cas9 system. Once the non-DS AMKL hPSC cell lines are generated, we confirm that they preserve their pluripotency by checking expression of pluripotency markers by flow cytometry and PCR. We also confirm their ability to differentiate into the three germ layers forming embryoid bodies. Using an *in vitro* differentiation system that recapitulates early human haematopoiesis, we compare non-DS-AMKL hPSCs and control hPSCs in order to determine at which point of the differentiation fails and the molecular mechanisms underlying. We analyse by multicolour flow cytometry the generation and evolution over time of different cell populations: mesodermal cells, hemato-endotelial progenitors, hematopoietic progenitors and megakaryocytic precursors. We use colony-forming assays (CFU) to determine the generation and functionality of hematopoietic progenitors.
Results: Here we report the generation and characterization of human non-DS AMKL models based on pluripotent stem cells that express oncogenic fusion proteins RBM15-MKL1, NUP98-JARID1 and CBFA2T3-GLIS2.
Summary/Conclusions: These models will serve as platforms to discover and understand the cellular and molecular alterations caused by these oncogenes, and their impact in the generation of hematopoietic cells during development. With this information we will have a better understanding of the origin and development of paediatric non-DS AMKL, so we will be able to design new therapeutic approaches for these children.

E894

CHARACTERIZATION OF HEMATOLOGIC MALIGNANCIES WITH ANCHORED MULTIPLEX PCR AND NEXT-GENERATION SEQUENCING

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Background: Hematologic malignancies can be driven by a diversity of mutation types, including single nucleotide variants, copy number variants, gene fusions, insertions and deletions and changes in gene expression profiles. However, comprehensive detection of these mutation types from a single clinical sample is challenging, as specific assays are required to detect each mutation type. Next-generation sequencing (NGS) enables comprehensive detection of all mutation types from whole genomes and transcriptomes. However, low detection sensitivity, high input requirements and high costs render these approaches impractical for routine detection of mutations from clinical sample types. Anchored Multiplex PCR (AMP™) is a target enrichment strategy for NGS that uses molecular barcoded (MBC) adapters and unidirectional gene-specific primers (GSPs) for amplification.
Aims: Our goal was to develop AMP-based NGS assays to simultaneously detect multiple mutation types from DNA and RNA, as well as relative gene expression levels and copy number alterations (CNA) relevant in hematologic malignancies. In particular, we sought to develop methods to detect novel gene fusions, internal tandem duplications (ITDs) and mutations in CEBPα.
Methods: We developed AMP-based Archer® VariantPlex™ and FusionPlex® assays to enable NGS-based detection of mutations from DNA and RNA, respectively. Open-ended amplification permits identification of novel gene fusions with FusionPlex and complex mutation types such as ITDs with VariantPlex assays. MBC adapters ligated to RNA and DNA fragments prior to amplification enable relative gene expression and CNA analysis.
Results: We show instances of gene fusion detection from open-ended amplification in RNA, including a novel fusion, RUNX1-G6PD, in a case of acute unclassifiable leukemia. Furthermore, unidirectional GSPs provided bidirectional coverage of a BCR-ABL1 fusion, which was detected with reads originating from ABL1 as well as BCR. Using our optimized bioinformatics algorithm and the VariantPlex assay, we accurately and reliably detected ITDs of varying sizes and insertion points, with simultaneous point mutation detection, in AML-positive blood samples. Furthermore, we show multiple mutations in various AML-positive sample types, including mutations in CEBPα. Finally, MBCs used in AMP enabled NGS-based expression profiling for identification of Diffuse Large B-Cell Lymphoma subtypes in a small cohort of samples.
Summary/Conclusions: Our results demonstrate that AMP-based NGS enables comprehensive detection of multiple mutation types as well as gene expression levels relevant in hematologic malignancies. Importantly, AMP enables identification of known and novel gene fusions at nucleotide resolution, detection of ITDs and characterization of relative gene expression levels and CNAs.

E895

ASXL1 MUTATIONS IN AML ARE ASSOCIATED WITH SPECIFIC CLINICAL AND CYTOGENETIC CHARACTERISTICS

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Background: Mutations of *ASXL1* are considered early founder events in AML leukemogenesis. They are included in the definition of the "chromatin-splicesome" genomic class of AML and among the high risk genetic prognosticators in the 2017 ELN recommendations.
Aims: We aimed to study the frequency of ASXL1 mutations in a cohort of newly diagnosed AML patients and to look for correlations with conventional cytogenetic findings and baseline characteristics.
Methods: Three hundred and sixty AML patients diagnosed between 2005 and 2014 were studied. Conventional cytogenetic analysis was performed on unstimulated bone marrow cells cultured for 24 and 48 hours. Molecular analysis of *ASXL1* exon 12 mutations was performed by PCR and subsequent direct Sanger sequencing in diagnostic bone marrow or peripheral blood samples.
Results: Median age of the whole cohort was 63 years (11-95) and 56% of patients were male. Eighty two patients (22.8%) had secondary AML (sec-AML) with prior diseases being MDS (63), CMML (4), PV/ET (9), MF (2) and CML (4). Karyotypic analysis was successful in 352 (97.7%) AML samples of which 252 (71.6%) exhibited clonal karyotypic abnormalities. *ASXL1* mutations were detected in 52 patients (14.4%). The most common mutation was c.1934 dupG in 44/52 (84.6%). ASXL1 mutated patients were significantly older with median age 72 *vs* 61.5 years in the unmutated (p=0.0001). Three of 61 patients (4.9%) aged ≤40, 10/97 aged 41-60 (10.3%) and 39/198 aged >60 (19.7%) were mutated (p=0.005). ASXL1 mutation frequency was similar in male (15.4%) and female patients (13.2%). *ASXL1* mutations were significantly more frequent in sec-AML patients (32.9%) than in *de novo* AML (9%, p<10^{-3}). ASXL1^{mut} cases tended to have higher peripheral white cell count at diagnosis (median 29 *vs* 11.5 x10^{9}/L). Frequency of *ASXL1* was similar in patients with normal (13%) and abnormal karyotypes (15.5%) (p=0.55). No association with complex (16.7 *vs* 14.3, p=0.63) or monosomal karyotypes (16.4 *vs* 14.5%, p=0.72) was found. *ASXL1* mutations were less common in patients with WHO defined MDS-related cytogenetic abnormalities (10.5% *vs* 16.6%, p=0.14). Moreover, we observed an inverse relationship of *ASXL1* mutations with -7/del(7q) or -5/del(5q) as a group (8.9% *vs* 16.8%, p=0.07). ASXL1 mutations were significantly more frequent among cases with trisomy 8 (25% *vs* 12.8%, p=0.02) and patients with chromosome 11 aberrations (23.7% *vs* 13.7%, p=0.1).None of the 12 patients with inv(16)/t(16;16) was mutated while 2/16 (12.5%) with t(8;21) had ASXL1

mutations. Moreover, ASXL1 mutations were detected in 3 of 12 patients with aberrations of 3q (25%), 2/9 (22%) with trisomy 13, 2/11 (18%) with t(9;22) and only 1 of 22 patients with t(15;17). Multivariate logistic regression showed that independent predictors of the presence of ASXL1 mutations were older age (OR 1.43 per decade, 95% CI 1.13-1.79), chromosome 11 aberrations (OR 2.69, 95% CI 1.09-6.63), and sec-AML (OR 4.44, 95% CI 2.3-8.57), whereas -7/del(7q) or -5/del5q predicted for lower frequency (OR 0.32, 95% CI 0.13-0.75).
Summary/Conclusions: Our results support the association of ASXL1 mutations in AML with advancing age and sec-AML. Association with trisomy 8 did not retain significance in multivariate analysis. Chromosome 11 aberrations emerged as an independent predictor. Despite the strong link with secondary AML (majority of cases post MDS), our data show inverse relationship with -7/del(7q) or -5/del5q. In addition, ASXL1 mutations were not positively associated with MDS-related cytogenetic abnormalities, complex or monosomal karyotypes.

E896

Abstract withdrawn.

E897

A COMPREHENSIVE DNA TEST FOR THE DETECTION OF TRANSLOCATIONS IN ACUTE LEUKEMIA

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Background: Patients with acute leukemias carry a wide range of chromosomal abnormalities, which affect their prognosis and treatment options. Currently, over 500 different translocations are reported to be involved in the disease progression. Traditional methods to detect chromosomal abnormalities involve a combination of techniques such as karyotyping, FISH, array and RT-PCR. However, these methods are laborious and at times inadequate. Targeted Locus Amplification (TLA), a new targeted next generation sequencing technology can overcome these shortcomings. It is based on proximity ligation (crosslinking) of DNA and outward oriented probes for enrichment and can therefore identify chromosomal translocation partners regardless of their identities.
Aims: Here we present a TLA multiplex panel in combination with next generation sequencing as a first tier screening tool in detecting translocations in acute leukemias.
Methods: A multiplex TLA panel was designed using primer sets covering known break-point regions of the 17 most frequently reported genes involved in acute leukemia's. TLA was performed on five different cell lines carrying translocations detectable by our panel. [t(12;21), t(6;11), t(11;19)t(8;13), t(6;9), t(17;19)]. Various combinations of cell line mixtures in multiple dilution series were used to determine the specificity and sensitivity of the panel, and to set sample quality thresholds during analysis. Samples were processed using standard TLA protocol (de Vree et al, 2014). Targets were enriched by PCR amplification with the multiplex panel and subjected for sequencing on Illumina Nextseq 500. To facilitate an easy analysis workflow a semi-automated data analysis was developed. This includes a quality control step, labelling samples with no coverages at the anchor regions after filtering at more than half the number of target regions as failed. These were not interpreted. Only peaks outside other anchor regions were considered as false positive peaks. Peaks present in other anchor regions were interpreted as possible artefacts and labelled as needing extra confirmation. In these series until now, up to 10% aberrant cells were detected with no false positives as no translocations other than expected for cell lines were detected. Bone marrows of 36 patients suspected of acute leukemia were taken for routine genetic diagnosis (Karyotyping, FISH and or RT-PCR) and TLA. Sample analysis was performed randomized and blinded. TLA outcome was then compared with results from routine genetic testing.
Results: From a total of 36 patients three samples did not meet the required sample quality for further analysis. In the remaining 33 patients our TLA multiplex panel confirmed the presence of translocations on 16 samples. This includes a cryptic translocation involving the *ETV6-RUNX1* fusion gene, t(12;21)(p13;q22) in five pediatric ALL samples, not detected with karyotyping but RT-PCR, confirming the TLA findings. In fifteen samples no translocation was detected, concordant to (cyto)genetic findings. Three translocations were missed due to insufficient sequence reads on the partner chromosome. In addition, in one sample one translocation partner was also missed, located in the telomeric region of the chromosome and therefore resulting to nonspecific mapping of the sequence reads. An additional finding, involving a three way translocation t(9;22;11), missed by cytogenetics was detected by our panel. Two new findings have yet to be confirmed with FISH.
Summary/Conclusions: Our TLA panel showed concordant results for 29 out of the 33 successful sequenced samples. No false positives were found, while an additional translocation was detected. Our panel is able to detect (cryptic) translocations without prior knowledge of the fusion partner Therefore, the TLA multiplex panel is suited as a first tier screening tool in acute leukemia. A prospective study, comparing the diagnostic yield of the TLA panel with current tests, can establish whether the TLA panel is applicable as a routine procedure.

E898

ALTERATIONS IN NECROPTOSIS PATHWAY AFFECT PROGNOSIS OF PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Necroptosis is a type of necrotic cell death involving several genes transcription and activation of molecular mechanisms as death receptors, interferon, toll-like receptors, intracellular RNA and DNA sensors. The process is leading by the family of receptor-interacting protein kinase (*RIPK3*, *RIPK2*, *RIPK1*) and the *MLKL* substrate. Losses of *RIPK3* or *MLKL*, as well as deficiency in apoptosis, could allow tumor cells to escape the immune-mediated cells death (ICD).
Aims: We want to investigate the role of necroptosis deficiency in correlation with chemotherapy resistance and its impact as prognostic factor in AML.

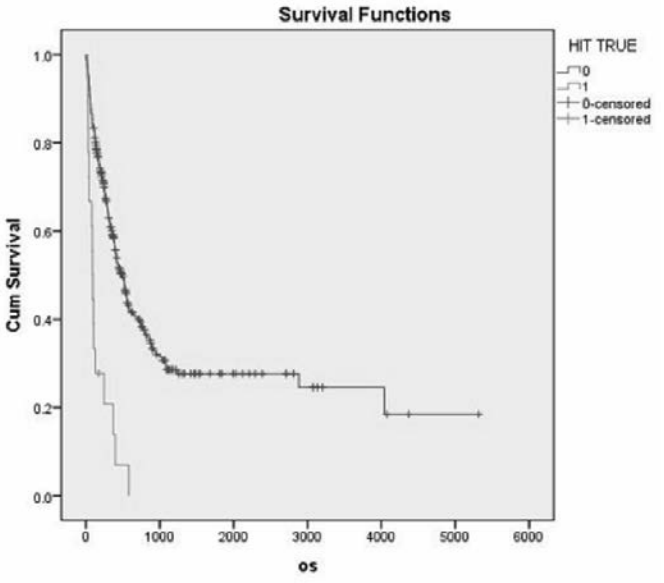

Figure 1.

Methods: We performed SNP Arrays (Cytoscan HD and SNP 6.0, Affymetrix) on a cohort of 300 non-M3 AML patients at diagnosis and we analyzed the Overall Survival (OS) of our patients with deficiency on necroptosis pathways. Survival was analyzed with Kaplan-Mayer method and Log-Rank test. We further analyze the relevance of different prognostic factors by the use of COX-Hazard Ratio statistical analysis.
Results: We found that 18 patients presented a loss of *RIPK1* or *MLKL* (nobody presented losses in *RIPK3/RIPK2*) and 13/18 patients were older than 65 years old. The Overall Survival (OS) of patients with alterations in these genes is significantly lower than control group, with a median OS of 3 *vs* 6 month respectively (p<.0.001). With Fisher Exact Test we further demonstrate that copy number loss of *RIPK1* or *MLKL* are associate to loss of *TP53* or *FANCA* genes, complex karyotype and advanced age. COX-Hazard Ratio model with *RIMK1* or *MLKL* loss, *BRCA1* loss, *TP53* mutation, *FANCA* loss, secondary disease and diagnosis karyotype considered as categorical variable show that necroptosis deficiency (HR 1.98, CI 95% 1.04-3.78) *TP53* mutation, and secondary AML are independent negative prognostic factors in an optimal model.
Summary/Conclusions: Our study shows that losses in necroptosis pathways are an uncommon alteration in AML, prevalent in old population. Moreover, we hypothesize that the loss of genes involved in necroptosis could be a real mechanism of tumor immune-escape and could be a rational to select patients that high probability to be resistant at chemotherapy promoting ICD mechanism.

Acknowledgment: ELN,AIL,AIRC, progetto Regione-Università 2010-12, FP7 NGS-PTL project,HARMONY.

E899

NGS ANALYSIS AND IMPACT OF VARIANT ALLELIC FREQUENCY AT RELAPSE AND REFRACTORINESS STATUS IN AML PATIENTS

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Background: A high number of patients with acute myeloid leukemia (AML) present resistance at treatment, which is associated with clonal persistence or evolution. The generation of high-depth sequencing data allowed to quantify variant allelic frequencies (VAF) and permitting estimation of the size of tumor clonal populations in each AML sample, and to perform an estimation of clonal evolution at relapse or refractory case according at diagnostic.
Aims: To evaluate the predictive impact of the fluctuation Variant Allelic Frequency in resistance to treatment cases in AML.

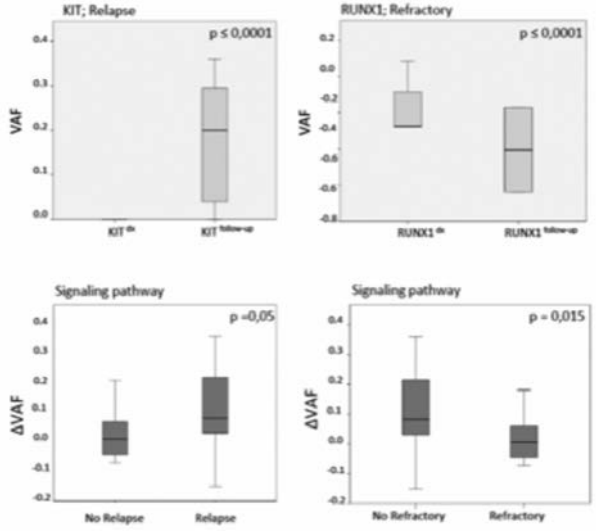

Figure 1. On top, representation of VAF at follow-up respect to the diagnosis in the most significant gene at relapse (KIT) and refractory (RUNX1). On bottom, representation of the fluctuation of VAF in patients with mutated gene in signaling pathway (EPOR, FLT3, JAK2, KIT, LNK or/and MPL) related to resistence to treatment event.

Figure 1.

Methods: We performed a custom-targeted sequencing panel of 32 genes (all coding regions) implicated in leukemia prognosis, including ASXL1, CBL, DNMT3A, EPOR, ETV6, EZH2, FLT3, HRAS, IDH1, IDH2, JAK2, KDM6A, KIT, KRAS, LNK, MLL, MPL, NRAS, PHF6, PRPF40B, PTEN, RUNX1, SF1, SF3A1, SF3B1, SRSF2, TET2, TP53, U2AF35, VHL, ZRSR2 and CALR, by *Ion Torrent Proton System-Thermo Fisher. P*rimary tumor-refractory (n=8) and primaty tumor-relapsed (n=17) samples pairs from 25 AML patients treated according *PETHEMA AML* clinical protocols were sequenced; in addition FLT3-ITD was detected by GENSCAN and NPM1 mutation was detected by PCR. We analyse the evolution of level of VAF, to measure the prevalence of somatic mutations between diagnosis and resistance status (relapse or refractory).
Results: Mutations in signalling pathway (EPOR, FLT3, JAK2, KIT, LNK or/and MPL) and GTPases pathway (KRAS, NRAS, HRAS) present significant ΔVAFs increases in relapse samples, p=0.05 and p=0.039 respectively. See figure 1. Furthermore, mutations in IDH2, JAK2 or KRAS show ΔVAF trend increases. Also, mutations in signalling pathway shows significant ΔVAF decrease in primary refractory samples, p=0.015; mutations in JAK2, KMT2A or SF3A1 shows ΔVAF trend decreases. Regarding to mutational profile we found significant differences in the VAF evolution in relapse samples, with VAF increment in IDH1 (p=0.031), KDM6A (p=0.018), KIT (p=0.000), PRPF40B (p=0.019), SF1 (p=0.033) and SF3A1 (p=0.044). Also, we found significant differences in the VAF evolution of primary refractory samples, reduced in KMT2A (p=0.016), RUNX1 (p=0.000) and the methylation pathway (DNMT3A, EZH2, KMD6A, MLL, TET2, IDH1 and IDH2; p=0.043). Mutations in TET2, U2AF1 or SF3A1 show VAF trend decreases. No correlation was found between VAF and% blasts, nor did VAF fluctuation with blasts fluctuation.
Summary/Conclusions: These results show VAF increases of specific mutations as KIT correlates with relapse status. However, in this cohort new specific mutations were not found neither VAF increases, even the opposite being as VAF decreases of specific mutations as RUNX1 correlates with primary refractoriness status. Furthermore, the variable frequency signalling pathway (EPOR, FLT3, JAK2, KIT, LNK or/and MPL) play a critical role in resistance status, increasing variant allelic frequencies of mutations in relapse and decreasing in refractoriness.

This work was supported by the grant: PI13/02387.

E900

PRECLINICAL EVIDENCE THAT TRAMETINIB ENHANCES THE RESPONSE TO TYROSINE KINASE INHIBITORS IN ACUTE MYELOID LEUKEMIA

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Background: Acute Myeloid Leukemia (AML) is the most common type of acute leukemia in adults and the second in children in whom overall survival is less than 35% and 60% respectively. Activating mutations of FLT3 are now recognized as the most common molecular abnormality in this disease, and the poor prognosis of patients harboring these mutations renders FLT3 an obvious therapeutic target. Although different tyrosine kinase inhibitors (TKI) have been used for this purpose, their ability to extend progression-free and overall survival is limited by drug resistance. This strategy could be improved by rationally combining TKIs with other agents. In this work, we have explored bone marrow samples from a FLT3-AML patient before and after TKI treatment by phosphoproteomics and observed enhanced activity of Ras-Raf-MEK-ERK1/2 pathway as a possible mechanism for TKI resistance.
Aims: To validate the role of ERK1/2 during TKI resistance *in vitro* and *ex vivo* and to search suitable combinations that allow overcome/avoid resistances in preclinical models of the disease.
Methods: Resistance mechanisms were studied *in vitro* in MOLM13 ($FLT3^{WT/ITD}$) after generating resistance by two different methods: by subculturing with increasing doses of sorafenib or by treating them with high doses of sorafenib, and recollecting alive proliferative (resistant) cells after CFDA and Anexinn labeling. Phosphoproteomic analyses were carried out by LC-MSMS after IMAC enrichment or by western blot techniques. Drug sensitivity assays with trametinib (MEK inhibitor) and three TKIs (sorafenib, pazopanib, midostaurin) were read after 48 hours or 72 hours of treatment *in vitro* or *ex vivo* respectively. The efficacy of the combinational treatments was characterized by the cell viability assay using WST8, and analyzed with Graphpad Prism software. Synergy effects were measured with Calcusyn software.

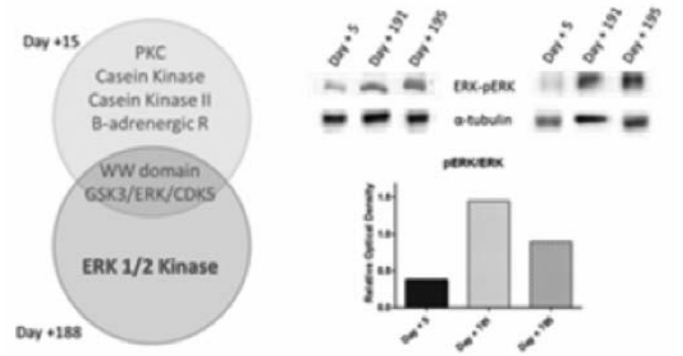

Figure 1.

Results: As it is presented in figure 1, ERK1/2 pathway was more activated after TKI treatment in the FLT3-AML patient during sorafenib-resistance development. The same fact was confirmed in MOLM13 sorafenib-resistant culture and in living proliferative cells recollected after sorafenib treatment. Different doses of trametinib, sorafenib, pazopanib and midostaurin in monotherapy were tested in MOLM13 cell line determining their IC50 values. Sinergy effects of combining trametinib with the three TKIs were analyzed with Calcusyn software and normalized isobolograms were represented. All combinations showed CI values less than 0.5 (Ci<0.5). The inhibition levels of four pathways (PI3K, STAT5, ERK1/2 and MAPK14) implicated in TKIs resistance were studied by western blot and the combination of midostaurin plus trametinib was the only one that inhibited all of them. Trametinib efficacy in the MOLM13 sorafenib-resistant culture was evaluated and confirmed that remained effective. Trametinib plus midostaurin combination was tested in OCI AML-3 cell line ($FLT3^{WT/WT}$) showing high efficacy and strong synergy (CI<0.5) too. Finally, we have assayed these drugs *ex vivo* in three AML samples showing the same effectiveness as *in vitro*, with IC50 values ranging from 0.2μM to 0.9 μM for midostaurin and 3 nM to 29 nM for trametinib and Ci values less than 0.5.
Summary/Conclusions: In conclusion, we provide preclinical evidence that combining a TKI, especially midostaurin, with a MEKI, such as trametinib, is a rational and efficacious treatment regimen for AML. As trametinib has previously shown good results when combined with pazopanib in clinical trials for other kind of tumors, we expect similar results in AML.

E901

IDENTIFICATION OF NOVEL THERAPEUTIC DRUGS IN DISTINCT PEDIATRIC AML SUBTYPES BY TARGETING EPIGENETIC REGULATORS

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Background: Treatment protocols for pediatric acute myeloid leukemia (AML) are chemotherapy-based, including high-dose cytarabine. While >90% of patients reach clinical remission, there is still a high relapse rate of ~30%, with overall survival rates of 60-70%. Therefore, better risk-classification at diagnosis and alternative treatment strategies are warranted. There is increasing evidence that epigenetic deregulation is involved in the initiation and progression of cancers, including AML. Epigenetic processes are required for hematopoiesis and epigenetic regulators are frequently translocated (MLL) or mutated (EZH2) in AML. Following this, deregulated epigenetic pathways could be used for targeted therapy and provide an alternative approach to improve pediatric AML therapy.
Aims: To identify new therapeutic drugs in pediatric AML by using an 80-compound screen containing inhibitors of epigenetic regulators, including histone writers (which deposit post-translational modifications (PTMs) on histones), readers (binding of PTMs) and erasers (removal of PTMs).
Methods: Cell lines used in this study are THP-1 (t(9;11)), Kasumi-1 (t(8;21)) and CMK (Down's syndrome with GATA1 mutation), reflecting distinct pediatric AML entities and a differential response to treatment with cytarabine. Cells were treated for 72hrs followed by analysis of cell viability and apoptosis based on Hoechst, Draq7 and Calcein Green staining. The effect of three candidate compounds were further investigated in triplicates at several concentrations for their effect on cell viability (Annexin V/PI staining), cell cycle, morphology, and apoptosis in the cell lines, normal myeloid precursor cells derived from cord blood, and pediatric AML patient cells representing distinct AML subtypes.
Results: From the 80 epigenetic compounds tested in THP-1, Kasumi-1 and CMK cells, we observed significant effects following treatment with the HDAC 4/5 inhibitor LMK235, the pan-HDAC inhibitor NSC3852, and the pan-bromodomain inhibitor Bromosporine. Dose-response curves showed differential cytotoxicity of the compounds and suggested LMK235 as most effective. Cell proliferation was inhibited by LMK235 at an IC50 of 0.1uM, 0.13uM and 0.425uM in Kasumi-1, CMK and THP-1, resp. While inhibition by LMK235 resulted in an immediate response of apoptosis, Bromosporine-treated cells retained in G1 before undergoing apoptosis and, interestingly, NSC3852 treatment resulted in an increase of cells in S-phase and G2/M. Among the differential effects of the compounds in the cell lines, we also observed differences in sensitivity. In line with previous studies, THP-1 cells were more resistant, illustrated by a 10-fold increase in concentration required for NSC3852-induced apoptosis. Interestingly, upon treatment with Bromosporine, Kasumi-1 and CMK cells showed a similar response, while Kasumi-1 cells were significantly more sensitive to NSC3852-induced effects. These data are currently validated in pediatric AML patient cells.
Summary/Conclusions: Treatment of three distinct pediatric AML cell lines with the epigenetic compounds LMK235, NSC3852 and Bromosporine resulted in cell line-specific effects, regarding compound sensitivity, and compound specific effects, including cell cycle regulation and induction of apoptosis. Our data suggests a potential role for epigenetic compounds, with specificity for molecular subtypes, in the treatment of clinically and biologically distinct pediatric AML subtypes.

E902

ALVOCIDIB SYNERGIZES WITH CYTARABINE AND DAUNORUBICIN (7+3) IN PRECLINICAL MODELS OF ACUTE MYELOID LEUKEMIA

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Background: Although survival of patients with acute myeloid leukemia (AML) has increased in the years 1975-2011, the 5-year, overall survival of patients with AML remains unacceptably low at an estimated 26% (2011). '7+3' treatment (cytarabine 100-200mg/m^2 + anthracycline [daunorubicin 60-90mg/m^2/day or idarubicin 12mg/m^2/day]) remains the standard induction therapy in AML patients, and has persisted largely unchanged for more than 30 years. There is a significant unmet clinical need for improved therapeutic options in patients with AML. Alvocidib is a CDK9 inhibitor currently in development for the treatment of patients with AML. Alvocidib has previously been studied as part of the ACM regimen, a combination regimen incorporating the time-sequential (TST) administration of alvocidib, cytarabine, and mitoxantrone, in multiple Phase 1 and 2 clinical trials. The ACM regimen has achieved significant improvements in complete response (CR) rates *versus* 7+3 in previously untreated intermediate and high-risk AML patients. The rationale for time-sequential administration of the ACM regimen was developed with an incomplete understanding of alvocidib's mechanism of action. TST was based on the expectation that alvocidib would synchronize cells, capitalizing on the S-phase specific activity of cytarabine in the combination. However, rather than cell-cycle inhibition, alvocidib potently induces apoptosis by inhibiting the expression of key anti-apoptotic proteins via CDK9/RNA polymerase II, including MCL-1. With this understanding, we reasoned that alvocidib would also enhance the activity of the 7+3 regimen.
Aims: These studies sought to interrogate the preclinical activity of alvocidib in the context of the 7+3 regimen in models for AML.
Methods: CellTiter-Glo and Caspase-Glo was used for all cell viability and apoptosis assays interrogating alvocidib and 7+3 activity in cell lines, following manufacturer's protocol. We used RT-PCR to measure mRNA expression of MCL-1 and other markers in response to drug treatment. Protein levels were interrogated using standard immunoblotting techniques. To determine the efficacy of an alvocidib/7+3 combination on tumor growth *in vivo*, we performed a MV4-11 xenograft mouse study.
Results: Single agent IC_{50} values of alvocidib, cytarabine, and daunorubicin range in AML cell lines from 2.2 nM to as high as 567 nM in viability assays. In apoptosis (Caspase-Glo) assays, however, we observed modest induction with single agent cytarabine, and good induction with single agent daunorubicin or alvocidib. In the combination, however, we observed very strong synergy with more than two-fold enhanced induction of apoptosis in some treatment groups. As has been previously described, we report here too that alvocidib treatment reduced the expression of MCL-1 protein and mRNA in a time and concentration-dependent fashion in AML cells. We observed this in the 7+3 treatment context as well. In an MV4-11 xenograft model, we observed 21.1 and 48.5% tumor growth inhibition (%TGI) following single agent treatment of daunorubicin or cytarabine, respectively. 1.25mg/kg alvocidib yielded 60.0%TGI. The combination of alvocidib, cytarabine, and daunorubicin, however, resulted in tumor regression, yielding a 116.2%TGI.
Summary/Conclusions: Though multiple clinical studies have demonstrated the increased efficacy of the ACM regimen over 7+3 in AML, these studies cannot correctly capture the clinical contribution of alvocidib alone as they do not offer a direct comparison. The current study attempted to interrogate the contribution of alvocidib to the 7+3 induction regimen, in preclinical models. These results provide a clear rationale for a clinical study directly comparing the triple combination to 7+3 alone. Taken together, our results suggest that a combination of alvocidib, cytarabine, and daunorubicin might be a potential clinical regimen in treating frontline AML, offering patients additional treatment options in treating their disease.

E903

COMBINATION OF INTERFERON-ALPHA AND VALPROIC ACID IN ACUTE MYELOID LEUKEMIA CELLS IN VITRO AND IN VIVO

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Background: Interferon alpha (IFNα) monotherapy is effective in selected myeloid neoplasias and is proposed to act through mechanisms that may be additive to the action of valproic acid (VPA), a histone deacetylase (HDAC) class I and IIA inhibitor with effect in approximately 20% of acute myeloid leukemia (AML) patients. Both drugs are found to have direct anti-cancer effects targeting apoptosis, differentiation and proliferation, as well as indirect effects targeting the immune system.
Aims: As several IFNα formulations are commercially available, we wished to explore the differences between two such drugs, recombinant IFNα-2b and human IFNα-Le, in relevance to AML treatment.
Methods: Flow cytometry and Hoechst staining was used to investigate apoptotic potential of the IFNα therapeutics, whilst phospho-flow cytometry and difference gel electrophoresis in combination with mass spectrometry unraveled IFNα signaling pathways. For *in vivo* effectivity analyses two orthotopic rodent models were implanted with leukemic cells and treated with VPA, IFNα-Le or both drugs.
Results: To investigate the anti-leukemic effects of IFNα we combined the two therapeutics with VPA *in vitro* using the human MOLM-13 cell line (wild type for FLT3 ITD and TP53). Results showed that IFNα-Le was more efficient compared to IFNα-2b in inducing apoptosis, whilst both were synergistic in combination with VPA. Investigating IFNα signaling pathways using phospho-specific flow cytometry we found IFNα-2b and IFNα-Le to have an identical stimulation profile in MOLM-13 cells, except from p-STAT6 Y641 that was higher expressed by IFNα-2b. The phospho-proteome was further explored using difference gel electrophoresis (DIGE) and mass spectrometric analyses to find a potential explanation to the difference in apoptosis-inducing effects between the two drugs. Here we found protein folding (LCP1, HSPA8, TCP1, CCT6A), cell stress (AKR1B1, HSP90AB1) and cell death (PKM2, PARK7, HSPB1, HSPA5, ANXA5, PRDX2) to be differently regulated between IFNα-2b and IFNα-Le, and also identified the presence of a dose-dependent effect on protein expression by IFNα-2b and IFNα-Le. Further, we investigated the potential synergistic anti-leukemic effects of VPA and IFNα-Le *in vivo* using a MOLM-13^{Luc+}immunodeficient NOD/Scid IL2 g$^{-/-}$ orthotopic xenograft mouse model, and the

immunocompetent brown Norwegian myeloid leukemia (BNML) syngeneic rat model. VPA mono-treatment increased survival from a median of 34 days to 38 days in the MOLM-13^{Luc+}mouse model, and from 21 days to 50 days in the BNML rat model. Additionally, the IFNα-Le ($0.8x10^6$ IU/kg) and VPA (400mg/kg) combination treatment indicated a tendency to increased survival in the BNML model. However, IFNα-Le monotherapy ($1x10^6$ IU/kg) decreased survival in the MOLM-13^{Luc+} model.

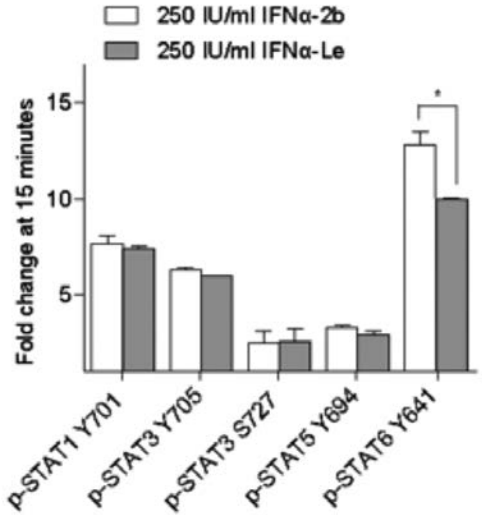

Figure 1.

Summary/Conclusions: IFNα does not add beneficial effects to VPA treatment in the two *in vivo* orthotopic models tested, possibly due to immune constitution and tumor load.

E904

KEVETRIN: PRECLINICAL STUDY OF A NEW COMPOUND IN ACUTE MYELOID LEUKEMIA

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Background: Acute Myeloid Leukemia (AML) is a heterogeneous disorder defined by clonal expansion of immature myeloid cells that infiltrate bone marrow and other tissues. AML therapeutic strategies remain unchanged since 1970 and the majority of patients often eventually relapse and die due to disease progression. Tumor protein p53 transcription factor is a key regulator of several cellular pathways, such as DNA repair, cell cycle, apoptosis and angiogenesis. It is mutated in 8-14% of AML cases and its mutations are commonly associated with a complex karyotype. Kevetrin is a new molecule compound, proposed by Cellceutix, with the ability to target both wild type and mutant p53 tumors.
Aims: The aim of this project is to explore cellular and molecular alterations induced by Kevetrin, focusing on its role in the p53 pathway.
Methods: Kevetrin was kindly provided by Cellceutix, dissolved and stored at 4°C in sterile water in a 600 µg/ml stock solution, and diluted in medium immediately before use [concentration range in use 15-60µg/ml]. Cell lines, MOLM-13 and KASUMI-1, were cultured in RPMI 1640 supplemented with 20% heat inactivated fetal bovine serum, 2mM L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin. After 24 and 48 h of treatment MTS, Annexin-V, TUNEL, JC-1 and Active Caspase-3 assays were performed according to manufacturer's instructions. Proteins were separated by polyacrylamide gel electrophoresis and transferred to 0.2 µm polyvinylidene fluoride membranes. Quantitative analysis was performed with Quantity One software. Statistical analysis was carried out using the paired and unpaired two-tailed Student's t tests. p values <0.05 were considered as significant.
Results: Our data indicate that Kevetrin exposure induces cell growth arrest, a great drop of mitochondrial membrane potential and a remarkable increment of Caspase-3 cleaved form, features that contribute to apoptotic cell death in the two cell lines. Cellular changes can be associated with a dose and time-dependent effect in the *TP53* mutated cell line (KASUMI-1) but not in the wild type one (MOLM-13), in which we can observe an activity only after 48 h at the higher concentration. Regarding molecular alterations in KASUMI-1 we found a great p53 down-regulation, probably due to Hsp90 reduction, resulting in a less marked formation of the Hsp90-p53 oncogenic complex. We also found a down-regulated p53 active form (Ser15), a reduced expression of p53 targets, p21 and PUMA, and a down-regulation of SIRT-3, that cannot exert its inhibitory activity on p53. The MOLM-13 cell line showed a great p53 reduction, probably related to SIRT-3 up-regulation and Hsp90 down-regulation. Regarding p53 active form, we noticed slight variations in protein expression, suggesting a physiological response of the protein to cellular damage. In accordance with p53 activity, we observed a great up-regulation of p21, probably associated with a drug resistance mechanism; in contrast, PUMA protein was highly down-regulated, suggesting a p53-independent mechanism of action or a feedback regulation of the apoptotic process, after Caspase-3 activation (Figure 1.). In order to better understand drug's mechanism of action we are performing gene expression profiling after 48h of treatment with Kevetrin 60µg/ml.

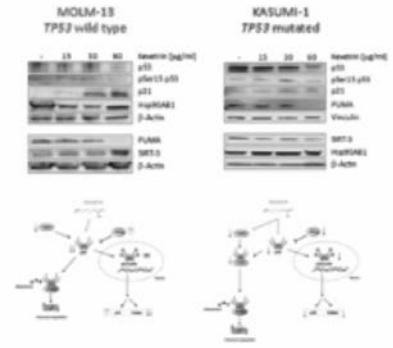

Figure 1.

Summary/Conclusions: Our results suggest Kevetrin is a promising new drug in AML patients treatment, both in wild type and, even more, in *TP53* mutated tumors, through different molecular mechanisms, giving more therapeutic alternatives in the treatment of this disease.

E905

CLEARANCE OF 'DRIVER-COSMIC' MUTATIONS POST CR1 WITH PERSISTING RUNX1_L56S IS UNLIKELY TO CONTRIBUTE TOWARDS DISEASE PROGRESSION IN AML

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Background: Clinical significance of gene variants in AML is well established (Papaemmanuil E *et al,* NEJM 2016) and is increasingly being implemented into routine diagnostic algorithms. Although 80% of patients achieve morphological remission after induction chemotherapy, long-term relapse free survival is a meagre 50% (Walter RB *et al*, JCO 2010. Monitoring of disease kinetics, is therefore, very critical.
Aims: To study the kinetics of gene variants post-induction chemotherapy in AML patients.
Methods: 130 follow-up samples from 45 *de novo* AML patients [median age-60 yr & median FU period- 18.6 mo] were screened for gene variants using TruSight Myeloid panel (Illumina, CA, USA) covering 54 genes with relevance in myeloid diseases. Gene variants at Variant allele frequency (VAF) of ≥10% at diagnosis and VAF of ≥1.5% during follow-up; both with target coverage of ≥300 reads were considered. Bone marrow (BM) or peripheral blood (PB) was obtained at presentation (BM:44; PB:1) and follow-up (BM-130). Gene variants in 95 samples from 40 MDS patients were also evaluated for progression to secondary AML. Public databases-Catalogue of Somatic Mutations In Cancer (COSMIC), dbSNP and 1000 genome (>2%) were used to classify gene variants as either Drivers (D), variants of unknown significance (VUS) and germline polymorphisms (SNP). P-value was generated with 2-tailed Fisher Exact (GraphPad Software, Inc, USA).
Results: Of 45 AML patients 19 achieved complete morphological remission (CR), 21 had a relapse and 5 had refractory disease with a median of 4 mutations/patient in each subgroup. Driver mutation was identified in 38 patients; 82% of who had persistence until clinical end-point. While 17 of 18 relapse patients retained a driver only 9 of 15 patients in remission retained it (Table 1). 8 of the 9 patients had a 'driver with COSMIC and SNP' (D-C/S) reference that persisted, while all 'driver with COSMIC only' (D-C) disappeared post-induction. This suggests that drivers with both COSMIC and SNP reference may not always contribute towards disease progression. We also found that D-C mutations persist in 85.7% of relapse pat.ients compared to only 11% of patients in remission (P-value: 0.001). Additionally, D-C mutations were retained in all 13 relapse patients with intermediate risk cytogenetics while complete clearance was observed in all 6 patients who were in sustained remission (P-value: 0.001). Further investigation of genes with D-C/S mutation in the remission cohort (8x) revealed that 4 patients had persistent DNMT3A-25457242, 1 had DNMT3A-25457243, 2 had RUNX1-36259324/L56S and 1 had CBL-119149011. As DNMT3A mutations are considered to occur in pre-leukemic

stem cells contributing to clonal haematopoiesis (Askush *et al*, Nature 2014; Genovese *et al*, NEJM 2014); this led us to study the distribution of RUNX1 gene variants in an additional 119 AML diagnostic samples. 34 patients (21%) harboured RUNX1 mutation, of which 5 had RUNX1_L56S that were often associated with D-C mutations (4 of 5 cases). Finally, we evaluated kinetics of D-C in 40 MDS cases of which 34 had chronic MDS and 6 had secondary AML (sAML). No significant difference was observed in the number of patients with persistent D-C mutation in the 2 subgroups (chronic MDS: 16 of 19 (84.2%); sAML: 5 of 5 (100%); P-value: 1.000).

Table 1.

AML Cohort (Total no. of patients: 45)				
Clinical Outcome		Morphological remission	AML relapse	Refractory disease
No. of patients in each clinical outcome category		19	21	5
Mean Age (Range) (in years)		55.8 (32-71)	57.3 (30-77)	63.8 (43-82)
No. of patients ≤60 years		10 of 19	10 of 21	2 of 5
Time to clinical end-point(Range) (in months)		5.2 (3-15)	17.8 (3-66)	4 (3.7-22.7)
Cytogenetics	Good	1	1	2
	Intermediate	12	16	0
	Poor	3	2	3
	Missing data	3	2	0
Median no. of mutations per patient at presentation		4	4	4.4
No. of patients with D-mutation at presentation		15	18	5
No. of patients with Dx D-mutation that was retained until clinical end-point		9 of 15*** (60%)	17 of 18 (94%)	5 of 5 (100%)
No. of patients who retained Dx D-mutation and acquired new D-mutations at clinical end-point		0	8 of 17	0
No. of patients who lost Dx D-mutation and/or acquired new D-mutations at clinical end-point		6 of 15 (40%)*	1 of 18** (6%)	0
No. of patients with Dx D-mutation that dissappeared and then reappeared at clinical end-		0	5 of 17	0
No. of patients without D-mutation at presentation		4	3	0
Of these how many acquired a D-mutation at clinical end-p		0 of 4	0 of 3	0
No. of patients with 'D-C' mutation persistent until clinical end-		1 of 9 (11%)^^	12 of 14 (85.7%)	2 of 3 (66.7%)
No. of patients with intermediate risk cytogenetics and D-mutation at Diagnosis		10 of 13	14 of 16	1 of 1
No. of patients with intermediate risk cytogenetics and persistent D-mutation at clinical end-point		6 of 10 (60%)	13 of 14 (93%)	1 of 1 (100%)
No. of patients with intermediate risk cytogenetics and persistent 'D-C' mutation		0 of 6 (0%)	13 of 13 (100%)	1 of 1(100%)

Table 1: Characteristics of patients and their gene variants in the three clinical subgroups of AML. SNP: Single Nucleotide Polymorphism; Dx: Diagnostic; D-mutation: driver mutation; D-C mutation: driver with COSMIC only

Summary/Conclusions: Clearance of 'Driver-COSMIC only' mutations while RUNX1_L56S persists is unlikely to contribute towards disease progression in AML.

Acute myeloid leukemia - Clinical

E906

PROGNOSTIC SIGNIFICANCE OF FLT3 STATUS, CYTOGENETIC, ECOG AND 50% BLAST DECREASE IN PRIMARY REFRACTORY OR EARLY RELAPSED AML PATIENTS BEFORE SALVAGE THERAPY

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Background: Prognosis of relapsed/refractory acute myeloid leukemia (R/R AML) is unfavorable with a long term overall survival around 10%. Thus, management of R/R AML represents one of the most difficult challenges. Because allogeneic-Hematopoietic Stem Cell Transplantation (allogeneic-HSCT) is considered as the best treatment for this category of patients, to determining which patient will benefit from this cumbersome strategy is a crucial issue. A better understanding of the mutational status, cytogenetic, histological and clinical findings of early R/R AML patients and their outcomes could help treatment decisions, particularly for those who allogeneic-HSCT is considered as the best therapeutic option.
Aims: The objective of this study is to determine prognostic factors and develop a prognostic score using usual mutational status, cytogenetic, histological and simple clinical variables in R/R AML patients before salvage treatments.
Methods: In this retrospective study in two hematological departments (Hospices Civils de Lyon and CHU of Toulouse), we evaluated clinical, biological, histological, cytogenetic and current mutational status of early R/R non APL AML patient between age from 18 to 70 years. Univariate and multivariate analysis were performed and we developed a prognostic score based on the independent prognostic parameters from Cox model.
Results: From January 2009 to May 2016, 58 patients presenting early relapse and primary refractory AML were analyzed. Overall Survival (OS) and Progression Free Survival (PFS) median were 9 and 2 months respectively. In univariate analysis, cytogenetic findings (unfavorable groups), unfavorable ECOG (>2), FLT3 positive status and <50% blast decrease (between induction and R/R assessment) independently predicted poor OS and were identified as significant prognostic parameters of OS (p=.037, p=.0084, p=.0452, p=.0071 respectively). In multivariate analysis, these last four criteria confirmed their worst prognostic impacts (p=.015, p=.017, p=.026, p=.015 respectively) and were used to create a five groups prognostic score. Better OS were statistically observed for patient with score 0 or 1 compared to 2, 3 or 4 (2-years OS 48% and 11% respectively, p= 0.0104) using a log-rank regression. When data were censored to allogeneic HSCT, the scoring system revealed a relevant difference with favorable OS for those with a score 0-1 compared to score 2-3-4 (2-years OS 64% and Not Reached respectively, p=.001) (Figure 3).

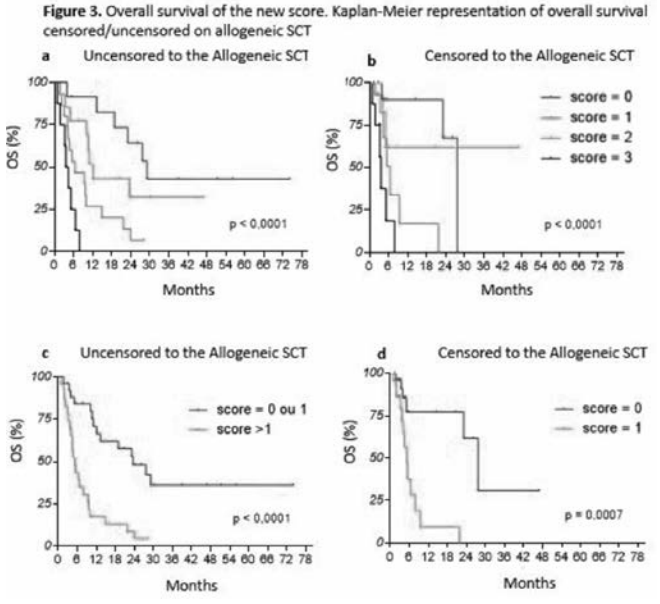

Figure 1.

Summary/Conclusions: Our prognostic score based on simple and usual data: FLT3 status, cytogenetic, ECOG and percentage blast decrease found distinct groups with statistically different outcomes. Basically, the higher is the score, the worst is the OS. This new score is a valuable, simple and useful score for the therapeutic salvage management of AML patients presenting early relapse and primary refractory.

E907

PRELIMINARY RESULTS FROM A PHASE 1 STUDY EXAMINING THE NOVEL BCL-2 INHIBITOR S55746/BCL201 AS SINGLE AGENT IN PATIENTS WITH ACUTE MYELOID LEUKEMIA OR HIGH RISK MYELODYSPLASTIC SYNDROME

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Background: Novel and effective therapeutic options for patients (pts) with advanced acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) are limited. Targeting the prosurvival molecule BCL-2 is clinically efficacious in various hematological malignancies. S55746/BCL201 is a novel, selective and potent inhibitor of BCL-2, with demonstrated antileukemic activity in preclinical models.
Aims: To evaluate the safety, recommended phase 2 dose (RP2D), pharmacokinetic (PK), pharmacodynamic (PD) and preliminary activity of S55746/BCL201 in patients with AML [relapsed/refractory (R/R) or ≥65 years unfit for intensive chemotherapy (IC)], or MDS failing prior therapies.
Methods: A phase I study (EUDRACT 2014-002559-24, NCT02920541) is underway to investigate S55746/BCL201 as a single agent in 5 European and Australian centers. S55746/BCL201 was initially administered in fasting conditions, once daily (21-day cycles), until disease progression, unacceptable toxicity, or investigator's or patient's decision. Pts giving informed consent received S55746/BCL201 at escalating dose levels according to a modified continual reassessment method for dose allocation.
Results: As of 23 February 2017, 34 pts have received S55746/BCL201 at doses ranging from 100 to 1300mg/day (median time on treatment: 43 days, range 1 to >374); 28 pts were R/R AML, 2 pts were elderly AML unfit for IC, and 4 pts had MDS failing prior therapies. Median age was 70 years (range 19-80), median number of prior therapies 2 (range 0-6), ECOG ≤2, and median WBC 3 G/L (range 0-30). Among the AML cohort, European LeukemiaNet risk (Döhner 2010) was adverse in 53%, intermediate-I in 20%, and intermediate-II in 17%. Preliminary PK results in fasting pts showed that exposure increased linearly but with some inter-individual variability. Most common (≥20% of pts) non-hematological adverse events (AEs), all grades, included diarrhea (27%), hypokalemia (27%), nausea (21%), and vomiting (21%). The most frequent grade ≥3 AEs were hematological [anemia (35%), thrombocytopenia (32%), febrile neutropenia (21%), and neutropenia (18%)], hypokalemia (18%), and sepsis (15%). Of 12 pts (38%) with AEs possibly related to study drug, the most frequent were diarrhea (3 pts), muscle spasms, thrombocytopenia, and anemia (2 pts each). One 74-year-old pt had grade 5 cardiac failure considered drug-related after 6 cycles of treatment (900mg). Non-related fatal AEs were reported in 7 pts (including sepsis, hemorrhagic stroke, pneumonia, and disease progression). There were no reported episodes of clinical or laboratory TLS. No DLT was reported and MTD has not been reached. Of 26 AML pts evaluable for response (at least 1 cycle completed), one achieved a CRi (complete remission with incomplete blood count recovery lasting 10 months) and one a PR (partial remission lasting 3 months before proceeding to allogeneic stem cell transplant). In MDS, 4 out of 4 pts had stable disease (lasting 1 to >7 months). Bone marrow blasts decreased in 50% of all evaluable pts, with the nadir reached (87%) within the first two cycles (Figure 1).

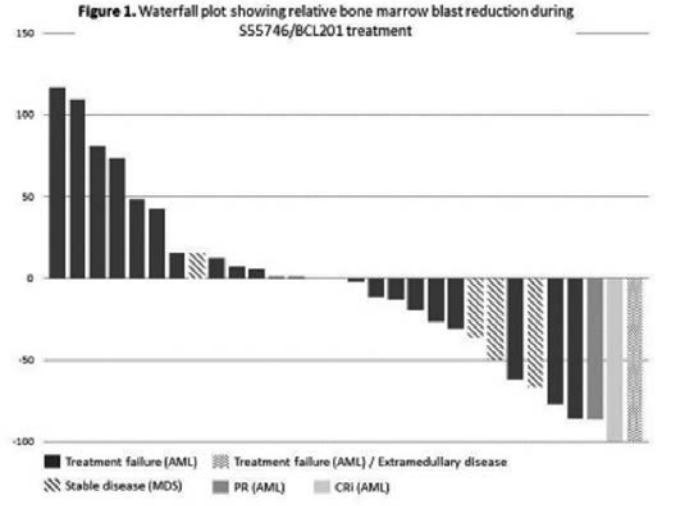

Figure 1.

Summary/Conclusions: Initial findings suggest that S55746/BCL201 has acceptable tolerability and clinical activity in advanced AML and MDS. Based on non-compartmental pharmacokinetic food interaction results from another study, demonstrating that S55746/BCL201 Cmax and AUC increased about 6-fold with food, dose escalation has started in patients with drug intake during a meal.

E908

DISSECTING THE CLINICAL HETEROGENEITY OF NUCLEOPHOSMIN-1 (NPM1) MUTATED ADULT ACUTE MYELOID LEUKEMIA: THE CONTRIBUTION OF FLOW-CYTOMETRIC DETERMINATION OF MINIMAL RESIDUAL DISEASE

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Background: Acute Myeloid Leukemia (AML) with mutations of the gene encoding Nucleophosmin-1 (NPM1) identifies a subgroup of patients with favorable prognosis according to the 2008 WHO classification. However, recent evidences (Papaemmanuil, NEJM 2016) suggest that the coexistence of additional gene mutations (e.g. DNMT3A, IDH1, IDH2^{R140} and TET2) may determine an inferior clinical outcome as compared to favorable risk AML and precludes a reliable outcome prediction. The presence of minimal residual disease (MRD), as determined by quantization of NPM1 mutated transcripts, provides powerful prognostic information independent of other risk factors (Ivey, NEJM 2016).
Aims: The aim of our study was to investigate if detection of MRD by multiparametric flow cytometry (MFC) might represent an alternative tool to discriminate different prognosis within the NPM1 mutated AML group, in a setting where an extensive gene profiling at diagnosis or a quantitative determination of NPM1 transcripts in remission would not be available.
Methods: We analyzed a series of 69 AML patients with NPM1 mutations; all the patients were in complete remission (CR) after intensive induction cycle of EORTC-GIMEMA protocols. The frequency of NPM1 mutated cases was not different among patients below (48/142, 34%) or above (21/61, 34%) the age of 60 years, respectively. Twenty out of 65 patients (31%) carried a concomitant FLT3-ITD mutation; 51/66 (77%) NPM1 mutated cases had a normal diploid karyotype. Upon full hematological recovery after consolidation cycle, counting, by MFC, ≥$3.5x10^{-4}$ (0.035%) residual leukemic cells (RLCs) in the bone marrow (BM) was regarded as a condition of MRD positivity.
Results: Among NPM1 mutated patients, the rate of MRD negative CR was significantly lower (5/69, 7%) as compared to NPM1 WT ones (39/134, 29%), respectively (p<0.001). Although there was not a statistically significant difference, probably due to the low numbers, MRD negative/NPM1^{mut} patients had a lower Cumulative Incidence of Relapse (CIR) as compared to MRD negative/NPM1^{wt} (25% *vs* 60%). We also evaluated the impact of autologous (AuSCT) or allogeneic (ASCT) transplantation on the outcome of MRD positive/NPM1^{mut} patients. The overall survival (OS) was significantly higher for patients submitted to ASCT (no=14) as compared to those (no=15) submitted to AuSCT (93% *vs* 33%, p=0.011). This was confirmed even after excluding from the analysis FLT3-ITD^{mut}patients. When all the meaningful clinical variables were challenged in multivariate analysis (MRD, type of transplant, age >60 yrs, karyotype), the type of transplant (ASCT *vs* AuSCT) was the only variable that significantly influenced OS and DFS (p=0.001 and 0.003, respectively).
Summary/Conclusions: In conclusion, although quantitative RT-PCR represents the gold standard, MFC determination of MRD also confirms that the quality of remission is critical to discriminate patients with a different outcome among NPM1^{mut} patients. In fact, these patients have a low chance to become MFC MRD negative and in a situation of MRD positivity, a very poor outcome can be substantially improved only by a timely use of an allogeneic procedure.

E909

EXPRESSION OF IMMUNE CHECKPOINT MOLECULES (PD-1, PD-L1, AND PD-L2) ON BONE MARROW T CELLS IN ACUTE MYELOID LEUKEMIA

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Background: Immune checkpoints constitute a mechanism by which tumors escape from the host immune system and involve the programmed death-1 (PD-1) receptor and its ligands, PD-L1 and PD-L2. In a tumor microenvironment, overexpression of PD-1, an inhibitory receptor on the surface of T cells, can lead to dysfunction of antitumor effector cells. Recently, investigators have detected overexpression of PD-1 for patients with acute myeloid leukemia (AML) who experienced relapse following allogeneic stem cell transplantation

(SCT). However, evidence regarding T cell phenotypes for patients with AML is sparse.
Aims: The authors evaluated patients with AML to determine expression levels of checkpoint molecules (PD-1, PD-L1, and PD-L2) according to diagnosis and treatments (chemotherapy [CTx] and SCT). The purpose of this study was to identify optimal candidates for checkpoint blockade therapy for AML.
Methods: Bone marrow (BM) samples were obtained from 195 AML patients in different stages of the disease. Samples were stratified by at time of diagnosis (n=69) and treatment response (complete remission [CR] after CTx, n=30; persistence after CTx, n=29; relapse after CTx, n=7; normocellular marrow with trilineage regeneration [NMTR] after SCT, n=19; persistence after SCT, n=18; and relapse after SCT, n=23). BM samples also were collected from 23 patients with no evidence of hematologic malignancies (control group). Flow cytometric analysis of PD-1 expression on T cells and PD-L1/PD-L2 expression on leukemic cells was performed by means of a FACSCanto II system (Becton-Dickinson, Sunnyvale, CA, USA).
Results: There were no differences in levels of PD-1 expression on CD8+ and CD4+ T cells at time of AML diagnosis, compared with controls. However, PD-1 expression levels on CD4+ T cells were significantly correlated with time since diagnosis. For patients at time of diagnosis, PD-1 expression on CD8+ and CD4+ T cells was significantly different compared with patients who experienced relapse after SCT (*P*=.017 and *P*<.0001), persistence after SCT (*P*=.025 and *P*<.0001), and NMTR after SCT (*P*<.0001 and *P*<.0001). In contrast, no difference in PD-1 expression was observed between patients at time of diagnosis and patients after CTx (Figure 1). For CD4+ T cells, a significant difference was found between SCT and CTx groups, and PD-1 expression levels of groups that experienced relapse (*P*<.0001) or persistence (*P*<.0001) after SCT were significantly higher than those of patients in the CTx groups. PD-L1 and PD-L2 expression on leukemic cells at time of diagnosis was higher in secondary AML transformed from myelodysplastic syndrome than in *de novo* AML (*P*=.0001 and *P*=.039). Although PD-L1 and PD-L2 expression levels for patients at time of AML diagnosis did not differ from groups that experienced relapse or persistence after SCT, PD-L1 and PD-L2 levels for diagnosed patients did differ from those of patients who experienced persistence after CTx (*P*=.038 and *P*=.023).

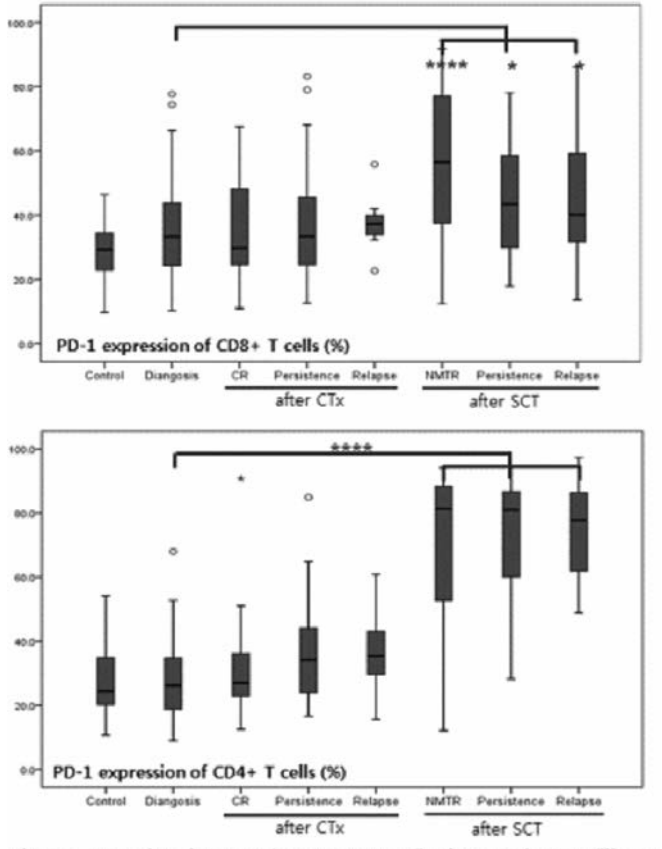

Figure 1. Expression of PD-1 on bone marrow T cells of AML patients at different stage of disease. Statistical differences were calculated to Diagonsis. *$P \leq 0.05$; **** $P \leq 0.0001$

Figure 1.

Summary/Conclusions: The present study suggests that the PD-1/PD-L1 pathway may constitute an immune-escape mechanism in AML. PD-1 expression in CD4+ T cells increased with time since diagnosis. Patients who underwent SCT exhibited overexpression of PD-1, which suggests that SCT and/or chronic stimulation of leukemic cells might induce more PD-1 expression by T cells. Blockade of the PD-1 immune checkpoint may represent an immunotherapeutic strategy for patients with AML relapse or AML persistence after SCT.

E910

ACUTE LEUKEMIA IN HIV PATIENTS : EPIDEMIOLOGY, THERAPEUTIC STRATEGY AND PROGNOSIS

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Background: Data on HIV patients with acute leukemia (AL, acute myeloid leukemia (AML) or non Burkitt acute lymphoid leukemia (ALL)) are very poor especially on their outcome. Treatment of acute leukemia usually depends of patient-related prognostics factors and disease related prognostic factors. Because HIV patients are considered frail, they are always excluded of therapeutics protocols. There are no guidelines for their treatments.
Aims: Our aim was to precise the epidemiology, the best therapeutic strategies as well as patient's prognostics, and to compare their outcome to those of seronegative patients with AL.
Methods: We conduct a retrospective national multicentric study. HIV positive patients with a diagnosis of AML or non Burkitt ALL between January 2000 and February 2016 were included. We compared HIV patients' outcome to those of seronegative patients with AL after a propensity score matching.
Results: 47 HIV patients with a diagnosis of AL (42 AML and 5 ALL) were included. AL incidence in HIV patients (HIVP) is not different than in general population but AL occurred earlier (49.29 years [44.21 ; 57.47]) and secondary AL are more frequent (42.55%). With a global and multidisciplinary approach these patients can be treated with intensive chemotherapy resulting on good efficiency (complete remission (CR)=84.38%)) and tolerance. Based on a multivariable model, only absence of CR was associated with hazard of death (p=0.01). 8 patients (17,02% ; 7 AML and 1 ALL) received an hematopoietic stem cell transplantation. HIVP with AL 2 years overall survival (OS) was 29% CI95% [15 ; 54] for AML and 40% CI95% [14 ; 100] for ALL. There was no difference in OS between our HIVP and seronegative controls with AL after propensity score matching (HR=1.347 [0.6486-2.796]; p=0,42).

Table 1.

Parameters	Value	N	Stats	N	Stats
		5	ALL	42	AML
Age		5	36.8 [34.66;42.75]	42	50.07 [46.24;58.16]
Sex	F	0	0%	11	26.19 %
	M	5	100%	31	73.81 %
CD4 at AL diagnosis		2	254.5 [220.8;288.2]	37	347 [210;571]
VL at AL diagnosis		4	2.6 [0;5.54]	37	0 [0;2.17]
De novo AL	No	3	60%	17	40.48 %
	Yes	2	40%	25	59.52 %
Hyperleukocytosis	No	4	100%	35	83.33 %
	Yes	0	0%	7	16.67 %
	NA	1		0	
Unfavorable cytogenetic risk	No	1	33.33 %	23	62.16 %
	Yes	2	66.67 %	14	37.84 %
	NA	2		5	
Therapy	NA	0	0%	1	2.38 %
	LD chemo or 5-aza	0	0%	6	14.29 %
	ATRA ATO	0	0%	4	9.52 %
	Intensive therapy	5	100%	27	64.29 %
	Best supportive care	0	0%	4	9.52 %
CR	No	1	20%	14	33.33 %
	Yes	4	80%	24	57.14 %
HSCT	No	4	80%	35	83.33 %
	Yes	1	20%	7	16.67 %

Summary/Conclusions: Our study shows that HIV status has no prognostic impact on AL patient's outcome. HIV patient with acute leukemia should thus be included in clinical trials to improve and standardize their therapeutic management.

E911

TEN-DAY DECITABINE AS INDUCTION THERAPY FOR OLDER PATIENTS WITH ACUTE MYELOID LEUKEMIA FIT FOR INTENSIVE CHEMOTHERAPY

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Background: Currently, there is no consensus regarding optimal treatment for older patients with acute myeloid leukemia (AML). Decitabine for 5 consecutive days produced a complete remission (CR) rate of 17.8% in older patients with newly diagnosed unfit AML. Ten-day regimen of decitabine induces a higher response in newly diagnosed older patients with AML considered unfit for intensive chemotherapy. But this 10-day regimen has not been tested in older fit AML before.
Aims: To investigated the efficacy and safety of the10-day decitabine regimen in older fit AML prospectively.
Methods: Twenty-one older patients (>60 years old) with newly diagnosed intermediate or adverse cytogenetic risk group AML, considered fit for intensive chemotherapy, were enrolled in a prospective clinical trial. These patients refused to take intensive chemotherapy. All patients were treated with at least one course of 10-day decitabine (20mg/m^2 daily). Patients who achieved less than 5% bone marrow blasts were subsequently treated with 5-day decitabine courses as maintenance therapy. Median age was 64 (range 60-74) years. There are was 5 patients with (23.8%), 10 (47.6%), 6 (28.6%) in favorable, intermediate and, poor-risk group, respectively, based on the NCCN guideline. All patients had an Eastern Cooperative Oncology Group performance status of 1.
Results: The overall response rate (ORR) was 57.1%, including 52.4% CR. There are no significant differences between responders and non-responders, in the following parameters, including age, LDH, DNMT3a mutation, white blood cells count in peripheral blood, or bone marrow blasts percentage. Nineteen patients experienced neutropenia during induction regimen. The lowest median white blood cell count and neutropenic time is $0.02´10^9$(0-3.05 ´10^9), 15(0-24) days, respectively. The common non-hematologic toxicities were febrile neutropenia and infections. Median overall survival (OS) of all patients was 20.7 months. One-year and two-year OSrate were 71.4% and 45.4%, respectively. Patients who responded to treatment had significantly longer OS than non-responders.
Summary/Conclusions: This indicates that the 10-day decitabine regimen may be an optimal management for older AML patients who are in intermediate or adverse cytogenetic risk group and fit for chemotherapy.

E912

INDOXIMOD IN COMBINATION WITH IDARUBICIN AND CYTARABINE FOR UPFRONT TREATMENT OF PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA (AML): PHASE 1 REPORT

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Background: AML cells can acquire immune evasion and tolerance through overexpression of indoleamine 2,3-dioxygenase (IDO), which exerts immunomodulatory effects through tryptophan (Trp) catabolism and kynurenine production. By degrading Trp, IDO shifts the balance from a Trp-rich environment, which encourages T-cell proliferation and activation, to a Trp-poor environment leading to immune system suppression. We hypothesized that incorporation of indoximod, an inhibitor of the IDO pathway, into conventional remission induction and consolidation would be well tolerated without adding significant toxicity and may improve clinical outcomes of patients (pts) with newly diagnosed AML.
Aims: The primary objective of the phase 1 portion of the trial is to characterize the regimen limiting toxicities (RLT) as well as the recommended phase 2 dose (RP2D) of indoximod in combination with standard of care (SOC) chemotherapy for frontline AML treatment. A key secondary objective is to determine the minimal (or measurable) residual disease (MRD) status, as measured by multicolor flow cytometry, in pts with AML who receive indoximod in combination with SOC at the end of induction, after completion of the 1st cycle of consolidation, and before maintenance or proceeding to allogeneic stem cell transplantation (allo-HSCT).
Methods: This is a phase 1b / randomized phase 2a trial of indoximod in combination with 7+3 remission induction consisting of cytarabine (100mg/m^2/day continuous infusion for 7 days) and idarubicin (12mg/m^2/day for 3 days) and high dose cytarabine (HiDAC) consolidation, as SOC, in pts with newly diagnosed AML (NCT02835729), with a 3+3 design for the phase 1 portion. Indoximod is given orally every 8 hours starting on day 8 of induction onward. Indoximod is held on days that pts receive HiDAC consolidation, and it is discontinued 4 weeks prior to allo-HSCT. The phase 1 consists of 4 dose levels [400mg (-1), 600mg (0, starting dose), 1000mg (1), 1200mg (2)] in combination with SOC.
Results: Twelve pts have been enrolled, as of March 1, 2017. Median age is 53 (range 18-69) years, and 4 pts are female. According to 2017 European Leukemia Net risk stratification, 9 had favorable risk (3 with *DNMT3A* and 2 with low allelic ratio (<0.5) of *FLT3*-ITD) and 3 had adverse risk. No RLT was observed with the 1st and 2nd dose levels. The most frequently reported adverse events (regardless of attribution), were febrile neutropenia, diarrhea, nausea and vomiting, dyspnea, hypotension, and hypoxia. Three pts are no longer on study: 1 (dose level 0) due to inability to swallow indoximod after hypoxic respiratory failure during induction, 1 (dose level 1) withdrew consent for personal reasons after only 2 doses of indoximod, and 1 (dose level 2) was taken off due to eligibility. The remaining 9 pts are still on study; 3 pts in dose level 2 are currently receiving induction and are not evaluable. Five of 6 (83%) evaluable pts in dose levels 0 and 1 achieved complete remission (CR) after induction. All 5 pts demonstrated no evidence of MRD at levels <0.02% (MRD-neg) post-induction and remained MRD-neg post cycle 1 of HiDAC. One pt in dose level 1 with favorable risk (normal karyotype, mutations in *DNMT3A*/*NPM1*/*NRAS*) had primary refractory disease. The pt who was unable to swallow indoximod had favorable risk (normal karyotype, mutations in *DNMT3A*/*NPM1*) and achieved morphologic CR but had MRD at the end of induction, and ultimately relapsed after 2 cycles of HiDAC consolidation.
Summary/Conclusions: Indoximod does not appear to add significant toxicity to standard remission induction and consolidation in pts with newly diagnosed AML. Initial data suggest a high rate of MRD-neg after one cycle of induction chemotherapy.

E913

PHASE I/II STUDY OF MEK INHIBITOR (MEK-162; BINIMETINIB) IN PATIENTS WITH RELAPSED AND/OR REFRACTORY MYELOID MALIGNANCIES

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Background: Activation of the mitogen-activated protein kinase (MAPK) signaling (RAS/RAF/MEK/ERK pathway) promotes growth and inhibits apoptosis of hematopoietic cells. Inhibition of MEK/MAPK pathway has shown antiproliferative effects in acute myeloid leukemia (AML) cell lines and AML blasts. MEK-162 is an oral, potent, selective allosteric, ATP non-competitive inhibitor of MEK1 and 2.
Aims: To study the efficacy and safety of MEK-162 in patients with advanced myeloid malignancies.
Methods: Patients with relapsed/refractory AML, not candidates for intensive chemotherapy, and patients with high risk myelodysplastic syndrome (MDS) who were resistant/intolerant to standard treatment including stem cell transplant were treated with MEK-162 twice daily every 28 days. Patients in the expansion phase had to be RAS mutated. The primary endpoint was overall response rate (ORR=CR + CRi) after 1 cycle of therapy. Survival was estimated using the Kaplan-Meier method. Safety analysis included all patients who had received at least 1 dose of MEK-162. MEK-162 dose escalation followed a 3+3 design; phase 2 had built in futility/toxicitiy boundaries. 45mg twice daily is the final dose level for expansion phase.
Results: Sixteen patients were treated (escalation=7; expansion=9): 14 AML and 2 MDS. Median age was 62 years (31-85). 56% were male; 94% had a performance status of 1-2. Median number of prior therapies was 4 (1-6). 3/16 (19%) patients had complex karyotype. 11/16 (69%) patients were RAS mutated. 10/16 patients completed a minimum of 1 cycle of MEK-162 therapy and were evaluable for response (3 at 30mg and 7 at 45mg dose). ORR was 10% (CRi in 1/10 patients). Median number of cycles administered were 2 (1-4). Median duration on therapy was 1.1 months (0.1-3.4). Median overall survival is 3.2 months (0.3-7.6). Common G3/4 toxicity included neutropenia (56%), fatigue (13%), nausea/vomiting (13%) and electrolyte abnormalities (19%). No dose limiting toxicity was reported.
Summary/Conclusions: MEK-162 shows a tolerable safety profile with an ORR of 10%. The study is currently on-going. Additional studies involving combination of MEK-162 with RAF and PI3 kinase inhibitors are ongoing.

E914

HAPLOIDENTICAL TRANSPLANTATION IS SAFE AND EFFECTIVE FOR OLDER PATIENTS WITH AML/MDS

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Background: Acute myeloid leukemia (AML) is more common in the older population. Haploidentical stem cell transplantation (haploSCT) is a potentially cur-

ative treatment option for patients with AML and allows transplantation for patients without an HLA matched donor. Recently, the use of post-transplant cyclophosphamide-based (PTCy) GVHD prophylaxis has improved outcomes of haploSCT; however, outcomes of haploSCT in older patients remain unclear.
Aims: Here we evaluated outcomes of older patients with AML/MDS who underwent haploSCT.
Methods: We retrospectively analyzed outcomes of all 43 patients ³55 years with AML/MDS who underwent a haploSCT at our institution after year 2009. All patients were treated with fludarabine-melphalan (FM)-based conditioning regimen (melphalan 100 or 140mg/m^2) plus thiotepa 5mg/kg or 2GyTBI. Characteristics of these patients are presented in **Table 1**.

Figure 1. OS estimates according to cytogenetics and donor age generated by Cox regression adjusted for disease status at transplant.

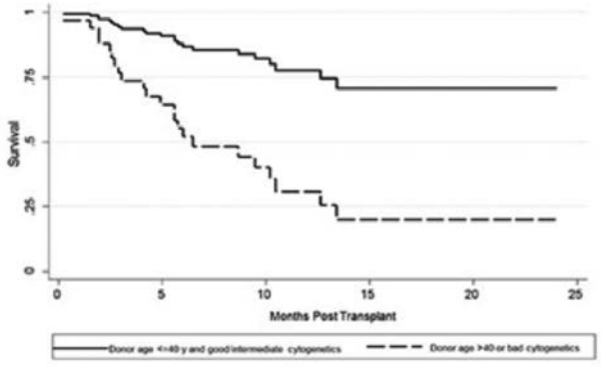

Figure 1.

Results: Median age was 61 years (range 55-69), 22 patients (51%) were in CR1/2, 16 patients (37%) had poor-risk cytogenetics, and median HCT-CI was 2 (range 0-11). Reduced melphalan regimen (100mg/m^2) was used in 29 pts (67%). Donors were children in 35 (81%) or siblings 10 (19%) patients.
Median follow-up was 19 months (range 6-49). One patient died prior to engraftment. Forty-two patients engrafted the donor cells (100%). Median time to neutrophil and platelet engraftment was 19 (13-28) and 28 (15-117) days. Day 30 chimerism was 100% donor in 38 patients (88%). The cumulative incidence (CI) of grade 2-4 and 3-4 aGVHD at 6 months post-transplant was 35% and 5% while CI of cGVHD at 2 years post-transplant was only 9%. The 2-year overall survival (OS) and progression-free survival (PFS) was 42%, and relapse rate was 24%. Cumulative non-relapse mortality (NRM) was 21%, 30% and 34% at day 100, 1 year, and 2 years post-transplant. Patients in CR1/2 had 2-year NRM and relapse rate of 23% and 14%, and OS was 61%. The 2-year OS for patients in CR1/2 with intermediate/favorable-risk cytogenetics was 73%. In multivariate analysis, favorable predictors for OS were CR1/2 (HR:0.4, p=0.05), good /intermediate cytogenetics (HR:0.2, p=0.01), and donor age greated than 40 (**Figure 1**).

Table 1.

Characteristic	N (%)
Median age	61 (55-69)
Median follow-up	19 (6-49)
Disease	
AML	25 (58%)
MDS/AML	8 (19%)
MDS	10 (23%)
Cytogenetics	
Poor	16 (37%)
Intermediate	24 (56%)
Good	3 (7%)
Conditioning	
RIC	29 (67%)
Stem cell source	
BM	42 (98%)
Disease Status	
CR1/2	22 (51%)
Other	21 (49%)
HCT-CI	Median 2 (range 0-11)
Donors	
Child	35 (81%)
Sibling	8 (9%)
Donor age	Median 37 (20-62)
	28 (65%)
Sex mismatch	
Female donor/Male	13 (30%)
recipient	30 (70%)
Other	

Summary/Conclusions: HaploSCT with PTCy-based GVHD prophylaxis is safe and effective for older AML/MDS patients. Lack of an HLA matched donor is not a contraindication to proceeding to a haploidentical transplant in older AML/MDS patients. In addition to remission status and cytogenetics, we found that younger donor age was significantly associated with improved survival in older AML/MDS patients undergoing haploidentical transplantation.

E915

OPTIMIZATION OF MINIMAL RESIDUAL DISEASE EVALUATION IN ACUTE MYELOID LEUKEMIA TO DRIVE POST REMISSION THERAPY

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Background: Among Acute Myeloid Leukemia (AML) patients achieving hematological complete remission (CR) the persistence of detectable disease assessed with highly sensitive techniques as Multicolor-Flow-Cytometry (MFC) or PCR-based molecular analysis retains a negative prognostic value. However, a consensus on the most informative time-points (TP) and sensitivity cut-offs for MRD assessment has not been reached.
Aims: The aim of the present study was the evaluation of the prognostic impact of MFC and molecular MRD assessment by identifying TP, MFC positivity cut-off values and molecular MRD markers with the highest prognostic impact.
Methods: One hundred and ten consecutive AML patients treated in our center between 2004 and 2014 were retrospectively analyzed. As previously described, all patients had received a fludarabine-containing induction.Median age was 47 years (range 18-65). Median follow up was 59 months. Three different MRD TP have been considered: TP1, after induction I; TP2, after induction II; TP3, after consolidation therapy for patients who did not undergo hematopoietic stem cells transplantation (HSCT). For patients who underwent HSCT, TP3 coincided with pre-transplant MRD evaluation. MFC-MRD evaluation had been performed through 4-colour MFC analysis (and 8-colour from 2013). To define MFC-MRD positivity two cut-offs were compared: a threshold of 2.5×10^{-4}residual leukemic cells (>0,025%) or a threshold of 1 x 10^{-3} residual leukemic cells (>0.1%). For patients carrying NPM1-gene mutation NPM1 expression levels at TP1, TP2, TP3 (NPM-MRD) were analyzed. A reduction >3.5 log of NPM1 transcript at TP1 was considered optimal as per our published experience. For patients presenting WT1 over-expression at diagnosis WT1-MRD was evaluated at TP1, considering WT1 negativity with a cut-off of WT1 copies/10^4 ABL lower than 250.
Results: CR rate after induction I and II was 82.7 and 85.5%, respectively. The percentage of MFC-MRD neg<0.025 and<0.1% increased from TP1 to TP2. The probability of achieving MFC-MRD negativity at TP2 was only influenced by ELN risk group (p <0.05). In the whole cohort, 2 years OS was 60.2% (median not reached). Multivariate Cox-Proportional Hazard model showed that MFC-MRD >0.1% at TP2 was the strongest predictor of higher risk of death, whereas MFC-MRD <0.025 at TP1 was the strongest predictor of long survival (Figure 1, a-b). However, patients with MFC-MRD >0.025 at TP1 displayed a heterogeneous outcome. In this subgroup WT1-MRD at TP1 was able to identify patients with the higher risk of death (Figure 1c). Thirty-five patients carried NPM1-mutation. Two-years OS for NPM1-mut patients showing more or less than 3.5 log transcript reduction at TP1 was 94.1% *vs* 58.3%, respectively (p 0.039); 2 years OS for patients achieving NPM1-MRD negativity at TP2 was 90.5% *vs* 42.9% (p 0.003). MFC MRD analysis in the NPM1-mut cohort led to results comparable with the whole cohort. Multivariate analysis showed that NPM1-MRD at TP1 was the strongest predictor for OS in this group.

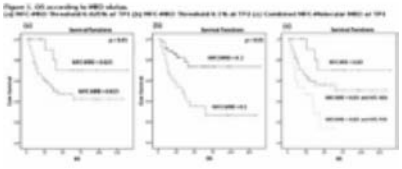

Figure 1.

Summary/Conclusions: Our data show that MRD assessment at different time-points during treatment retains a strong prognostic impact in AML and that different cut-offs at different time-points can give different and useful prognostic information that may drive post-induction therapy. MFC MRD evaluation at TP2 with 0.1% cut-off is the most useful for patients risk stratification. However, the evaluation of MFC-MRD at TP1 with 0.025% cut-off can early identify a group of patients with a significantly low risk of relapse. At the same TP, MFC and WT1 MRD integration allows a better risk stratification. MFC MRD is accurate also in NPM1-mut patients; however, in this cohort NPM1-based MRD evaluation is the most accurate predictor of prognosis.

E916

THE NUMBER OF CD34+CD38+CD117+HLA-DR+CD13+CD33+ CELLS INDICATES POST-CHEMOTHERAPY NEUTROPHIL RECOVERY IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Hematopoietic recovery is considered to associated with the number of multipotent hematopoietic stem cells in the bone marrow, as observed in functional assays involving stem cell transplantation. However, there is little evidence related to hematopoietic recovery in non-transplantation settings, which is accomplished by endogenous hematopoietic cells. A recent study suggested that progenitors are the main contributors during this steady-state hematopoiesis, which differs from exogenous transplantation. And our previously data revealed that, CD34+CD38+CD117+HLA-DR+CD13+ CD33+ cells (P cells), a kind of progenitor cell, is significantly decreased in patients with delayed neutrophil recovery after chemotherapy compared with that without delayed count recovery.
Aims: To further examine a potential impact of P cells percentage on hematopoietic recovery.
Methods: The data of 223 patients diagnosed with *de novo* AML was analyzed retrospectively. All these patients enrolled in our previously registered prospective randomized clinical trial AML 2010-01(201002024). We reviewed the data from bone marrow flowcytometry before the first and second course of consolidation therapy, in which the CD34+CD38+CD117+HLA-DR+CD13+CD33+ progenitor cell percentage in the bone marrow was analyzed. Platelet recovery time and time of neutropenia were counted for the evaluation of hematopoietic recovery ability after chemotherapy.
Results: We found that less P cell percentage was significantly associated with prolonged neutropenia recovery time after the first and second courses of consolidation chemotherapy (p=0.001; p=0.028, respectively). We also observed similar results regard to platelet recovery time after the first course of consolidation chemotherapy (p=0.001). Univariate analysis showed that P cell percentage, rather than gender, age, WHO classification and cytogenetic subgroup, were associated with neutrophil recovery after chemotherapy. Multivariate analysis demonstrated that P cell percentage is an independent factor affecting neutrophil recovery capability for both first and second courses (p=0.015; p=0.036, respectively).
Summary/Conclusions: Our results indicate that CD34+CD38+CD117+HLA-DR+CD13+CD33+ cells before each course of chemotherapy is associated with chemotherapy related hematopoietic reconstitution capacity independently. These findings may help better understand endogenous hematopoietic reconstitution and modify future chemotherapy regimens based on progenitor cell percentages.

E917

MICRORNAS (MIRS) IN HIGH RISK PEDIATRIC ACUTE MYELOID LEUKEMIA (AML) AS PREDICTION TOOLS FOR RELAPSE INCIDENCE

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Background: Despite recent progresses made in the treatment of acute myeloid leukemia (AML) of childhood, the cure rates of high-risk subtypes remain low. Indeed, patients harboring FLT3-ITD mutations or 11q23 translocations (MLL rearrangements) are still characterized by a poor prognosis, mainly due to leukemia recurrence. Since microRNA (miRs) are small RNA molecules controlling normal hematopoiesis whose deregulation is fundamental in leukemia's pathogenesis, a possible role as predictors of relapse should be considered.
Aims: Our purpose is to identify, at time of diagnosis, significant miRs signatures able to predict the risk of relapse for patients with high-risk AML, such as FLT3-ITD and MLL mutated. Moreover, these signatures would help us in identifying new molecules for novel targeted therapy and to deeply characterize different deregulated pathways among FLT3-ITD and MLL rearranged patients.
Methods: A total of 20 AML bone marrow (BM) derived samples collected either at diagnosis (ND) and at relapse (RL) together with 8 healthy controls (HCs) were studied (total: N=48). Informed consent has been obtained from either parents or legal guardians according to the Declaration of Helsinki. RNA was extracted, cleaned up and Nanostring microRNA profiling was performed. Statistical and bioinformatics analysis were performed using nSolver™ (NanoString Technologies; Seattle, WA, USA) and R-based software. All the presented results imply a P<0.05 where not mentioned.
Results: Comparing all AML samples with HCs, we found 16 up- and 509 down-regulated miRs. Similarly, FLT3-ITD (n=11) *vs* HCs, showed 17 up- and 361 down- regulated miRs, while MLL-rearranged (n=9) *vs* HCs showed 16 and 428 up- and down- regulated miRs, respectively. A trend towards down-regulation of the whole cohort was detected and a block in miRs maturation occurring in the 2 molecular subsets was supposed. Finally, a FLT3-ITD *vs* MLL-rearranged analysis produced a signature in which 20 miRs were up- and 18 down- regulated, a putative signature which could characterize high-risk AML. ND *vs* HCs analysis identified 17 up- and 397 downregulated miRs, confirming a tendency toward downregulation, as well as in RL *vs* HCs analysis, in which we found 12 and 374 up- and down- regulated miRs, respectively. RL *vs* ND comparison showed a total of 16 up- and 15 down- regulated miRs. In the attempt to identify a signature predictive of recurrence at time of diagnosis, we compared ND and RL samples, revealing 301 miRs that maintained their deregulation in the 2 subgroups, while 113 and 85 were uniquely found in ND *vs* HCs and RL *vs* HCs, respectively. Remarkably, miR-34a-5p (P<0.0001) was the recurrent and most statistically significant upregulated miR in both ND and RL samples. Moreover, upregulated miR-10a-5p and miR-99a-5p (P<0.0001), and downregulated miR-9-5p (P<0.0001) were the most statistically significant miRs in the FLT3-ITD and MLL-rearranged sets respectively, underlying putative unique elements distinguishing the two clinical subsets.
Summary/Conclusions: Our results suggest the presence of different microRNA signatures in pediatric AML carrying FLT3-ITD and 11q23 translocations [t(9;11) and t(10;11)]. The identifications of new targets linked to this miRs would be useful for further studies focused on finding molecular-based therapy. Interestingly, miR-34a-5p was recurrently found upregulated either in ND and RL groups, but not in the comparative analysis between ND *vs* RL, suggesting a potential involvement in the mechanisms at the base of both onset and relapse in these subtypes of high-risk AML.

E918

CYTOKINE RECEPTORS AND SOLUBLE ADHESION MOLECULE LEVELS ARE ASSOCIATED WITH PROGNOSIS OF NEWLY DIAGNOSED AML

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Background: The outcomes of acute myeloid leukemia (AML) treatment are beleaguered by the high resistance of malignant clones to therapy. Cytokines and adhesion molecules have been studied as markers of immune system activation in many diseases including AML. Further knowledge gained from baseline cytokine levels assessment may help to improve treatment outcomes.
Aims: The aim of this study is to evaluate baseline levels of selected cytokines, cytokine receptors and adhesion molecules and their relationship with prognosis in newly diagnosed AML patients.
Methods: A total of 75 AML patients, age 52.9±13.0 years, median 58.5 years, 44 female, were studied in the period 2010-2015. Only patients with minimal follow-up of 1 year were included. All patients were induced with "3+7" induction chemotherapy consisting of Cytarabin 100mg/m^2 per day for 7 consecutive days and Daunorubicin 90mg/m^2 for the first 3 days of therapy in younger patients. Since the beginning of 2015, the induction dose of Daunorubicin used has been 60mg/m^2 even in younger patients, according to recent evidence-based data modifications. Those who failed to achieve CR were given FlAG-Ida salvage followed by allogeneic stem cell transplantation in younger and fit patients. In CR, the patients were treated either with HiDAC consolidations alone, or followed by allogeneic stem cell transplantation in indicated cases. A total of 39 patients underwent allogeneic stem cell transplantation. We evaluated serum levels of the following 29 analytes: interleukins (IL-1α,IL-1β,IL-2,IL-3,IL-4,IL-5,IL-6,IL-7,IL-8,IL-10,IL-12,IL-13,IL-15), Epidermal Growth Factor, Granulocyte Macrophage Colony Stimulating Factor, Interferon-γ, Macrophage Inflammatory Protein-1α, Monocyte Chemotactic Protein-1, Tumor Necrosis Factor-α (TNF-α), Vascular Endothelial Growth Factor, E-selectin (E-SEL), P-selectin (P-SEL), Intercellular Adhesion Molecule-1 (ICAM-1), Vascular Cell Adhesion Molecule-1 (VCAM-1), Matrix Metalloproteinase-9, soluble IL-2 receptor-α (sIL-2Rα) and soluble receptors for IL-6 (sIL-6R) and TNF-α type I and II (TNFR-1,2). All biomarkers were measured by biochip array technology on Evidence Investigator analyzer (Randox). Associations of these markers with complete remission (CR) achievement, 1 year progression-free survival (PFS), and 1 year overall survival (OS), were evaluated. Statistical analysis was performed in STATISTICA 2.0.
Results: CR by induction therapy was achieved in 53 cases (70.7%). Those who failed to achieve CR by induction therapy had higher IL-7 levels, which was not significant after Bonferroni correction (*P*=0.0913). Inferior PFS was associated with higher sIL-2Rα (*P*=0.0525). Inferior OS was significantly associated with higher P-SEL (*P*=0.0003), higher sIL-2Rα (*P*=0.0029), higher age (*P*=0.0356) and possibly with higher TNFR-1 (*P*=0.0611). Age has not correlated with any evaluated analyte. TNFR-1 correlated with TNFR-2 (P<0.0001), but not with TNF-α. The sIL-2Rα did not correlate with IL-2. Only IL-6 and ICAM-1 were significantly influenced by CRP levels.
Summary/Conclusions: Better understanding of the cancer microenvironment is a sine qua non for development of new treatment approaches. Our results show that P-SEL, sIL-2Rα and TNFR-1 may be associated with treatment outcome and thus should be further investigated as possible therapeutic targets.
The work was supported by a long-term organisation development plan 1011 (FMHS) and by MH CZ – DRO (UHHK, 00179906).

E919

MRD-DRIVEN CHOICE OF CONSOLIDATION AND MODULATION OF INDUCTION AND CONSOLIDATION INTENSITY RESULTED IN A SIGNIFICANTLY IMPROVED OUTCOME OF YOUNGER AML PATIENTS IN THE LAST THREE YEARS

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Background: In the last decades no effective new drugs have been introduced and AML induction therapy is still based on a combination of an anthracycline and cytarabine. The MRC group has, however, reported a progressive increase of cure rates in younger patients. Our group has recently showed that the outcome can be improved by a fludarabine-containing induction (FLAI5, with fludarabine administration in first course only), followed by a risk- adapted consolidation.
Aims: The aim of the present study was to evaluate if the disease free survival (DFS) and the overall survival (OS) of younger (<65 years) AML patient treated in our center had shown any modification in four consecutive periods of treatment (< 2008; 2008-2010; 2011-2013; 2014-2016) and to recognize factors possibly leading to this result.
Methods: We reviewed the outcome of 145 consecutive AML patients aged 65 or less and uniformly treated according to the above mentioned strategy. Minimal residual disease (MRD) evaluation was performed by flow cytometry (MFC), assessment of WT1 expression levels and, where applicable, evaluation of recurrent abnormalities such as NPM1 mutation.

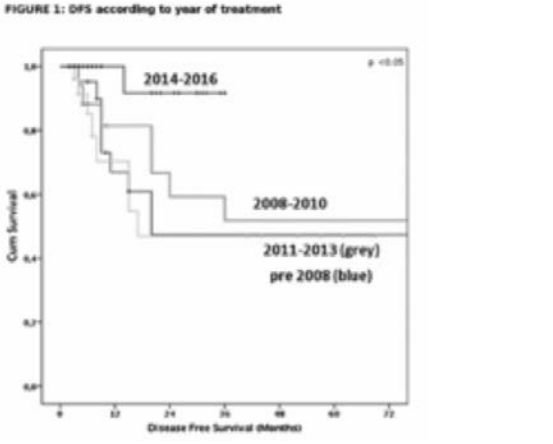

Figure 1.

Results: The cohorts of patients treated in the four periods had a comparable age and risk distribution. Notably, although the median follow up of the 4 cohorts of patients is different, patients treated in the last 3 years showed a significant improvement in DFS (Fig 1), in comparison with previously treated patients. When we reviewed our experience, we found that some changes we introduced in the therapeutic management, possibly contributed to improve outcome. Beside classical risk factors, the time from hematological recovery after the first induction (induction 1) and the start of the second induction course (induction 2) proved to be significantly related to DFS and OS probability. An interval shorter than 15 days resulted in significantly higher toxicity, whereas a time longer than 25 days was associated with an increased relapse probability. Patients being treated in the last three years had a median time from recovery after induction 1 to start of induction 2 of 17 days, compared to 22 days in the other cohorts ($p<0.05$). Furthermore, after 2013, MRD information after induction 1 was added as a prognostic factor and ELN low and intermediate risk patient with negative MRD after induction 1 were no more scheduled for early allogeneic stem cell transplant (HSCT), but received an higher dose of Ara-C in each of the three consolidation cycles (12g/sqm cumulative dose *vs* 8g/sqm). Among 8 intermediate risk patients who were MFC MRD negative post FLAI and did not proceed to HSCT in first complete remission (CR1), only one relapsed whereas among 5 intermediate risk patients who underwent HSCT in CR1 because of MRD positivity no relapses have been observed. Starting from 2014, patient in CR1 not scheduled for HSCT who showed MRD reccurrence underwent salvage therapy before overt hematologic relapse, followed by HSCT consolidation. MRD-directed therapy allowed all treated patients to achieve MRD negative remission before HSCT. Finally, the improved outcome may be associated with a reduced incidence of invasive fungal infections (IFI) due to the introduction of prophylaxis with posaconazole. The lower rate of IFI contributed to the reduction in the delay between chemotherapy courses.
Summary/Conclusions: Our experience shows that, even without the contribution of new drugs, more appropriate utilization of HSCT, tailored on early MRD assessment, MRD directed salvage therapy and posaconazole prophylaxis of IFI led to a relevant improvement of outcome.

E920

EFFECTIVENESS OF TREATMENT ACUTE MYELOID LEUKEMIA IN THE ELDERLY USING CLADRIBINE WITH LOW-DOSE ARAC

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Background: Treatment of acute myeloid leukemia(AML) in the elderly, unfit patients is a challenge for clinical hematologists. Therapeutic management in this group of patients causes intolerance and numerous complications including early deaths. A standard treatment of low dose cytarabine (LD-AraC) or using hypomethylating therapy is not satisfying enough. Polish Adult Leukemia Group's (PALG) studies showed, that addition of cladribine to daunorubicine and cytarabine increases the complete remission rate and improves overall survival in younger patients with AML. We also proved effectiveness of cladribine combined with high dose AraC and mitoxantron in relapsed and refractory AML (1, 2). Cladribine, enhances the concentration of Ara-CTP, an active metabolite of Ara-C in leukemic cells (3). Recent data indicate that cladribine has also hypomathylating properties.
Aims: The aim of our study was to evaluate the efficacy and toxicity of cladribine in combination with LD-AraC in older AML patients.
Methods: Patients with newly diagnosed AML (excluding APL), older than 60 years, unfit for standard induction chemotherapy, were enrolled to our study. The patients were given two cycles of cladribine 5mg/m^2 i.v on days 1-5 and low-dose cytarabine 40mg/m^2 s.c on days 1-10 every 28 days followed by 2 cycles of cladribine 5mg/m^2 i.v on days 1-2 with LD-AraC (40mg/m^2 s.c 1-10 days). Responding patients were treated with a prolonged maintenance consisting of LD-AraC (40mg/m^2 1-10 day). The treatment was continued until progression.
Results: Twenty-four patients have been enrolled with median age 70 years (range 62-84). In our cohort 20 patients had newly diagnosed AML, 3 secondary and 1 therapy related AML. Cytogenetic risk: good risk 5 patients, intermediate 12, poor risk 3 patients, 4 patients were unclassified. The overall response rate (CR+PR) was 84%. 13 out of 24 (54%) patients achieved complete remission (CR) and 7 (30%) achieved partial remission (PR). The median number of cycles to obtain CR was 2 (range 1-3). 16% of patients do not responded to treatment. The regiment was well tolerated without 4-week and 8-week mortality. The main reason of death were: heart failure (n=2), renal failure (n=1) and progressive disease (n=4). We didn't observe grade 3. and 4. nonhematologic adverse events. With a median time of follow-up 14 months, the median overall survival was 12 months.
Summary/Conclusions: The combination of cladribine plus low dose AraC is effective and well tolerated regiment in elderly AML patients unfit for standard chemotherapy.

E921

SMALL CUSTOMIZABLE NGS BASED TARGET CAPTURE PANELS DETECT VARIANTS IN CLINICAL SPECIMENS AT FREQUENCIES AS LOW AS 0.5%

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Background: The use of large scale hybridization panels in early stages of clinical trials for novel therapies elicits a plethora of information for targeted biomarkers. However, as theraputic targets are further characterized large panels generate an overly broad set of data, compromising sensitivity in the selected biomarker subset. Therefore, once biomarker targets are identified, the use of smaller hybridization panels can facilitate specific variant detection by analyzing specific genomic regions of interest with greater sensitivity than larger gene panels and greater breadth than that available via PCR-based assays. Modifications of laboratory methods for small scale panels allow for the maintenance of high analytic quality with finely targeted panels. Our small panels (~10kb) focus on 1-4 genes, allowing for high-multiplexing of samples on sequencers, and reduced costs/processing times without compromising accuracy.
Aims: To demonstrate the sensitivity, linearity, concordance with other assays, and clinical applications of small NGS target capture panels.
Methods: Two separate next generation sequencing-target capture assays were developed with bioinformatics software under ISO13485 design control. One panel contained 3 genes, including fms related tyrosine kinase 3 (*FLT3*); the second covers only *CD274* (*PD-L1*). Libraries were made, hybridized with baits, and sequenced using the Illumina MiSeqDx. Validation was carried out by spiking in fixed amounts of mutant DNA into wild type DNA to establish the linearity and sensitivity of the assays. Sequencing libraries were generated by capturing with baits from either one or both panels. Sequencing data was analyzed using proprietary software developed by Invivoscribe. Eight AML clinical samples were cross validated for *FLT3* mutations by this small panel, amplicon based NGS assay, and capillary electrophoresis (CE) assay.

Results: DNA from 24 cell lines was assessed using both panels, confirming variants previously detected using other methods. A validation was run on the 3-gene panel using a series of contrived samples generated from cell lines containing between 0.5% and 25% variant allele frequencies for expected variants. Initial validation indicates that these small panel assays can detect mutations down to 0.5% variant allele frequencies. Assay linearity for *FLT3*/TKD detection from 0.25% to 12.5% or for *FLT3*/ITD detection from 0.5% to 25% is excellent (R^2= 0.996 and 0.998, respectively). Average sequencing coverage was high, ranging from 5,265x to 7,680x. Comparison of *FLT3* analysis of the small panel to amplicon based NGS assay and CE, *FLT3*-ITD showed complete concordance in clinical samples - and showed a strong linear relationship between calculated VAFs,and detected ITD sizes. There was also complete concordance for *FLT3*-TKD mutations in clinical samples.
Summary/Conclusions: Small hybridization panels are cost effective in detecting low-frequency variants from smaller subsets of genes while using far less DNA than individual PCR-based biomarker assays would require. Additionally, preliminary data shows great accuracy on clinical samples. These smaller assays focus on the most pertinent genes for a targeted therapy, and have the potential to greatly assist in understanding the molecular backgrounds of responders, super-responders, and non-responders, information which can help improve patient outcomes. Developing these assays with bioinformatics using the international ISO13485 design control standards makes them suitable for regulatory approval worldwide.

E922

EFFICACY BY OUTPATIENT *VS* INPATIENT ADMINISTRATION OF CONSOLIDATION: SUBGROUP ANALYSIS OF A PHASE 3 STUDY OF CPX-351 *VERSUS* 7+3 IN OLDER ADULTS WITH NEWLY DIAGNOSED, HIGH-RISK ACUTE MYELOID LEUKEMIA

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Background: The CPX-351 liposomal formulation delivers a synergistic 5:1 molar ratio of cytarabine and daunorubicin preferentially to leukemia cells. CPX-351 has demonstrated significantly improved overall survival (OS) *versus* cytarabine/daunorubicin (7+3) in a randomized, open-label, phase 3 study in patients aged 60 to 75 years with newly diagnosed, high-risk AML (Lancet, *et al.* ASCO 2016). In contrast to the 7+3 regimen, which includes cytarabine continuous infusion for 7 days, CPX-351 is administered as a 90-minute infusion and thus has the potential to be given in the outpatient setting.
Aims: The current analysis of the phase 3 trial assessed the number of patients getting treated in the outpatient setting and their outcomes.
Methods: Enrolled patients were randomized 1:1 to receive 1 to 2 induction cycles of CPX-351 or 7+3; patients with complete remission (CR) or CR with incomplete platelet or neutrophil recovery (CRi) could receive up to 2 consolidation cycles (CPX-351: 65 units/m^2 [cytarabine 65mg/m^2 + daunorubicin 28.6mg/m^2] on Days 1 and 3; 7+3: cytarabine 100mg/m^2/day x 5 days + daunorubicin 60mg/m^2 on Days 1 and 2). The site of administration was not protocol defined.

Table 1.

	Inpatient		Outpatient	
	CPX-351	7+3	CPX-351	7+3
Consolidation 1, n/N (%)	24/49 (49)	30/32 (94)	25/49 (51)	2/32 (6)
Median OS, months	14.72	9.26	25.43	6.87
Hazard ratio (95% CI)	0.55 (0.25, 1.21)		0.10 (0.01, 1.11)	
Consolidation 2, n/N (%)	9/23 (39)	12/12 (100)	14/23 (61)	0/12 (0)
Median OS, months	Not reached	14.31	26.32	–
Hazard ratio (95% CI)	0.45 (0.09, 2.36)		–	

Results: Few patients received induction as outpatient therapy (CPX-351 n=3/153 and 7+3 n=1/151 in each cycle). A total of 49/153 patients in the CPX-351 arm and 32/151 patients in the 7+3 arm received consolidation. In contrast to the induction cycles, a substantial proportion of patients received consolidation with CPX-351 in the outpatient setting (consolidation 1: n=25/49 [51%]; consolidation 2: n=14/23 [61%]) compared with the 7+3 chemotherapy arm (n=2/32 [6.2%] and n=0/12 [0%], respectively). For the CPX-351 arm, this resulted in a reduction in the mean number of treatment days spent in the inpatient setting (consolidation 1: 1.4 days; consolidation 2: 1.2 days) compared with 7+3 (5.3 and 5.5 days, respectively), and a reduction in the mean percentage of time spent by patients treated with CPX-351 consolidation in the inpatient setting (consolidation 1: 47.6%; consolidation 2: 39.1%) compared with the 7+3 arm (93.8% and 100%, respectively). CPX-351 consolidation was associated with numerical improvement in median OS *versus* 7+3, irrespective of administration in the inpatient or outpatient setting (Table). Median OS was not diminished when CPX-351 was administered in the outpatient (consolidation 1: 25.43 months; consolidation 2: 26.32 months) *versus* inpatient setting (consolidation 1: 14.72 months; consolidation 2: not reached). CPX-351 was associated with a manageable safety profile that was generally comparable to that of 7+3 chemotherapy, with no clinically meaningful differences in the rates of grade ≥3 treatment-emergent adverse events, including febrile neutropenia (68% *vs* 71%, respectively) and infections (55% *vs* 51%).
Summary/Conclusions: Some patients can successfully receive CPX-351 consolidation as outpatients without diminished efficacy, potentially reducing hospitalizations associated with treatment administration.

E923

MOLECULAR GENETIC TESTING PATTERNS FOR PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA (AML) ENROLLED IN THE CONNECT® MDS/AML DISEASE REGISTRY

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Background: Recurrent mutations in AML-associated genes have prognostic value and may help guide treatment decisions. Molecular genetic testing patterns for AML in clinical practice are largely unknown. Previous results of the CONNECT MDS/AML Disease Registry (George *et al.* ASH 2016; abstract 3548) showed suboptimal adherence to WHO 2008 recommendations for AML diagnosis in a cohort of patients with newly diagnosed AML in clinical practice.
Aims: To report a detailed analysis of patterns of molecular genetic testing in patients with newly diagnosed AML in community and academic settings.
Methods: The CONNECT MDS/AML Disease Registry (NCT01688011) is a US prospective, observational cohort study of patients with newly diagnosed AML (≥55 years) or myelodysplastic syndrome (MDS). All clinical decisions are made by the treating clinicians. Data are collected, using an electronic data capture system, at screening, enrollment, and approximately quarterly throughout the duration of the patient's participation in the registry. All patients provided informed consent. Enrollment is ongoing. The current analysis evaluated the percentage of patients with AML who had undergone molecular genetic testing recommended by NCCN guidelines (*NPM1*, *FLT3-ITD*, *CEBPA*, *IDH1*, *IDH2*, *DNMT3A*, and *KIT*). Chi-square tests evaluated effects of several variables on likelihood of molecular genetic testing.
Results: Between 12 Dec 2013 and 8 Dec 2016 (data cutoff), 259 patients with AML were enrolled at 86 sites. Molecular genetic testing was reported in 67% (173/259) of patients. Likelihood of testing varied, respectively, for academic *vs* community sites (76% [70/92] *vs* 62% [103/167], *P*=.018), normal *vs* abnormal karyotype (77% [79/103] *vs* 59% [79/133], *P*=.006), age <65 *vs* ≥65 (83% [65/78] *vs* 60% [108/181], *P*=.0003), and Medicare *vs* other insurance (61% [83/137] *vs* 74% [90/122], *P*=.025). In patients who had undergone molecular genetic testing (n=173), the mutations tested varied substantially. All of the NCCN-recommended molecular genetic tests were reported in 9% (15/173) of patients, including 8% (6/79) of those with normal karyotype. Of the seven NCCN-recommended tests, *NPM1* (77%) and *FLT3-ITD* (76%) were most often reported and *DNMT3A* least often (16%).
Summary/Conclusions: Early data from the CONNECT MDS/AML Disease Registry reveal that despite molecular testing reported in 67% of patients with newly-diagnosed AML, a majority do not receive guideline-recommended testing. This prospective registry is uniquely positioned to capture changes in testing patterns as guidelines are established.

E924

PHASE 1, OPEN-LABEL, RANDOMIZED STUDY TO EVALUATE THE EFFECT OF CYTOCHROME P450 (CYP) 3A4 INHIBITION ON THE PHARMACOKINETICS (PK) AND SAFETY OF QUIZARTINIB (Q) AND ITS ACTIVE METABOLITE, AC886

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Background: Q is a potent, selective FMS-like tyrosine kinase 3 (FLT3) inhibitor currently being investigated in Phase 3 studies in AML patients (pt) with FLT3 internal tandem duplication (ITD) mutations. Early studies showed concentration (conc)-dependent QTc prolongation and identified conc of Q, but not its active metabolite, AC886, was a significant predictor of the QTc prolonging effect(Levis, *et al.* ASH 2016). Q and AC886 are both primarily metabolized by CYP3A4. Because CYP3A4 inhibiting drugs are frequently required in the

course of AML treatment, a drug interaction study was performed to assess PK when Q is co-administered with CYP3A4 inhibitors.
Aims: The primary aim was to determine the effect of ketoconazole (K), a strong CYP3A4 inhibitor, and fluconazole (F), a moderate CYP3A4 inhibitor, on PK of Q and AC886. The secondary aim was to assess the tolerability and safety of Q co-administered with K or F.
Methods: This was an open-label, randomized, parallel-group study. Healthy subjects (HS) age 18 -55 years (yr) who provided informed consent were randomized 1:1:1 to receive K 200mg twice daily (BID), F 200mg BID, or placebo (P) BID on Days(D) 1-28. A single 30mg dose of Q was administered to all HS on D8. Plasma Q and AC886 conc were measured D8-28, using a validated liquid chromatography-tandem mass spectrometry method. PK parameters were determined using noncompartmental analysis. Steady-state (SS) drug conc, following repeated once daily dosing, were predicted using non-parametric superposition. An analysis of variance (ANOVA) was performed to assess the CYP3A4 inhibitory effect of K and F on the PK.
Results: 93 HS were enrolled (31 per arm), and 89 received Q. 75% were male, median age 32 yr (18-53). Relative to Q+P, co-administration of Q+K or Q+F increased the geometric mean (GeoMean) C_{max} of Q by 17% and 11%, and GeoMean $AUC_{0\text{-}inf}$ by 94% and 20%, respectively (Table 1 below). The GeoMean C_{max} and $AUC_{0\text{-}inf}$ of AC886 were decreased by 60% and 15%, respectively, for Q+K, and were increased by 3% and 14%, respectively, for Q+F. Apparent clearance (CL/F) of Q was 50% lower and $t_{1/2}$ of Q and AC886 were 46% and 96% longer, respectively in Q+K *vs* Q+P. CL/F of Q was 17% lower and $t_{1/2}$ of Q and AC886 were 10% and 28% longer, respectively, in Q+F *vs* Q+P. AC886 is a minor component in circulation relative to Q (approximately 25%). An increase of 86% in simulated SS Q C_{max} and 96% in SS Q $AUC_{0\text{-}24}$ was predicted following repeat daily dosing of 30mg Q+K *vs* Q+P, while a modest decrease in AC886 exposure (<20%) was predicted. The most common (≥5%) adverse events were headache (7.5%) and diarrhea (5.4%), with the majority being Grade 1/2. There were no clinically significant hematology, clinical chemistry, QTc, or vital sign observations, and no deaths or serious adverse events.

Table 1.

Table 1: Statistical Comparisons (ANOVA) of Quizartinib PK Parameters

CYP3A4 Inhibitor	PK Parameter of Quizartinib	Quizartinib + Inhibitor			Quizartinib + Placebo			Ratio of Geometric LS Mean (%)	90% CI	p-value
		n	Geometric LS Mean	SE	n	Geometric LS Mean	SE			
Ketoconazole	$AUC_{0\text{-}inf}$ (ng·hr/mL)	28	18,706	1116	29	9626	564.4	194.33	(169.08, 223.35)	<0.0001
	C_{max} (ng/mL)	29	121.4	5.422	29	103.9	4.639	116.87	(105.22, 129.82)	0.0156
Fluconazole	$AUC_{0\text{-}inf}$ (ng·hr/mL)	28	11,549	689.2	29	9626	564.4	119.98	(104.39, 137.90)	0.0323
	C_{max} (ng/mL)	28	115.7	5.261	29	103.9	4.639	111.44	(100.23, 123.90)	0.093

Abbreviations: ANOVA = analysis of variance; LS = least square; SE = standard error; PK = pharmacokinetic
Note: LS Mean: Least-square mean. Ratio of Geometric LS Mean (Q + Inhibitor/Q + Placebo)

Summary/Conclusions: Co-administration of Q with K or F was well tolerated and safe. Overall, there was an approximate 2-fold increase in Q exposure when Q was co-administered with K, which is considered clinically significant. The increase in Q exposure when Q was co-administered with F was within 20% and is not considered clinically relevant. Given the relationship between Q conc and QTc prolongation, these results support reducing Q doses by approximately one-half when taken concomitantly with a strong CYP3A4 inhibitor. No dose reduction is needed when Q is co-administered with a moderate or weak CYP3A4 inhibitor. This approach has been implemented in two ongoing Phase 3 trials of Q in FLT3-ITD mutated AML.

E925

SYSTEMATIC LITERATURE REVIEW AND INDIRECT COMPARISON OF GLASDEGIB PLUS LOW DOSE ARA-C *VERSUS* A HYPOMETHYLATING AGENT FOR ACUTE MYELOID LEUKEMIA PATIENTS INELIGIBLE FOR INTENSIVE CHEMOTHERAPY

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Background: In a randomized phase 2 study, glasdegib (GLAS), an oral, smoothened inhibitor, combined with Low Dose ARA-C (LDAC), showed significantly better overall survival (OS) *vs* LDAC alone in previously untreated acute myeloid leukemia (AML) patients ineligible for intensive chemotherapy (NIC). Hypomethylating agents (HMAs), azacitidine (AZA) and decitabine (DEC) are considered current standard of care in this population.
Aims: To conduct an indirect treatment comparison (ITC) comparing OS for GLAS+LDAC *vs* AZA and DEC, respectively.
Methods: Embase, MEDLINE, Cochrane database, and conference abstracts (ASCO, ESMO and ASH) were systematically searched through 12/2016 for relevant randomized controlled trials (RCTs) of GLAS, AZA and DEC in AML patients ineligible for IC. Classical frequentist ITC using the Bucher method was used to indirectly compare OS hazards ratios with 95% confidence intervals (CI) using LDAC as the common comparator.
Results: Four studies met inclusion criteria: two studies comparing AZA to LDAC: Fenaux 2010; Dombret 2015; one study comparing DEC to LDAC: Kantarjian 2012, and one study comparing GLAS+LDAC to LDAC: Cortes 2016 (Table). Fenaux 2010 study was excluded due to population differences: baseline median bone marrow blasts at 23% in Fenaux 2010 *vs* 49% in Cortes 2016. The remaining AZA and DEC studies were generally comparable in patient baseline characteristics to the GLAS study: age and cytogenic risk: age 75/73/76 years old, poor cytogenic risk 34%/37%/39%, in AZA/DEC/GLAS+LDAC, respectively. In the ITC, with LDAC as the common comparator, GLAS+LDAC compared favorably with indirect HR for OS *vs* AZA and DEC being 0.51 and 0.56, respectively, (Table 1).

Table 1.

Interventions	Study	N	OS months	HR (95% CI)
GLAS vs. LDAC	Cortes 2016	78 vs 38	8.8 vs. 4.9	0.50 (0.33-0.75)
AZA vs. LDAC	Faunaux 2010	14 vs 20	24.5 vs. 17.0	0.37 (0.12-1.13)
AZA vs. LDAC	Dombret 2015	154 vs 158	11.2 vs 6.4	0.90 (0.70-1.16)
GLAS vs. AZA	Cortes 2016 & Dombret 2015			**0.51 (0.31-0.85)**
DEC vs LDAC	Kantarjian 2012	242 vs 243	7.7 vs. 5.0	0.82 (0.68-0.99)
GLAS v DEC	Cortes 2016 & Kantarjian 2012		-	**0.56 (0.35-0.91)**

Summary/Conclusions: Using ITC, treatment with GLAS+LDAC showed significantly better OS HR than AZA and DEC in previously untreated AML patients ineligible for treatment with IC. Limitations of current analysis include mixed IC & NIC population for the AZA trial, and mixed comparator arm of both LDAC and BSC for the DEC trial. Analyses using patient-level data matching baseline characteristics across studies may enable more robust ITC.

E926

CLINICAL OUTCOMES OF CHILDHOOD ACUTE MEGAKARYOBLASTIC LEUKEMIA: THE CHILDREN CANCER HOSPITAL EGYPT 57357 EXPERIENCE

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Background: Acute megakaryoblastic leukemia is a rare subtype of pediatric AML occurring in both Down and non-Down syndrome patients. Down syndrome patients with M7 subtype have an excellent prognosis while non-Down syndrome patients have poor outcomes. Heterogenous cytogenetic abnormalities have been described with M7 AML and the impact of different prognostic factors on outcomes is yet to be determined.
Aims: To evaluate the prognostic significance of various cytogenetic abnormalities and minimal residual disease (MRD) by flow cytometry after induction I and correlate them with the clinical outcomes of patients with acute megakaryoblastic leukemia.
Methods: We retrospectively analyzed the data of 80 non-Down syndrome patients diagnosed with M7 AML treated at CCHE between January 2007 through December 2016. Three treatment protocols were used.
Results: The median age at diagnosis was 1.7 years (range 0.2-15). The median time to diagnosis was 1 month. The overall (OS), event free survival(EFS) and cumulative incidence of relapse at 2 years were 53.4%, 42.9% and 28.4% respectively. Sixty one patients had abnormal cytogenetic abnormalities including Trisomy 19 (n=20), 13q (n=3), Trisomy 8 (n=12), Complex karyotype (n=28), t(1;22) (n=12), MLL gene rearrangement (n=9), Trisomy 21 (n=24) but none of these had an impact on outcomes. Out of the 80 patients 56 were in complete remission post induction I. Twenty two patients had MRD<0.1% after induction I. In the univariate analysis patients with MRD <0.1% post induction I had a better OS and EFS with a lower cumulative incidence of relapse however these findings did not reach a statistical significance.
Summary/Conclusions: Acute megakaryoblastic leukemia in non-Down syndrome patients have poor outcomes irrespective of any cytogenetic abnormalities. Future direction to determining tumor biology based on molecular pathways in this disease is being considered.

E927

IDENTIFICATION OF RESISTANCE ASSOCIATED CPG METHYLATION CHANGES IN ACUTE MYELOID LEUKEMIA PATIENTS UNDERGOING INDUCTION CHEMOTHERAPY

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Background: Acute myeloid leukemia (AML) is a heterogeneous disease associated with epigenetic alterations that can be targeted with demethylating agents to induce CR in a subgroup of patients. However, there are currently no predictive markers that reliably distinguish responder from non-responder patients. In this analysis we assessed DNA methylation changes in a group of refractory patients with AML treated either with the hypomethylating agent azacytidine followed by intensive chemotherapy or with intensive chemotherapy alone in order to identify the alterations and genes involved.
Aims: The exploration of whole genome methylation changes of azacytidine and chemotherapy treatment in refractory patients with AML guides treatment refinement.
Methods: Patients from the AML-AZA trial of the Study Alliance Leukemia were randomized to receive either azacytidine followed by chemotherapy or chemotherapy alone. Cells were harvested at baseline and 15 days after chemotherapy from 16 of the 105 patients receiving the combination and from four of the 109 patients randomized to receive chemotherapy only. Genome wide DNA methylation was analysed using a 450K Illumina array (Illumina, San Diego, USA). With a signature derived by differential blasts within diagnosis to day 15, patients with a reduction of blasts clustered together by methylation of all the selected CpG sites, as did those with an increase of blasts on both day 0 and 15, besides paired samples of day 0 and day 15 frequently clustering together as well. This led us to refine blast independent analyses. We excluded methylation changes correlating with the percentage of blasts (p=0.14, exploratory regression among blast change and median methylation change day 0 to day 15, each), since these are likely to reflect the increased lymphocyte counts on day 15 in the unsorted samples used for analysis. Motifs most strongly impacted by methylation changes were detected using the Homer software (Salk institute, San Diego, USA). Methylation changes were compared between the two groups to identify the changes associated with the use of azacytidine prior to chemotherapy.
Results: In the Azacytidine plus Chemotherapy treated group, a total of 389 differentially methylated regions (DMRs), most of which were single CpGs, were identified, 176 of which were hypermethylated and 213 hypomethylated. The most highly represented hypermethylated loci were *INSM1* (p=1e-17, 6.25% of 176 DMRs), *KLF13* (p=1e-14, 7.95%), *HIC2* (p=1e-11, 5.11%), while those most commonly hypomethylated were *NRF1* (p=1e-15, 2.82% of 213 DMR's), *MYB* (p=1e-14, 3.76%) and *STAT1* (p=1e-14, 1.88%). The chemotherapy alone group yielded 7181 DMRs, 5752 of which were hypermethylated and 1429 hypomethylated. The genes most commonly hypermethylated in these patients were *EHF(ETS)* (p=1e-226, 32.79% of 5752), *CEBPE* (p=1e-90, 10.34%), and *Jun-AP1* (p=1e-45, 6.10%), while those most commonly hypomethylated were *RUNX1* (p=1e-24, 28.34% of 1429 DMRs), *TCF4* (p=1e-21, 8.40%) and *SMAD3* (p=1e-17, 1.05%). Median overall survival did not differ between the two treatment groups, with 153 days for chemotherapy and 143 days for azacytidine/chemotherapy patients.
Summary/Conclusions: Methylation changes associated with azacytidine and chemotherapy of refractory patients were particularly found in genes previously associated with cancer and AML. DNA hypermethylation was more common after chemotherapy alone. This finding suggests that DNA hypermethylation of specific loci may be associated with therapy resistance. Hence, the methylation levels were detected from the most resistant cells. Of note, upon Azacytidine treatment more hypomethylated loci were observed. This potentially indicates DNA hypomethylation in vivo.

E928

OVER-EXPRESSION OF ZEB2-AS1 LNCRNA PREDICTS POOR OUTCOMES IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) is a fatal hematopoietic malignancy with poor clinical outcomes characterized by blasts infiltrated in tissues.
Aims: To determine whether the antisense lncRNA namely ZEB2-AS1 would be associated with clinical outcomes, we assessed its expression in retrospective cohort with 62 *de novo* AML cases.
Methods: Relative quantitative real-time PCR analysis was employed for detecting levels of ZEB2-AS1. SYBR Green RT-PCR was performed, followed by obtaining relative threshold cycle normalized to reference GAPDH gene. Cell migration, invasion, proliferation and apoptosis tests were used to analyze biological phenotypes of AML cells after knocking down ZEB2-AS1 lncRNA by small interfering RNAs.
Results: Results showed that expression of ZEB2-AS1 lncRNA was prominently high and closely correlated with adverse clinical outcomes in AML patients, based on either modified MRC or ELN risk stratification system. Univariate analyses indicated that patients with higher expression of ZEB2-AS1 lncRNA had significant shorter 3-year overall survival (OS) (0% *vs* 68.2%, p=0.036) and disease-free survival (DFS) (25.0% *vs* 69.8%, p=0.039). In addition, Patients with higher expression of ZEB2-AS1 lncRNA had significant lower complete remission (CR) rate in response to induction chemotherapy (75.0% *vs* 27.3%, p=0.031). In patients with low levels of ZEB2-AS1 lncRNA, patients treated by allogenic hematopoietic stem cell transplantation had significant longer OS (3-year OS, 75.8% *vs* 28.6%, p=0.037) and DFS (3-year DFS, 81.8% *vs* 28.6%, p=0.049) compared to that of chemotherapy.
Summary/Conclusions: Moreover, knockdown of ZEB2-AS1 lncRNA could effectively inhibit invasion and migration in AML cells, which was closely associated with down-regulation of ZEB2 and up-regulation of E-cadherin. Collectively, although independent prognostic value for survivals was not rigorously determined, ZEB2-AS1 lncRNA may serve as candidate to improve conventional risk stratification system and contribute to evaluating therapeutic responses in AML. Furthermore, ZEB2-AS1 lncRNA could effectively modulate leukemic phenotypes of invasion and migration which may be the potential for targeted-therapy in AML.

E929

INTENSIFICATION OF ANTHRACYCLINE DURING INDUCTION AND CONSOLIDATION IS SAFE AND WELL TOLERATED IN OLDER PATIENTS WITH ACUTE MYELOID LEUKAEMIA

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Background: AML in the elderly is more susceptible to treatment failure. Treatment related mortality in elderly patients with AML is decreasing over time, and receiving chemotherapy of adequate intensity is important in treating AML in these patients. The optimal induction and consolidation approach for patients in this age group is yet to be established, however data from the HOVON group has demonstrated the benefit of anthracycline intensification during indcution in patients aged 60-65 years, while locally the Australian AML12 study demonstrated the value of anthracycline intensification during consolidation in younger adults. We have implemented a novel combination of intensified anthracycline in combination with infusional cytarabine (AraC) during induction and in combination with intermediate-dose AraC during consolidation.
Aims: To demonstrate the safety and tolerability and provide preliminary efficacy evidence for anthracycline intensification during induction and consolidation in older adults with Acute Myeloid Leukaemia.
Methods: A retrospective pilot study was done on 76 consecutive patients above the age of 55 years with newly diagnosed AML between January 2010 to June 2016 at Alfred Hospital, Melbourne, Australia. All received the 7+3 induction regime (AraC continuous infusion at dose of 100mg/m^2/day on days 1 to 7, and idarubicin at a dose of 12mg/m^2/day on days 1 to 3), with a planned consolidation with IDAC+2 (AraC 1000mg/m^2 twice daily Day 1, 3, 5, and idarubicin 12mg/m^2/day Day 1-2). Outcomes were assessed according to the Cheson criteria with cytogenetic risk assessed by the refined Grimwade MRC criteria.

Table 1.

Table 1 – Comparison with Lowenberg et al NEJM 2009 for patients 60-65

Parameter	7+3 / IDAC+2 (n=22) (intention to treat population)	HOVOK/SAKK (n=299)
Complete Remission	81%	73%
EFS at 2-years	20%	14%
DFS at 2-years	20%	27%
OS at 2-years	58%	23%
Early death (30-days)	0%	11%

Results: 76 patients, with a median age of 62 years (range 55.4-70.6 years) received the 7+3 induction with a median overall survival of 590 (range 6-1996) days and overall response rate was 52 patients (68.4%). The event-free survival median is 109 days (range 6-1988) and the relapse-free survival median is 314 days (range 4-1947). There were 9 treatment-related deaths (11.8%) within 30 days following 7+3 induction. Of 41 patients who attained complete morphological remission after induction, 29 patients (70.7%) received the planned IDAC+2 consolidation with 17 (41.5%) receiving two consolidation cycles. Of those not receiving IDAC+2, 10 patients (24.4%) received an alternative consolidation regimen and 2 patients (4.9%) did not receive consolidation. Of those receiving IDAC+2 25 (86.2%) were intermediate cytogenetic risk and 3 (10.7%) were adverse. No treatment-related deaths occurred during consolidation with IDAC+2. 20 patients (26.3%) from the whole cohort received an allogeneic stem cell transplant (SCT), and 8 patients (27.6%) of those who received the IDAC+2 consolidation regimen proceeded to an allogeneic SCT. In all IDAC+2

consolidation cycles, the median days to neutrophil recovery was 26 days (range 18-72), platelet recovery 32 days (range 17-75), and the ICU admission rate was 12.8% (range 2-10 days). 18 patients (62.1%) receiving IDAC+2 consolidation suffered disease relapse. For patients receiving IDAC+2 consolidation the median OS was 727 days (range 113-1614 days) with an EFS of 388 days (range 109-1614 days). For patients aged 60-65 years the remission rate and survival outcomes were similar to those published by Lowenberg et al.
Summary/Conclusions: Anthracycline intensification was well tolerated with low treatment related mortality and rates of ICU admission along with acceptable time to count recovery. In patients aged 60-65 outcomes were similar to published data with high-dose daunorubicin. Despite this intensive post-remission therapy approach rates of disease relapse were high highlighting the need for novel therapeutic approaches in this patient group.

E930

PROGNOSTIC IMPACT OF IDH1 AND IDH2 MUTATIONS IN LOW AND INTERMEDIATE RISK AML: A MULTICENTER RETROSPECTIVE STUDY
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Background: Mutations in the isocitrate dehydrogenase 1 and 2 (*IDH1* and *IDH2*) genes are common in acute myeloid leukemia (AML) but, although investigated in several studies, their prognostic significance still remains controversial.
Aims: To evaluate the prevalence and prognostic impact of IDH1 and IDH2 mutations in adult AML patients with low and intermediate-1 and 2 risk (European Leukemia Net, ELN 2010).
Methods: We retrospectively evaluated IDH1 and IDH2 mutations in 99 low and intermediate risk patients with new diagnosed AML who underwent intensive induction chemotherapy in three Italian centers.
Results: Median age for all patients was 60 years. IDH mutations were detected in 25% of our patients. 7% were IDH1 R132, 16% were IDH2 R140 and 2% R172. Median WBC count was 12,66x10^9/L in IDH wild-type, and 24,71 x10^9/L in IDH mutated. Absolute neutrophil count was 3,1x10^9/L in IDH wild-type and 0,9x10^9/L in IDH mutated, and the difference was statistically significant (p<0,001). Median bone marrow blasts, platelets count, and LDH did not differ significantly. Cytogenetic risk group according to ELN 2010 showed favorable risk in 31,4%, and intermediate (I and II) risk in 68,6%. In favorable risk group IDH mutated patients were 12%, and 13% in the intermediate risk group. IDH expression was significantly correlated neither with NPM1 mutation nor with FLT3 mutation. There were no significant differences between induction therapies in IDH mutated and unmutated patients (overall 3+7 "like" regimens in 57 patients, FLAI like regimens in 42). There were not significant differences in CR rate after induction therapy, OS and PFS between IDH mutated and unmutated patients. Median OS was 595 days in IDH mutated and 467 in IDH wild-type (p=0,67) Median PFS was 319 days in IDH mutated and 406 days in unmutated (p =0,157). We further analyzed the impact of IDH mutation in different cytogenetic risk groups: OS and PFS were not significantly different between mutated and unmutated low cytogenetic risk patients. In intermediate I and II risk patients PFS was significantly different (177 days in IDH mutated *vs* 406 days in IDH wild type, p= 0.002), but OS was not. We then evaluated patients with normal karyotype and mutated NPM1: IDH mutations had no impact on OS and PFS.
Summary/Conclusions: In this multicenter retrospective study we found that lower absolute neutrophil count at diagnosis is significantly correlated with IDH mutations as already confirmed by other groups. In terms of prognosis we only demonstrated an advantage in PFS for umutated intermediate risk patients, suggesting a negative prognostic impact of the mutations which need to be confirmed in further studies.

E931

DECITABINE COMBINED WITH HAAG REGIMEN IS AN EFFECTIVE SALVAGE TREATMENT FOR ADVANCED ACUTE MYELOID LEUKEMIA
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Background: As relapsed or refractory acute myeloid leukemia (AML) are known to have poor prognosis, achieving complete remission (CR) and long-term survival become extremely challenging for these patients. Reasons for the dismal prognosis rely on more frequent resistance to conventional chemotherapy and higher treatment-related mortality. Therefore, novel therapeutic approach to treat these patients were developed.
Aims: To evaluate the clinical efficacy and safety of decitabine (DAC) in combination with HAAG regimen [homoharringtonine (HHT), cytarabine (Ara-C), doxorubicin (Acla) and recombinant human granulocyte colony stimulating factor (G-CSF)] for advanced patients with AML.
Methods: hirty-six patients with advanced AML receiving DAC combined with HAAG chemotherapy in our center from December 2012 to August 2015 were enrolled in this study. Eighteen of them were refractory or relapsed AML, and another 18 patients were those who didn't achieve CR after a course of induction chemotherapy. The therapeutic responses, side effects and long-time survival were retrospectively analyzed.
Results: After a course of treatment, the rate of CR and partial response (PR) was 58.3% (21/36) and 22.2% 8/36 respectively, while the overall response rate (ORR) was 80.6% (29/36) in the cohort. For the patients with refractory or relapse AML, CR was 61.0% (11/18), PR was 22.2% (4/18), and ORR was 83.3% (15/18). While for the other not getting CR after a course of induction chemotherapy, CR was 55.6% (10/18), PR was 22.2% (4/18), and ORR was 77.8% (14/18). Grade 4 hematological toxicities were observed in all patients, and 72.2% cases experienced infection. And all non hematological side effects were mild and well-tolerated. With a median follow-up of 7.5 (0.5-33.3) months, the 1-year overall survival (OS) rate was 43.3%, 24.2% for the refractory or relapsed AML patients, and 61.6% for those not achieving CR after a course of induction chemotherapy. The difference was significantly (*P*=0.01).
Summary/Conclusions: DAC combined with HAAG regimen is safe and effective salvage treatment for advanced stage AML patients.

E932

LESS-INTENSIVE TREATMENT LEADS TO DECREASED SURVIVAL IN UNMARRIED ACUTE MYELOID LEUKEMIA PATIENTS AND PATIENTS LIVING ALONE. A DANISH NATIONAL POPULATION-BASED COHORT STUDY
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Background: Marital status has been found to affect leukemia survival. Still, lack of individual-level socioeconomic data, cohabitation status, and treatment information prevented further investigation of underlying mechanisms. As treatment is changing towards outpatient-care, effects of social support may become even more important.
Aims: We investigated whether and how cohabitation and marital status affect chance of intensive remission-induction chemotherapy and allogeneic stem cell transplantation (HSCT), treatment response, and survival in acute myeloid leukemia (AML) patients using individualized socioeconomic and clinical data from Statistics Denmark and The Danish National Acute Leukemia Registry.

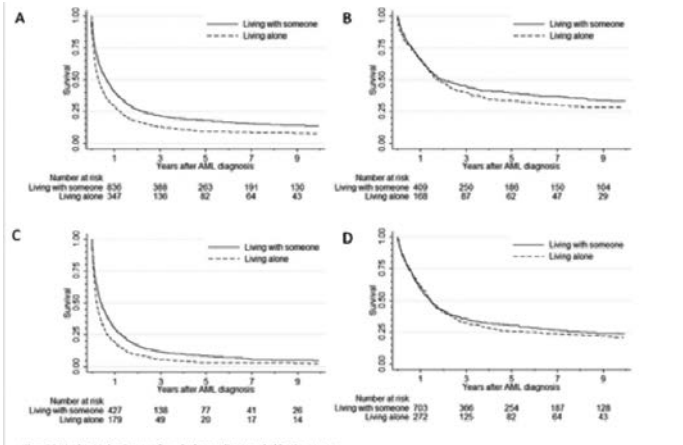

Figure 2. Kaplan Meier Curves of survival according to cohabitation status
Crude survival curves for 3243 patients by cohabitation status. In all patients (A), in patients <60 years (B), in patients ≥60 years (C), and in all patients selected for intensive therapy (D)

Figure 1.

Methods: We conducted a nationwide population-based cohort study and included all AML patients >25 years diagnosed in Denmark between 2000-2014 (follow-up ended Feb 2016). We compared chance of intensive chemotherapy, complete remission (CR) and chance of alloHSCT in CR1 by cohabitation and marital status (logistic regression, odds ratios; ORs). We used Cox regression (Hazard ratios; HRs) to compare survival. To help explain underlying mechanisms, results were given sequentially adjusted for: age, sex, income, education and occupation, and, additionally for clinical prognostic

markers. Results were given overall and stratified by age (<60/≥60 years) and sex. Kaplan Meier curves and Cox regression (Hazard ratios; HRs) was used to compare survival by cohabitation (living with someone, living alone) and marital status (married, divorced, widowed, unmarried).
Results: The study included 3243 AML patients. Patients living with someone (n=2056) were younger, more likely to be married, male, to be working, and to have a higher education than patients living alone. Comorbidity, white blood cell count, lactate dehydrogenase, and blast counts did not differ between groups, however patients living with someone tended to have better performance status at time of diagnosis. Patients living with someone were more likely to receive intensive chemotherapy than patients living alone when aged 60 years or older (41.2% *versus* 22.8%, adjusted OR 0.61 (CI=0.46-0.81)). In patients <60 years, never-married patients were less likely to receive intensive therapy (adjusted OR 0.43 (CI=0.19-0.99)) than married patients. In patients <70 years achieving CR, the chance of alloHSCT was reduced when living alone (11.8%, adjusted OR 0.47 (CI=0.28-0.78)), *versus* 19.0% in patients living with someone. In divorced/widowed, the chance was also reduced (7.6%, adjusted OR 0.38 (CI=0.20-0.74)) compared to married patients (19.3%). Crude survival by cohabitation is shown in Figure 1. Overall survival was inferior in patients ≥60 years living alone (adjusted HR 1.21 (CI=1.09-1.33)) and unmarried patients (never-married: adjusted HR 1.29 (CI=1.06-1.57), divorced/widowed: adjusted HR 1.11 (CI=1.00-1.23)) compared to married patients. In contrast, cohabitation and marital status did not affect treatment response (living with someone: CR 70.6%, living alone: CR 72.8%) or overall survival (adjusted HR 1.08 (CI=0.81-1.23)) in intensive therapy patients only.
Summary/Conclusions: Our study results indicate, that the effect of cohabitation and marital status on AML outcome, especially in patients ≥60 years, is explained by social support rather than by differences in income and occupation. Patients living alone do not present with more advanced disease or higher comorbidity burden than patients living with someone. Still, patients living alone and never-married patients are less likely to receive intensive chemotherapy affecting overall survival. Increased focus on what drives treatment decisions in patients lacking social support is important to improve survival in these patients.

E933

TREATMENT OF MOLECULAR RELAPSE IN ACUTE MYELOID LEUKEMIA WITH MUTATED NPM1 REDUCES TOXICITY OF SALVAGE TREATMENT AND IMPROVES DISEASE CLEARANCE

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Background: Acute Myeloid Leukemia with mutated NPM1 (NPM-AML) is characterized by a favorable prognosis. Most patients achieve hematological complete remission (CR) and are not considered eligible for an early allogeneic stem cell transplantation (HSCT). The importance of longitudinal NPM1 minimal residual disease (MRD) monitoring in NPM-AML is well recognized but no data are currently available on MRD-directed therapy in this AML subset. Since 2004 we have prospectically evaluated NPM1 MRD at precise time points to evaluate response to therapy and predict the risk of hematological relapse (HR).
Aims: The aim of this study was to set a standardized operative definition of molecular relapse and to evaluate the efficacy and feasibility of MRD-directed salvage therapy.
Methods: From January 2004 to January 2014, 36 consecutive younger intensively treated patients with NPM-AML achieving CR were included in the study. MRD assessment was performed on bone marrow (BM) samples after 1st and 2nd induction cycle, after each of the three consolidation cycles and then every three months for five years. If MRD positivity was found, a new analysis was to be repeated in 15 days. NPM1 mutation was measured on BM samples using MutaQuant® kit Ipsogen® from Qiagen. All Real-Time PCR were performed on DNA Engine Opticon 2. Until 2014 our policy included the treatment of leukemia in hematological relapse (HR). Salvage chemotherapy consistied in two MEC cycles and then patients proceeded to HSCT, if feasible. From January 2015 we decided to use a pre-emptive strategy, treating the molecular relapse. Four consecutive NPM-AML patients who showed MRD relapse received MRD-directed therapy so far, which consisted of one cycle of MEC.
Results: Among 36 patients, 13 showed HR, after a median of 24 months (range 14-52). All relapsing patients showed NPM MRD reccurrence prior to HR, with a median time of 4.5 months (range 1-8.4) from the first recurrence. Based on these data we defined MRD relapse as the reccurrence of NPM1 mutation, confirmed in 3 consecutive bone marrow samples repeated every 15 days, with a total increase of NPM1 expression levels of at least 2 logarithms (i.e at least from 0 to $100/10^4$Abl). All patients who fulfilled criteria for MRD relapse progressed to HR within 3 months (range 1.5-3). All 13 patients treated in HR received two cycles of MEC. Eight (62%) patients obtained hematological CR, and one patient died during therapy. Complete NPM-MRD clearance was achieved in 4/13 patients (31%). Starting from January 2015, 4 patients who met the MRD relapse criteria received pre emptive therapy, consisting of a single course of MEC. Four consecutive patients have been treated so far. Pre-chemotherapy and post-therapy disease burden, assessed by NPM levels, was significantly lower than in patients treated in HR (p <0.001, Figure 1). Both hematological and non-hematological toxicity was significantly lower than in patients treated in HR. Notably, all patients were able to achieve complete MRD clearance before HSCT and are alive and well at the time of the analysis.

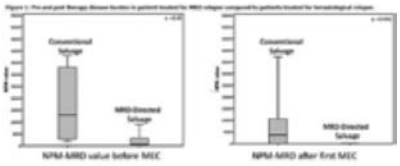

Figure 1.

Summary/Conclusions: Despite the good overall prognosis, a significant proportion of NPM-AML patients will relapse. Our preliminary data strongly support the feasibility and efficacy of MRD-directed therapy in NPM-AML. This strategy reduces the toxicity related to re-inductin and increases the proportion of patients achieving a MRD negative CR.

E934

MINIMAL RESIDUAL DISEASE AND LAIP CHANGES BY FLOW CYTOMETRY IN DE NOVO ACUTE MYELOID LEUKEMIA DURING CHEMOTHERAPY AND CLINICAL OUTCOMES

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Background: Minimal residual disease (MRD) detection by multicolor flow cytometry (MFC) in acute myeloid leukemia (AML) is widely explored by different researchers and it is an additional independent factor in clinical outcomes. The prognostic value of leukemia associated immunophenotype (LAIP) changes in not explored enough.
Aims: To investigate the amount and clearance of MRD reduction and LAIP changes in *de novo* AML during chemotherapy and compare the results with clinical outcomes.
Methods: In clinical prospective study since March 2016 till February 2017 50 patients (pts) *de novo* AML (f/m 32/18 m. age 44 (17-85)) were included. 14 pts by this moment completed basic chemotherapy (ChT) courses: «7+3» 2 induction and 2 consolidation. Among them favorable cytogenetics was in 4pts (t(16,21)-1, 16q22-1, t(8,21)-2pts), intermediate-7 (6-with normal caryotype, 1-t(17,22)), poor–3 (complex karyotype-2, 11q23-1pt). Bone marrow samples were studied in standardized panel with most common antibodies by 6-color MFC (BD FACSCanto II, USA) before the treatment, after 1st and 2nd courses of induction and after 2nd consolidation. Any amount of MRD >0 was assumed as MRD positivity. Besides MRD status we also explored LAIP changes in patients with CMR after 2nd ChT and in drug resistant cases.
Results: Leukemia associated immunophenotype (LAIP) was detected in all monitored patients at the diagnosis. Molecular markers were detected in 28,5% (2pts-with NPM1+FLT3+CEBPA+, 1-with FLT3+, 1-NPM1+). 2 pts had resistant AML after 2 courses (DR). 3 pts out of 7 with complete morphological remission (CMR) after 1st course had MRD positivity (0,03%, 1,61%, 8,3%), and these pts became MRD-negative after 2nd course. CMR was achieved after 2ndcourse in 5 more pts and MRD positivity was detected in 3 pts (0,033%, 0,523 and 3,9%) with intermediate cytogenetic risk. By the end of 4th course 11 pts stayed in CMR and we diagnosed 1 morphological relapse (patient with MRD-negativity and CMR after 2nd ChT). Two early relapses were also traced: both with persistent MRD during all period of ChT and CMR after the second ChT. All pts with MRD-negative status after first course are alive and in CMR (8 months from diagnosis). While monitoring, LAIP changes were distinguished in 7 pts. One from two with resistant AML lost CD65, another one acquired CD11b. 5 pts were in CMR after the second course and during ChT one of them gained CD56 and CD13, 2nd- lost CD65 and CD11b, 3rd – gained CD65, 4th gained CD11b after 2nd ChT, the last one didn't change LAIP. We detected relapse in 3 pts from this group and one – with increasing MRD after 4th course and cytopenic syndrome. We may suggest that LAIP changes during ChT reflect selection of more chemoresistant leukemia clone, followed by subsequent relapse.
Summary/Conclusions: 1. The most favorable group consisted of MRD negative pts after 1st course 2. LAIP changes are common in pts with less favorable prognosis.

E935

LENALIDOMIDE MAINTENANCE IN PATIENTS WITH HIGH RISK ACUTE MYELOID LEUKEMIA

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Background: New drug combinations and higher intensity therapy have led to significant improvements in complete remission (CR) rates for patients with acute myeloid leukemia (AML). However, relapsed disease remains a major source of failure. With the exception of allogeneic stem cell transplant (SCT), there are few options for post-consolidation maintenance of remission in high-risk patients. NK cells as part of the immune microenvironment are important mediators of immune surveillance in AML. Lenalidomide has demonstrated single-agent activity in AML and enhances NK cell activity and immune synapse formation in leukemia.
Aims: We designed a phase II clinical trial studying the efficacy of lenalidomide as maintenance therapy in AML patients with high-risk disease in remission, who were not being considered for SCT.
Methods: AML patients ≥18 years with a high-risk feature in 1st CR (CR1) or any patient in 2nd CR (CR2) who had received induction and at least 1 consolidation cycle were eligible for enrollment. Patients should be within 12 months of achieving CR, have PS ≤3, adequate kidney/liver function, ANC >0.5 and platelets ≥30. Patients were treated continuously with lenalidomide 10mg PO daily on D1-28 of a 28 day cycle for up to 24 cycles. All pts had baseline cytogenetic and molecular testing, and minimal residual disease (MRD) assessment by flow cytometry. After cycle 1, stepwise dose escalations were allowed to 20mg daily in pts who were tolerating their dose and have presence of minimal residual or morphologically detectable disease.

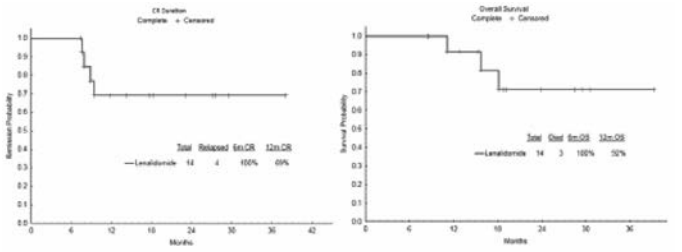

Figure 1.

Results: A total of 14 patients have been enrolled with a median age of 57.5 years (range, 23-67). All pts were in CR at the time of enrollment, with 12 pts (86%) in CR1 and 2 (14%) in CR2. Baseline pt characteristics are outlined in table 1. AML-related mutations detected at start of therapy include: CEBPA (n=5), NPM1 (3), FLT3 (3), IDH2 (2), NRAS (2), DNMT3a (2), and 1 each of JAK2, TET2, and EZH2. High risk features at the time of enrollment were as follows (some are overlapping): 5 (36%) with history of prior myeloid neoplasm or therapy related AML, 4 (29%) persistent MRD, 4 (29%) adverse mutational profile, 2 (14%) adverse karyotype, 1 (7%) primary refractory disease, and 2 (14%) CR2 status. Patients have received a median of 9 cycles (1-24) cycles of therapy. With a median followup of 19+ months (8.5-39), the 6- and 12-month estimated RFS were 100% and 69%, respectively. The 6- and 12-month estimated OS were 100% and 90%, respectively (Figure 1). The regimen was well tolerated. Cytopenias were mild and managed with dose adjustments. The most common grade 3 (no grade 4 toxicity) non-heme toxicities were 1 each of rash, fatigue, cough, and nausea, vomiting, and stroke.

Table 1.

Characteristic	Median (range)
Age	58 (23 - 67)
WBC [x10^9/L]	4.9 (2.5 - 9)
Platelets [x10^9/L]	119 (52 - 213)
LDH	459 (291 - 1125)
Albumin	4.2 (3.3 - 4.7)
Bilirubin	0.6 (0.2 - 1.1)
Creatinine	0.8 (0.4 - 1.2)

Summary/Conclusions: Lenalidomide is a safe and feasible maintenance strategy in high-risk AML patients who are not candidates for SCT. The study continues to surpass the pre-specified expected rate of relapse-free survival of high-risk patients based on a historical cohort. Studies evaluating dynamics of MRD on study are ongoing.

E936

POSTREMISSION THERAPY FOR AML WITH INTERMEDIATE RISK CYTOGENETICS IN FIRST COMPLETE REMISSION

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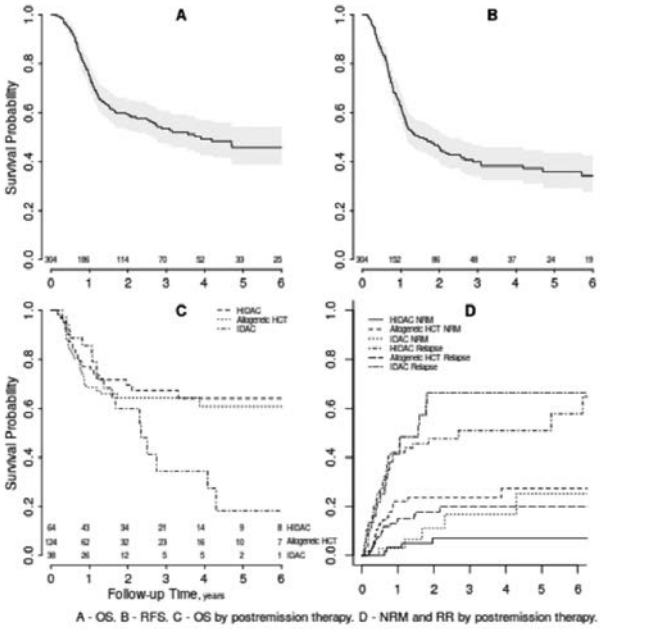
Background: Postremission therapy of AML with intermediate risk cytogenetics in first CR is based on chemotherapy with high dose cytarabine (HIDAC) or hematopoietic cell transplantation (HCT). Evidence from single trials with regards to optimal postremission therapy has been inconclusive, metaanalyses suggest a survival benefit of allogeneic HCT in first CR, except for patients with mutation of NPM1 without concomitant FLT3/ITD.
Aims: We analyzed retrospectively data from patients with AML with intermediate risk cytogenetics in CR1 with the aim to determine rates of completion of postremission therapy, rates and risk factors for early relapse and non relapse mortality (NRM), overall survival (OS) and relapse free survival (RFS) according to postremission treatment and describe causes of and risk factors for treatment failure.
Methods: Data on 304 patients in CR1 treated with curative intent between 2007 and 2016 in four centers participating in Czech Leukemia Study Group for Life were analyzed. All patients signed informed consent with data collection, analysis and publication. Cox regression was used to determine risk factors for OS and RFS, using time dependent covariates for postremission therapy. Age, WBC count, number of induction cycles, NPM1 mutation, FLT3/ITD, performance status, BMI, previous malignancy and extramedullary disease were included in models. Postremission therapy was completed after HCT or after three cycles of HIDAC without HCT in patients <= 60 years or two cycles of intermediate dose cytarabine (IDAC) in patients>60 years. Competing risk cumulative incidence estimates were calculated for NRM and relapse. Early relapse and NRM were defined as relapse/NRM before completion of postremission therapy.



A - OS. B - RFS. C - OS by postremission therapy. D - NRM and RR by postremission therapy.

Figure 1.

Results: Median age was 52(18-74) years. Median follow up time was 481(31-3384) days. Early relapse rate (RR) and NRM were 11.01% and 5.29%, respectively. Median OS after early relapse was only 128 days. Presence of FLT3/ITD mutation and high body mass index were associated with increased risk of early relapse on multivariate analysis (HR 14.88, 95%CI 3.24-68.43 and 2.34, 95%CI 1.3-4.2, respectively). Age increased risk of early NRM (HR 5.13, 95%CI 1.5-17.58 for age 55:35 years). 76% of patients completed therapy: 42% received allogeneic HCT in CR1, 21% completed three cycles of HIDAC and 13% completed two cycles of IDAC. 3-year OS and RFS of the whole cohort were 53.68% and 40.26%, respectively. OS was 67% in a group of patients who completed HIDAC, 34% in IDAC group and 64% in HCT group (p=0.28469). Cumulative incidence of NRM and RR 3 years after completion of therapy were 23% and 20% after HCT, 7.13% and 51% after HIDAC and 16.8% and 66.4% after IDAC, respectively, differences among groups were significant (p=0.00947 and p<0.00001). HCT reduced the risk of relapse in comparison to chemotherapy (HR 0.51, 95%CI 0.3-0.85). RFS was adversely influenced by concomitant FLT3 ITD/NPM1 mutation (HR 2.17, 95%CI 1.06-4.45). Increasing age had negative effect on OS (HR 1.65, 95%CI 1.13-2.42 for age 55:35 years). After HCT, HLA mismatch and TBI based myeloablative conditioning were associated with increased NRM [HR 6.32 (95%CI 1.89-21.14) and 6 (95%CI 1.88-19.2), respectively] in comparison to transplantation from HLA matched donors and busulphan based myeloablative conditioning.
Summary/Conclusions: The majority of patients within intermediate cytogenetic group in our analysis received allogeneic HCT. Patients who relapsed before completion of treatment had dismal outcome with very short OS. Allogeneic HCT decreased risk of relapse but led to increased NRM, reducing positive effect of HCT on OS. Risk of NRM was increased after TBI based myeloablative conditioning and after HCT from mismatched unrelated donors.

Supported by Ministry of Health of the Czech Republic, grant nr. 15-25809A.

E937

LONG TERM FOLLOW UP OF PATIENTS OVER 60 YEARS TREATED WITH INTENSIVE CHEMOTHERAPY FOR ACUTE MYELOID LEUKEMIA AND MYELODYSPLASTIC SYNDROMES

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Background: More and more data on patients over the age of 60 years treated with intensive chemotherapy are emerging, however, long term data with patient outcome after the initial 2-5 years are lacking. In 2007, we published a single center study on patients over the age of 60 years, suffering from acute myeloid leukemia (AML) or high risk myelodysplastic syndrome (MDS), treated with intensive chemotherapy (Knipp *et al.* Cancer 2007, 110:345-52). We now present long term follow up data of these patients, the first patient being treated in 1991, meaning 26 years ago.
Aims: To characterize the longterm outcome of elderly AML and high risk MDS patients treated with intensive chemotherapy after the usual 2-5 year follow up period.
Methods: We treated 160 patients aged 60 years or more suffering from high risk MDS and AML with intensive chemotherapy regimen between 1991 and 2004. None of the patients underwent allogeneic stem cell transplantation afterwards. We now performed a follow up of the surviving patients 10 years after publication of the initial study.
Results: In the initial study median survival from the start of induction therapy was 9.5 months (10 days to 157 months), with the median survival from diagnosis of 14 months (1 day to 157 months). At publication of the study in the year 2007, 20 patients were still alive, 18 of them presented with a low risk karyotype. 13 of these patients were in complete remission and 7 patients had relapsed. Since then 11 of the 13 patients who were in CR relapsed and died of their leukemia. One patient died of other causes and only one patient is still alive and well, currently at the age of 84. This patient initially presented with a normal karyotype, too. As a result the rate of long term survivors 5 years after treatment is 5.6% only.
Summary/Conclusions: Long term follow up data of elderly patients treated for AML and MDS with intensive chemotherapy is scarce. Our data show, that induction chemotherapy not followed by allogeneic stem cell transplantation does not result in a meaningful improvement of outcome. In addition, morbidity and lack of quality of life has to be taken into account. More data and studies on this subject are urgently needed in an aging population. In our population of 160 treated patients, 158 died of their leukaemia, only one patient died of another cause and only one single patient is still alive and well over a decade later.

E938

FLAG-IDA FOR RELAPSED/REFRACTORY ACUTE MYELOID LEUKAEMIA: A SINGLE CENTRE 5-YEAR STUDY

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Background: The treatment of relapsed/refractory Acute Myeloid Leukaemia (AML) remains a formidable challenge as the therapeutic options are limited. The regimen most commonly used in this setting, FLAG-Ida (Fludarabine, cytarabine, G-CSF and idaurubicin) is considered more toxic than standard Daunorubicin plus Cytarabine (DA) regimen, often associated with prolonged periods of bone marrow suppression and predisposition to severe infections.
Aims: In this study, we present a single tertiary centre experience in the use of this regimen with a view to identifying predictive factors for survival following FLAG-ida chemotherapy. The secondary aim of this project was to assess its efficacy and safety profile in the routine clinical setting.
Methods: We conducted a retrospective chart review of patients treated with FLAG/FLAG-ida chemotherapy regimen for relapsed or refractory acute myeloid leukaemia (including secondary AML) between 2011 and 2016 in a large tertiary hospital. Patients treated with FLAG/FLAG-ida as first line therapy were excluded. Important prognostic variables including age, cytogenetics, performance status, previous chemotherapy regimen, complete response rate and overall survival were collected in an anonymized format. Informed consent was obtained as part of routine clinical care.
Results: Fifty-four patients met the criteria for inclusion in this study. The median age of the patients was 53 (10-69) years. Eighteen percent (18%) received FLAG/FLAG-ida for primary refractory AML while the remainder were treated having relapsed after at least 1 previous regimen. The median time to relapse was 15 months. Complete remission was achieved in 70% of patients and 81% of these patients proceeded to have an allogeneic stem cell transplant. The median overall survival following FLAG/FLAG-ida chemotherapy was 16 months with 1-year and 2-year survival rates of 59% and 46% respectively. Approximately 6% therapy-related mortality was observed. The median overall survival in patients with early relapse (<12 months) was significantly shorter than those with late relapse (>12 months): 6 months and 20 months respectively (log-rank test p value: 0.04) (Figure 1). Complete remission rates were similar between relapsed and primary refractory AML patients.

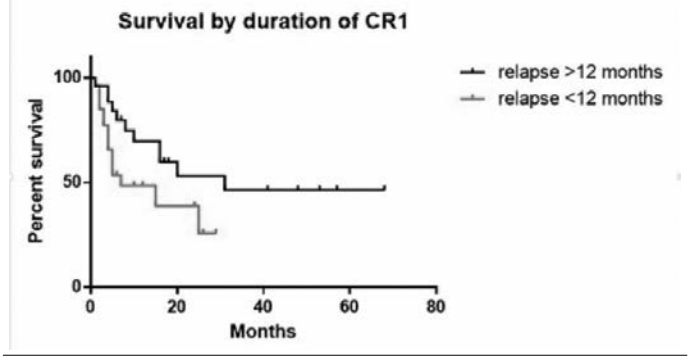

Figure 1.

Summary/Conclusions: FLAG-ida is an effective salvage regimen in patients with refractory or relapsed AML allowing the achievement of complete remission in the majority of cases. In this single-centre cohort, early relapse, within 12 months, from first line therapy was associated with an inferior survival following salvage therapy with FLAG-ida.

E939

A MULTICENTER, RETROSPECTIVE ANALYSIS OF ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA WHO WERE TREATED WITH DECITABINE

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Background: While acute myeloid leukemia (AML) is the disease of the elderly, treatment options has been limited for elderly patients. Decitabine is widely accepted as the treatment options for them. However, the efficacy has yet been evaluated in Asian population where difference of clinical manifestation or cytogenetics had been noted
Aims: In the current study, we conducted a multicenter, retrospective analysis on elderly AML patients from 8 tertiary institutes in Korea who were treated with decitabine in order to confirm whether the clinical outcomes of this agent are also acceptable in this population, and to provide further understanding of the disease nature of AML arisen in elderly patients.
Methods: Patients diagnosed with AML from 2013 to 2016 were included in the analysis. The inclusion criteria were as follows: (1) 65 or older patients with newly diagnosed, histologically confirmed AML (myeloid blast ≥20% either in the bone marrow or peripheral blood); (2) Treated with decitabine in a schedule of 20mg/m^2 for five days every 4 weeks in patients. The primary end-point of the study was OS. We compared our data to the data from another Korean retrospective analysis, in which elderly patients with AML were treated with best supportive care or intensive chemotherapy. (Int J Hematol 2014; 100: 141-151).
Results: A total of 80 patients were eligible for the analysis. The median age of patients was 74 years (range, 64 to 86 years) and 49 patients (61.3%) were male. Regarding the risk group, 6 (7.5%), 49 (61.2%), and 25 (31.3%) cases were classified as favorable, intermediate, and poor risk group, respectively. The patients had received median 3 (range 1-27) cycles of treatment and the median OS for all patients was 10.2 months. The median OS durations according to the cytogenetic risk group are as follows; 12.4 months (95% CI 11.4-13.4) for favorable risk group (N=6), 13.6 months (95% CI 8.7-18.5) for intermediate risk group (N=49), and 5.5 months (95% CI 1.4-9.6) for poor risk group (N=25) (p=.001). And when we categorized our cohort into two groups, that is ECOG-PS 0~2 *vs.* ECOG-PS 3 & 4, those with good performance status demonstrated improved survival (11.5 months (95% CI 6.6-16.4) *vs* 4.4 months (95% CI 2.4-6.4), p=.004). The OS curves according to prognostic factors are provided in **figure 1**. Next, we compared our data to another Korean retrospective analysis dealing with elderly AML patients who were treated with either best supportive care or intensive treatment. Although our cohort contains more patients with poor performance status and elderly patients, it seems that outcomes of decitabine treatment are fairly better than that of best supportive care (OS 3 months) and comparable to intensive chemotherapy (12.1 months).

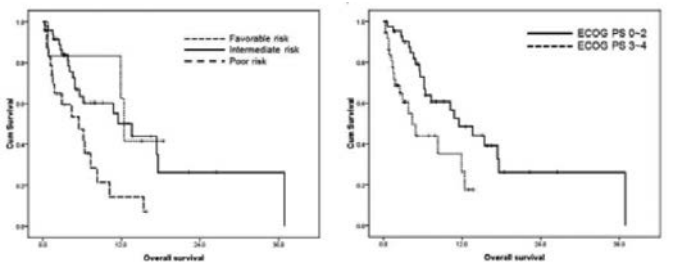

Figure 1.

Summary/Conclusions: While the treatment options for elder AML patients have been limited, our real world data suggest that decitabine could be an effective treatment of choice also in Asia

E940

DRUG-DRUG INTERACTION POTENTIAL OF GILTERITINIB IN HEALTHY SUBJECTS AND PATIENTS WITH RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA

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Background: Gilteritinib (ASP2215), a highly selective FLT3/AXL tyrosine kinase inhibitor with activity against both FLT3-ITD and FLT3-D835 mutations, is currently in development for the treatment of acute myeloid leukemia (AML). *in vitro* data suggest that gilteritinib is a CYP3A substrate as well as an inducer and weak inhibitor of CYP3A.
Aims: To evaluate drug-drug interaction potential with gilteritinib in healthy subjects and patients with relapsed/refractory (R/R) AML.
Methods: The effects of CYP3A4 inhibitors (itraconazole [ITZ] and fluconazole [FLZ]), as well as a CYP3A4 inducer (rifampin [RIF]), on the gilteritinib pharmacokinetic (PK) profile were assessed in an open-label, parallel-group study conducted in 81 healthy subjects. Gilteritinib was administered as a single 10mg dose alone on Day 6, or in combination with 200mg ITZ administered twice daily on Day 1 and once daily on Days 2–28, or in combination with once daily 400mg FLZ on Day 1 and 200mg FLZ on Days 2–28. When given concomitantly with ITZ or FLZ, gilteritinib was administered on Day 6. In an additional cohort, RIF 600mg was administered on Days 1–21 and gilteritinib was administered as a single 20-mg dose on Day 8. Additionally, the potential inhibitory effects of gilteritinib on the PK profile of a CYP3A4 substrate (midazolam) was assessed in a cohort of patients with R/R AML (n=9) in the Phase 1/2 CHRYSALIS study (NCT02014558). Patients received oral gilteritinib (300mg/d) and single oral midazolam (2mg) doses. Gilteritinib was administered on Cycle 1 Day 1 and continued once daily in 28-day cycles; midazolam was administered on Day -1 and Cycle 1 Day 15. Furthermore, in patients with R/R AML, gilteritinib trough concentration data for patients on strong (eg, voriconazole or posaconazole) or moderate (eg, FLZ) CYP3A4 inhibitors were compared with those for patients not using CYP3A4 inhibitors.

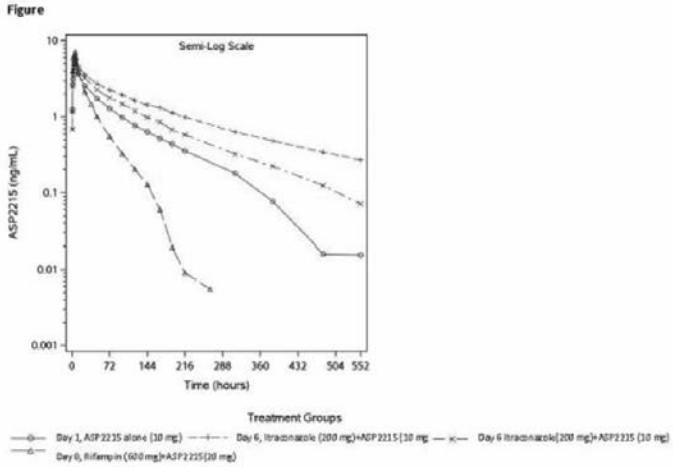

Figure 1.

Results: In healthy subjects, gilteritinib exposure (expressed as C_{max} and AUC_{24}) was higher (2.2-fold increase) in subjects who were coadministered gilteritinib with a strong CYP3A4 inhibitor (ITZ) than in subjects who were administered gilteritinib alone. Coadministration of gilteritinib with RIF, a strong CYP3A4 inducer, resulted in an approximate 70% decrease in gilteritinib exposure in healthy adult subjects (**Figure 1**). In patients with R/R AML, midazolam exposure was approximately 10% higher when administered with gilteritinib compared to midazolam alone as reflected by the geometric mean ratio and 90% confidence intervals of midazolam C_{max} (111.64%; 69.54%–179.25%) and AUC_{24} (109.46%; 49.82%–240.48%). Additionally, a <2-fold increase in gilteritinib exposure was observed in patients who were taking concomitant medications that were moderate or strong CYP3A4 inhibitors relative to patients who did not use a CYP3A4 inhibitor. The increased exposure in these patients, however, did not translate to differences in the incidence of drug-related safety events when compared across groups.
Summary/Conclusions: These data suggest limiting concomitant use of strong CYP3A4 inducers, such as rifampin, with gilteritinib. Furthermore, these data suggest coadministration of CYP3A substrates with gilteritinib is unrestricted. A comprehensive review of safety data in patients with R/R AML did not suggest that dose adjustment is warranted when gilteritinib is coadministered with strong CYP3A inhibitors. Although concomitant use of gilteritinib with strong CYP3A inhibitors (eg, ITZ or FLZ) may be permissible, precaution is warranted.

E941

A FLUDARABINE-BASED ACUTE MYELOID LEUKEMIA INDUCTION IS WELL TOLERATED UP TO 75Y OF AGE ALLOWS EARLY CONSOLIDATION AND LONG TERM SURVIVAL. A SINGLE CENTRE EXPERIENCE OF 136 CONSECUTIVE PATIENTS

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Background: For decades no effective new drugs or better anthracyclin cytarabin combinations other than the standard 3 + 7 regimen have been available for AML induction treatment. Fludarabine-based regimens have shown good efficacy in relapsed patients but raised concern about toxicity in the induction setting (Burnett JCO 2013, PMID 23940227) a modified regimen has shown better tolerance and good results in patients younger than 60 years (ys) (Guolo AJH 2016, PMID 27084986)
Aims: We report a single center, real life experience of unselected 136 consecutive AML patients treated since 2002 in our center with Fludarabine, Aracytin, Idarubicin with or without Etoposide: FLAIE up to 65ys or FLAI up to 75ys.
Methods: Patients were treated with the FLAIE or FLAI regimen followed by Idarubicin plus Aracytin as 2 step induction. Exclusion criteria for treatment were: acute promyelocytic leukemia, poor performance status and severe comorbidity. Post remission treatment included up to three cycles of high dose Aracytin, autologous (Auto) or allogeneic (Allo) stem cell transplantation according to cytogenetic and molecular risk stratification (CMR, Döhner Blood 2010 PMID 19880497) aiming for a curative strategy for all our AML patients.

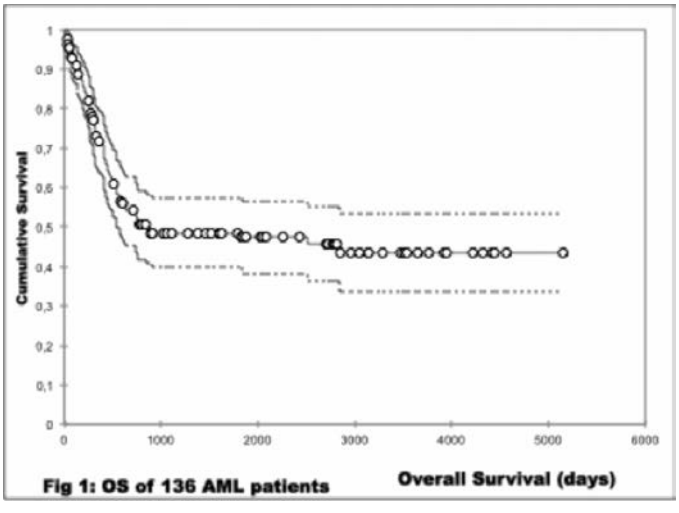

Figure 1.

Results: Median age at diagnosis was 55ys (18-75ys), median follow up was 18 months (range 3-172 months), 75% of patients (102/136) had *de novo* AML while 25% (34/136) had secondary AML mostly from myelodisplastic syndrome. 19% of patients (26/136) had good CMR risk disease, 45% of patients (61/136) had intermediate risk and 36% of patients (50/136) had high risk disease. Complete remission (CR) rate was 68% and was comparable to the majority of pub-

lished trial data, considering the proportion of high CMR risk (36%) and leukemia of secondary origin (25%) and the relatively high median age: 36% of patients (49/136) were above the 60ys old age limit of most AML protocols. In multivariate analysis CR rate was significantly affected by age below 50ys: p=0,011; good/intermediate CMR risk: p=0,011 and *de novo* AML: p=0,008. The induction death rate was 4% in line or slightly lower than published results, showing that the treatment was well tolerated; grade 3-4 non hematologic toxicity incidence was 9,6% allowing to proceed to consolidation in more than 70% of CR patients. Overall 80/136 patients (59%) were beyond 50ys, intensive condolidation with Allo or Auto was done in 34/80 patients (43%) confirming the feasibility of this therapeutic strategy. The Kaplan-Meier median probability of overall survival (OS) for the whole cohort was 28 months and factors significantly affecting OS were age below 50ys p<0,0001; *de novo* AML p<0,0003; good-intermediate CMR risk p<0,0002; intensive consolidation with Allo or Auto transplant p<0,0001 compared to chemotherapy alone. The mean probability of Leukemia free survival (LFS) was 88 months (median not reached). Patients above 50ys of age had worse outcome, the median probability of OS and LFS were 16,4 and 23,4 months respectively, this compares favorably with many published results. Chen Medicine 2016 PMID: 27472687 reported a median OS of 10,3 months in a large cohort of patients of similar age treated with intensive induction. Morevover we did not found a significant difference between the 50-59ys and 60-75ys age groups: median OS was 20,8 and 14 months (p=0,12) and median LFS was 15,9 and 23,6 months (p=0,71) respectively.
Summary/Conclusions: In our real life experience the FLAIE/FLAI regimen combined with intensive consolidation demonstrated good long term results both in terms of OS and LFS in patients younger than 50ys, this regimen was also feasible and manageble in patients beyond age of 60ys a difficult population to treat with a curative intention mainly because of concern of high toxicity of intensive induction regimens and higher incidence of poor risk prognostic factors.

E942

OVEREXPRESSION OF SOX4 CORRELATEDS WITH POOR PROGNOSIS OF ACUTE MYELOID LEUKEMIA

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Background: The SOX4 belongs to the SOX (Sry-related high-mobility group box) family and has been characterized as a transcription factor. Over the past decade, multiple functions of SOX4 have been unveiled, and the protein is now known to play important roles in embryonic development, cell fate decision, and cellular differentiation. Overexpression and amplification of SOX4 have been implicated in various cancers and are correlated with poor prognosis. In mouse models, previous studies demonstrated that the upregulation of Sox4 can be induced by and then cooperate with the aberrant expression of AML1-ETO, NUP98-DDX10, and PML-RARa; the overexpression of HOXA9, CREB, and Evi1, and the haplosufficiency of PU.1 to trigger leukemogenesis. Furthermore, a previous study that employed retroviral transduction of Sox4 and bone marrow transplantation techniques revealed that increased Sox4 expression may cooperate with the deregulation of Mef2c expression to induce myeloid leukemia in recipient mice. Sox4 gene was also reported to be as a direct target of C/EBPα. C/EBPα is known to inhibit the self-renewal of leukemic cells and to restore cellular differentiation. The overexpression of Sox4 that results from C/EBPα inactivation contributes to the development of a type of leukemia that is characterized by a distinct leukemia-initiating cell (LIC) phenotype. This work further indicated that Sox4 is a key oncogenic target and critical mediator of C/EBPα mutants in acute myeloid leukemia (AML), which suggests a potential novel therapeutic approach to the treatment of this disease. However, the clinical implications of SOX4 expression and its role of AML leukemogenesis are not well understood.
Aims: To evaluate the relationship between bone marrow (BM) SOX4 expression and clinicopathological parameters of *de novo* AML and to evaluate the prognostic value of SOX4 expression for AML patients.
Methods: From Mar 2009 to Dec 2011, a total number of 112 adult AML patients were enrolled in this study. This study was approved by the Institutional Review Board (IRB) of the National Taiwan University Hospital (NTUH) and written informed consent was obtained from all participants in accordance with the Declaration of Helsinki. Immunocytochemical staining was used to assess SOX4 expression in bone marrow leukemic cells. All statistical analyses performed for this study involved two-tailed Student's t-tests, Mann-Whitney U tests, one-way ANOVA, Chi-square test or Fisher's exact test and multivariate analysis with Cox proportional hazards regression models. Kaplan-Meier estimation techniques were used to plot survival curves and log-rank tests.
Results: We divided AML patients into two groups according to the intensity and extent of SOX4 expression as follows: low expression group (score 0-2, n=62) and high expression group (score 3-4, n=50), respectively. The various clinical manifestations of AML did not show significant differences in terms of SOX4 expression. However, AML patients with low SOX4 expression tended to have favorable-risk cytogenetic (P=0.0866). We did not observe significant differences between the high and low expression groups in terms of age, gender, hemograms, NPM1 mutation and FLT3/ITD. Additionally, of the 112 AML patients that underwent conventional intensive induction chemotherapy, 85 (75.9%) achieved complete remission (CR), and the high and low expression groups showed similar probabilities of achieving first CR (36/50, 72% *vs* 49/62, 79%, P=0.3219). However, high SOX4 expression were associated with increased relapse rates compared to low SOX4 expression (19/36, 52.8% *vs* 13/49, 26.5%, P=0.028). Furthermore, with a median follow-up period of 46.7 months (range: 0.3 to 70.9 months), SOX4 expression was associated with overall survival (OS) and disease-free survival (DFS) in all patients with *de novo* AML (P=0.008 and P=0.013, respectively), patients with non-M3 subtypes (P<0.001 and P=0.001, respectively), patients with intermediate-risk cytogenetics, (P=0.001 and P=0.005 respectively), or even in those with normal karyotype profile (P=0.022 and P=0.111, respectively). In multivariate analysis, high SOX4 expression was found to be an independent poor prognostic factor of OS (RR 1.924, 95% CI 1.020-3.628, P=0.043) irrespective of age, WBC count at diagnosis, karyotype profile and NPM1/FLT3-ITD status. A meta-analysis that we conducted using an on-line data cohort retrieved from PrognoScan (a database for meta-analysis of the prognostic value of genes; http://www.abren.net/PrognoScan/) revealed similar findings.
Summary/Conclusions: In the current study, we found that AML patients with low BM SOX4 expression had higher remission rates and longer overall survival than those with high SOX4 expression, regardless of age, WBC count at diagnosis, karyotype profile and NPM1/FLT3-ITD status. Our results also reveal that SOX4 is an independent prognostic factor of AML. In conclusion, we reveal that BM SOX4 expression could serve as an informative new biomarker for the clinical prognosis of AML patients.

E943

AN OPEN-LABEL, MULTICENTER, PROSPECTIVE, RANDOMIZED STUDY OF RECOMBINANT HUMAN THROMBOPOIETIN AS AN ADJUNCT AFTER INTENSIVE CONSOLIDATION CHEMOTHERAPY IN ACUTE MYELOID LEUKEMIA

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Background: Thrombocytopenia is a common problem in the management of patients with acute myeloid leukemia (AML) receiving induction and consolidation therapy. AML patients with platelet count of less than 20×10^9/l might have a high risk of bleeding complications and had to take dose modifications instead of intensive chemotherapy leading to increased disease-free survival and overall survival. Platelet transfusions have a short therapeutic effect and are associated with all types of transfusion reactions. Recombinant human thrombopoietin (rhTPO) has been shown to improve the megakaryocyte and platelet development in solid tumor patients and immune thrombocytopenia (ITP) patients refractory to glucocorticoid. We conducted this study to determine the availability of rhTPO in the platelet recovery after intensive consolidation chemotherapy with AML patients.
Aims: The aims of this study were to identify the effectiveness and safety of rhTPO in supportive care in patients with AML receiving consolidation chemotherapy.
Methods: *Patients:* Patients were eligible if they were 15□70 years of age who achieved completing remission after one course of IA induction therapy, and had platelet counts of less than 50×10^9/L after induction therapy, with an Eastern Cooperative Oncology Group (ECOG) performance status of 0~3. Patients with FAB M3 (acute progranulocytic leukemia) and FAB M7 (acute megakaryoblastic leukemia) were excluded from the study. All patients provided written informed consent according to protocol guidelines approved by the institutional review boards at their individual institutions. *Study design:* Patients received consolidation chemotherapy with DA, MA and intermediated-dose arabinosylcytosine(Ara-C), *et al.* When the platelet counts were less than or equal to 50×10^9/L, patients in study group received 15000u/day of rhTPO (trade name: TPIAO) administration subcutaneously and patients in control group not received rhTPO therapy. The administration of rhTPO continued until the platelet count was more than 100×10^9/L or for the maximum of 21 days. *Statistical analysis:* Baseline of patients' characteristics was summarized by using independent samples test and chi-square test. Other statistical data analyses were performed using the two-tailed Student's t test and were represented as means±SD of values. All differences were considered to be statistically significant when the P value was less than 0.05.
Results: There was no significant difference was observed in the main initial characteristics between study group (n=49) and control group (n=36), including age, gender and other baseline characteristics. No patient withdrew. Platelet transfusion and time required for platelet recovery were shown in **Table 1**. Platelet transfusions: The mean number and days of platelet transfusions for patients in study group were less than those in control group, but there were no significant differences of statistic status between the patients. Platelet recovery: 1. rhTPO might reduce the duration of platelet count less than or equal to 20×10^9/l and 30×10^9/l after chemotherapy. 2. rhTPO could increase the maxi-

mal and minimal platelet count after chemotherapy. 3. rhTPO might shorten the days of platelet count recover to at least 20×10^9/l from its nadir. The incidence of side effects were similar in both groups of the study.

Table 1.

Table 1. Platelet Parameters of Study Group Compared With Control Group

	Contral group N=36	Study group N=49	p value
Mean number of platelet transfusion(u)	23.08	19.24	0.154
Mean days of platelet transfusion	1.75	1.22	0.124
Mean minimal platelet count (10^9/l)	8.28	12.43	0.007
Mean maximal platelet count(10^9/l)	48.86	104.63	0.001
Mean duration time to platelet count <20×10^9/l(days)	9.36	5.63	0.008
Mean duration time to platelet count <30×10^9/l(days)	12.19	8.90	0.009
Mean duration time to platelet count <50×10^9/l(days)	13.92	11.63	0.061
Mean days to platelet recovery >=20×10^9/l from the lowest	5.28	3.59	0.048
Mean days to platelet recovery>=30×10^9/l from the minimal	7.15	5.98	0.259
Mean days to platelet recovery>=50×10^9/l from the minimal	7.08	7.56	0.690

Summary/Conclusions: rhTPO, administered as dose of 15000u/day when platelet count less than or equal to 50×10^9/l, might improve the recovery of thrombocytopenia of patients with acute myeloid leukemia in CR after consolidation chemotherapy. While there was no significant difference between study group and control group, there was a decreasing trend of platelet transfusion number and shorter time required for platelet transfusion for patients in study group.

E944

TREATMENT-ASSOCIATED SURVIVAL RATES IN OLDER PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML): A SYSTEMATIC LITERATURE REVIEW

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Background: AML patients ≥60 years old are more likely to experience complications following intensive induction chemotherapy and are at higher risk of unfavorable outcomes compared with younger patients. Information regarding optimal treatment approaches for older AML patients is limited.
Aims: Summarize outcomes associated with therapies among older AML patients, with a focus on treatment patterns and overall survival (OS) as reported in the literature.
Methods: Searches were conducted in Medline and Embase (Jan 2014–May 2016) and supplemented by conference abstracts (2015–2016). Eligibility included studies in English reporting on treatment regimens and outcomes associated with older AML patients or subgroups thereof, and conducted in the US, EU 5 (United Kingdom, Germany, France, Spain, Italy), or Japan. Only studies enrolling ≥50 patients were included.
Results: Twelve studies (in 19 publications) reporting on OS among older AML patients were included. Participants in most studies were newly diagnosed with AML; ages ranged from 60 to 93 years. Five non-comparative studies examining the effects of various treatment modalities were identified. Median OS in studies examining azacitidine (AZA) ranged from 10 to 12 months, whereas in studies examining induction chemotherapy or reduced intensity conditioning-hematopoietic stem cell transplantation, the median OS ranged from 6.85 months (95% CI: 3.7–13.5) to 16.4 months (95% CI: 12.6–24.6), respectively. Six comparative observational studies assessed the efficacy of different treatment regimens. Intensive chemotherapy (IC) was generally associated with longer median OS compared to other regimens. In one study, median OS for patients receiving IC, lower-intensity therapy (low dose cytarabine [LD-AraC])-(AZA, decitabine), or best supportive care (BSC) was 12.4 months (95% CI: 8.5–17.4), 11.5 months (95% CI: 9.2–13.9), and 2.6 months (95% CI: 1.9–3.1), with 3-year OS rates at 27%, 17% and 6% (p<0.0001), respectively. Another study assessed the efficacy of LD-AraC relative to IC, hypomethylating agents (HMA), and BSC. Patients appeared to have longer OS when receiving IC compared to LD-AraC (median OS: 12.4 *vs* 9.6 months; 3-year OS: 27% *vs* 12%; p=0.07), and those receiving LD-AraC compared to BSC had significantly improved OS (median: 9.6 *vs* 3.4 months, p=0.001). In this same study, while OS was longer with HMA than LD-AraC, this difference was not significant (median OS 16.1 *vs* 9.6 months; 3-year OS 22% *vs* 12%, respectively; p=0.1).Two studies assessed the efficacy of AZA *vs* moderate-IC, LD-AraC, or palliative therapy, alone or in combination. AZA had a significantly better survival rate *vs* LD-AraC in poor prognosis patients (p=0.015). Furthermore, 1-year survival was higher for AZA-treated patients (67.8%) compared to those not treated with AZA (36.9%) (p=0.004). The efficacy of AZA relative to other conventional care regimens (CCRs) including BSC, LD-AraC, or standard IC was also examined in a randomized clinical trial (n=488). Median OS at 1-year was significantly higher for AZA relative to CCR (10.4 *vs* 6.5 months). Results also showed that 1-year median OS was higher with AZA than CCR in all cytogenetic risk groups, normal risk (14.1 *vs* 10.0), intermediate risk (13.0 *vs* 10.1), and high risk (6.4 *vs* 3.2), respectively.

Table 1.

Summary/Conclusions: Among older AML patients, IC tended to be associated with improved OS compared with other CCRs. However, evidence from this review indicates that AZA could be an alternative treatment option for older AML patients, whether fit or unfit for IC.

E945

SYSTEMATIC REVIEW OF HEALTH STATE UTILITY VALUES FOR ECONOMIC EVALUATION OF ACUTE MYELOID LEUKEMIA

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Background: Cost-utility analyses undertaken to inform decision making regarding acute myeloid leukemia (AML) require a set of health state utility values (HSUVs) so that the time AML patients spend in different health states can be aggregated into quality-adjusted life-years (QALY).
Aims: This study reviews AML-related HSUVs that could be used in economic evaluation and assesses their advantages and disadvantages with respect to valuation methods used and AML clinical pathways.
Methods: Embase, MEDLINE, Cochrane database, and conference abstracts (ASCO, ESMO and ASH) were systematically searched from Jan 2000 through Nov 2016 for relevant studies that reported quality of life (QOL) and HSUV in AML. Identified relevant EORTC Quality of Life Core Questionnaire QLQ-C30 values were mapped to HDUV using previously published algorithm by Crott, *et al.* 2010. HSUV for induction, consolidation, complete remission (CR), relapse, stem cell therapy (SCT) treatment, SCT recovery and CR post SCT were identified.
Results: Ten relevant studies were identified. Six were cost effectiveness analyses utilizing HSUVs for calculation of Quality Adjusted Life years (QALY), one effectiveness analysis (incremental QALY), two QOL studies reporting specific AML utilities (either collected or mapped from QLQ-C30). An additional study reported QOL for patients undergoing SCT. Since no study reported HSUV for relapse, values from study of secondary AML patients who failed prior treatment for Myelodysplastic Syndrome, were used. Where multiple HSUVs were available, priority was made for collected values *vs* assumption. Identified HSUV are presented in Figure. AML treatment (both induction, consolidation and SCT) was associated with decreased HSUV, while post-treatment CR lead to increased HSUV.

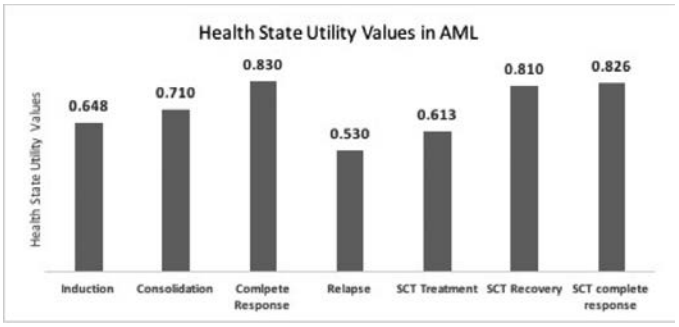

Figure 1.

Summary/Conclusions: There are relatively few methodologically robust HSUVs that can be directly used in economic evaluations concerned with AML. Careful interpretation of published values is advised considering difference in studies methodologies and patient populations. There is need to develop new HSUVs which improve on those currently available either by utilizing time trade off studies or by making greater use of condition-specific data and further use of mapping algorithms.

E946

ITALIAN REAL LIFE EXPERIENCE OF DECITABINE IN ELDERLY ACUTE MYELOID LEUKEMIA PATIENTS: INTERIM ANALYSIS OF MULTICENTRIC OBSERVATIONAL DEA65 STUDY.

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Background: Acute Myeloid Leukemia (AML) has a higher incidence among the elderly population. Older patients (pts) with AML have a worse prognosis and limited treatment options. Hypomethylating agent decitabine was recently approved by FDA and EMEA as first line treatment in AML pts older than 65 yrs and unfit to receive standard cytotoxic chemotherapy. Decitabine showed to be superior to supportive care or low dose cytarabine in controlled randomized clinical studies (Kantarjian, JCO 2012; Cashen, JCO 2010).
Aims: In July 2016 we approved a retrospective and prospective multicentric observational study to investigate efficacy and tolerability of decitabine at the approved schedule of 20mg/m^2 daily for 5 days of a 4-week cycle in real life (DEA65 study). The primary objective was the assessment of overall survival (OS). Secondary objectives were evaluation of adverse events (AEs) and response rate: complete remission (CR), CR with incomplete platelets or white blood cells (WBCs) count recovery (CRi), partial remission (PR) and hematological improvement with transfusion independence. We here present an interim analysis of the first 56 pts enrolled.
Methods: AML pts older than 65 yrs treated in first line with decitabine were enrolled in the study. At diagnosis and during follow-up, cytogenetic and molecular assessment was performed by each center according to local guidelines for AML management in elderly pts.
Results: Biologic and clinical data of 56 pts, with a median age of 73 yrs (range 65-90 yrs) are reported. Thirty-one patients (55,3%) had a secondary AML and 13/31 (42%) were progressed MDS previously treated with 5-azacitidine. Median WBCs count was 3050/µL (range 770-131500/µL) with 13/56 (23%) pts with WBCs>10000/µL. Cytogenetic analysis was performed in 52/56 pts, and in 24/56 (43%) molecular analysis including FLT3 and NPM1 mutations was performed. According to prognostication, 50% of pts had a high risk, 34% an intermediate risk, 9% a low risk AML and in 4/56 (7%) pts risk was unknown. Median OS was 7 months (range 1-19 months) with 34/56 deaths (60,7%) and a median of 6 cycles (range 1 to 19) of decitabine. Overall response rate was 60,7% (34/56 pts), of which 7/56 (12,5%) CR or Cri; 17/56 (30,4%) PR and 10/56 (17,8%) improvement of transfusion needs. According to response, median OS was 9,5 months (range 4-19) and 4 months (range 1-15) in responder *vs* not responder pts. Table 1 shows response rate according to pts characteristics. At present time 18/22 alive pts are still on treatment with decitabine. Regarding toxicity, 23/56 (41%) pts manifested a grade ≥3 AEs although severe comorbidities (cardiovascular and metabolic) pre-existed in 14/23 (60,9%). A total of 35 hospitalization episodes due to toxicity were recorded and 10/56 pts (17.8%) died due to serious AEs. Overall the most common non-hematologic AEs were pneumonia and fever.

Table 1.

Summary/Conclusions: This interim analysis of the use of decitabine in real life showed a superimposable OS to controlled international clinical trials. Safety profile was acceptable considering setting of pts and incidence of important comorbidities. Despite a similar OS, the comparison between our data and Cashen study (56 *vs* 55 pts) showed in our cohort, a poorer rate of CR+CRi with a negative impact of secondary AML, previous 5-azacitidine therapy, WBC >10000/µL as well as high cytogenetic risk. This apparent contradiction supports the idea that in elderly pts recovery of peripheral blood cells counts (PR+hematological improvement) is probably the most important factor influencing OS (Ferrara, Hemat 2016).

E947

ASPARAGINASE ERWINIA CHRYSANTHEMI EFFECTIVELY DEPLETES PLASMA GLUTAMINE, HAS CLINICAL ACTIVITY, AND IS WELL TOLERATED IN ADULT PATIENTS WITH RELAPSED OR REFRACTORY ACUTE MYELOID LEUKEMIA

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Background: Asparaginase-induced glutamine (Gln) depletion demonstrates anti-leukemic activity in preclinical studies of AML. We hypothesized that administration of asparaginase *Erwinia chrysanthemi* (Erwinaze) would lead to effective plasma Gln reduction and may be a feasible therapeutic approach for AML, because myeloblasts may be addicted to Gln.
Aims: The primary aim was to determine the dose of Erwinaze inducing plasma Gln levels ≤120µmol/L, with an acceptable safety profile, 48 hours (h) after the first intravenous (IV) dose and before each subsequent dose administered thrice weekly for 2 weeks in patients (pts) with relapsed or refractory (R/R) AML.
Methods: This was a phase 1, single-arm, pharmacokinetic investigator-initiated trial (NCT02283190, funded by Jazz Pharmaceuticals), with a 3+3+3 design with dose de-escalation/escalation rules that incorporate both safety and biochemical activity (nadir plasma Gln levels) of IV Erwinaze. There was no intrapatient dose adjustment. For safety, a 3rd cohort of three pts was to be added if 2 of 6 pts in the 1st and 2nd cohorts experience a dose limiting toxicity (DLT) at a certain dose level. If ≥3 of 9 patients experienced DLT, the trial was to be terminated. To evaluate Gln reduction ability of Erwinaze, the dose could be increased based on 48h trough plasma Gln in cohorts of 3, 6, or 9 pts per dose level. Correlative studies measured plasma Gln, glutamate (Glu) and asparagine (Asn) levels, plasma asparaginase activity and plasma and urine 2-hydroxyglutarate (2-HG) levels.
Results: Five pts were enrolled on study. Enrollment was then halted due to Erwinaze supply manufacturing complexities. Median age was 69 (range 20-83) years, 4 were male, 2 had prior MDS or CMML, 3 had high risk abnormal karyotype, 3 had isocitrate dehydrogenase (2 *IDH1,* 1 *IDH2*) mutations, and 3 had been treated with ≥2 lines of prior treatment. Erwinaze was administered IV (25,000 IU/m^2, dose level 0) for 6 doses MWF for 2 weeks to all pts. No DLT was observed. Anemia and electrolyte abnormalities were the most common adverse events. Plasma asparaginase activity ≥0.1 IU/mL was achieved in all pts at 48h trough, but in 3 pts it decreased to zero on day 8 (72h trough). Median trough plasma Gln, Asn and peak Glu levels (µmol/L) at 48h were 27.6 (range <12.5-227), 0 (range 0-0), and 704 (range 474-754), respectively. Asn remained undetectable for the entire 2 weeks. Gln levels increased significantly on day 8 (72h trough) compared to day 5, p<0.001. Four of 5 pts (80%, lower limit of 1-sided 95% CI: 34%) achieved at least one nadir Gln value <120 µmol/L. The fold reduction (FR) in Gln level at 3 days, relative to baseline, was 0.16 (p<0.001 for rejecting FR=1). One pt achieved partial remission (PR) and one achieved hematologic improvement (HI) after 6 doses of single agent Erwinaze. Both pts had plasma Gln levels <85 µmol/L on days 5, 10 and 12. Off study, after completion of Erwinaze, they have been treated with azacitidine. Both pts are still alive in complete remission (CR) and CR with incomplete count recovery (CRi) 13.3 and 13.4 months after the on-study date. Plasma and urine 2-HG levels did not change significantly. The 3 pts with *IDH* mutations tended to have higher plasma 2-HG levels (p=0.10).

Summary/Conclusions: To the best of our knowledge, this is the first clinical report demonstrating that an asparaginase product is capable of not only decreasing plasma Gln level to ≤120µmol/L but also depleting it to undetectable (*i.e.* <12.5µmol/L) levels in pts with AML. Two of 5 patients with R/R AML had clinical responses and are alive in remission. Given clinical activity of asparaginase in AML, we are to investigate mechanistically-designed asparaginase combination therapies.

E948

PROGNOSTIC SIGNIFICANCE OF SOX2, SOX3, SOX11, SOX14 AND SOX18 GENE EXPRESSION IN DE NOVO ACUTE MYELOID LEUKEMIA (AML) PATIENTS

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Background: Members of the SOX (SRY-related high mobility group (HMG) box) gene family encode a group of transcriptional factors with important functions in embryonic development. Also, *SOX* genes are aberrantly expressed in different types of cancer. However, their role in hematological malignancies, especially in acute myeloid leukemia (AML), remains elusive.
Aims: The aim of this study was to investigate the expression level of *SOX2*, *SOX3*, *SOX11*, *SOX14* and *SOX18* genes in *de novo* AML patients, and to evaluate their potential as prognostic markers.
Methods: Fresh bone marrow (BM) samples were collected from 50 non-APL AML patients at diagnosis (27 male, 23 female, median age 52.5 years, range 22-73) and from 8 healthy donors. Relative quantification analysis of *SOX* genes expression level was performed by RQ-PCR methodology, with *GAPDH* gene as endogenous control, and using comparative *ddCt* method with healthy controls as calibrator.
Results: The median expression level of *SOX2*, *SOX3*, *SOX11*, *SOX14* and *SOX18* in AML patients was 0.46 (0.01-226.13), 0.81 (0.01-1210.00), 0.35 (0.01-177.29), 0.98 (0.02-469.51) and 3.53 (0.18-332.00), respectively. This was not significantly different from the levels detected in healthy controls where the expression levels were 1.01 (0.32-2.54), 1.00 (0.45-5.73), 1.00 (0.19-2.83), 1.04 (0.38-2.38) and1.00 (0.48-12.29), respectively. As a cut-off value above which the patients were considered to be positive for *SOX2/3/11/14/18* gene expression we used median expression level of each *SOX* gene in healthy controls + 2SD. The percentage of patients who were positive for the expression of the studied genes ranged from 14% ($SOX2^+$ and $SOX11^+$), 20% ($SOX3^+$ and $SOX18^+$) to 28% ($SOX14^+$). A significant association with the presence of *FLT3-ITD* and *NPM1* mutations was detected in all but $SOX14^+$ patients. The same result was found concerning association with higher leukocyte count. There were no significant associations with any other presenting clinical parameters. As for the impact that SOX expression positive status that any of the analyzed genes had on the prognosis and outcome of the disease, we detected higher relapse rate in $SOX14^+$ patients (p=0.045). Significantly shorter disease-free-survival (DFS) was detected among $SOX2^+$, $SOX11^+$ and $SOX18^+$ patients (p<0.001; p=0.001; p=0.017, respectively). Although all of the SOX^+ patients had shorter overall survival (OS) time compared to SOX^- patients, the most prominent influence has been detected for the $SOX2^+$ patients (p=0.034).
Summary/Conclusions: This is the first study focused on examining the expression level of *SOX2/3/11/14/18* in AML patients. We have found that these genes are seldom overexpressed among patients in comparison with normal BM. However, in some patients, the expression of these genes is highly increased, and associated with a negative prognostic factors such as the presence of FLT3-ITD mutations and higher leukocyte count. Also, increased expression of these genes has been clearly associated with shorter DFS and OS. Although the exact function of these genes in the pathogenesis of AML is not yet known, our preliminary results show that their overexpression can have prominent prognostic significance in AML patients and therefore should be the subject of further investigation.

E949

ACUTE ANTHRACYCLINE INDUCED CARDIOTOXICITY IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Chemotherapeutic agents are associated with a wide range of cardiotoxic adverse effects. Anthracyclines and related drugs are some of the most implicated agents, with a well-recognized potential for the development of cardiomyopathy and heart failure. Chronic anthracycline induced cardiotoxicity can lead to cardiomyopathy, which may develop several years after treatment. Acute and subacute anthracycline induced cardiotoxicity is considered relatively uncommon, described mostly in patients treated for solid tumors or lymphomas. While anthracycline based regimens have been used to induce remission in newly diagnosed patients with acute myeloid leukemia (AML) for more than four decades, relatively little is known about the acute cardiotoxic effect of anthracyclines in this setting. Since many of these patients are candidates for hematopoietic stem cell transplantation (HSCT), an intensive intervention usually reserved for fit patients, even transient decrease in cardiac function might render them ineligible for this intervention, or might increase their transplant related morbidity.
Aims: To study the short-term outcomes of anthracycline exposure on cardiac function in patients with AML who are candidates for allogeneic HSCT. Because current AML-induction regimens use anthracyclines (most commonly daunorubicin) at a relatively high dose between 45 and 90mg/m^2/day for three consecutive days, we hypothesized that the incidence of post-induction cardiac injury in patients with AML might be high.
Methods: The medical records of 55 consecutive patients who had received induction chemotherapy and had undergone HSCT in our medical center were reviewed. Patients included in the study were those with echocardiographic data both prior to and post induction therapy. Median age at diagnosis was 59 years (range: 18-73) and 49% were males. Approximately half of the patients had *de novo* AML (N=29, 53%). 26 patients (47%) had either therapy related AML or AML secondary to a previous hematological disorder. Induction treatment included 7 days of cytarabine at a dose of 100mg/m^2/day and 3 days of daunorubicin at a dose of 45mg/m^2/day (N=2, 3.6%), 60mg/m^2/day (N=34, 61.8%) or 90mg/m^2/day (N=15, 27.3%).
Results: Selected patient characteristics are summarized in Table1. Post-induction echocardiogram studies demonstrated a significant cardiac deterioration in left ventricular ejection fraction (EF) (defined as 10% or more absolute decrease from baseline EF) in 25.5% of the patients (N=14). Higher doses (90mg/m^2/day) of anthracyclines were associated with higher rates of cardiac function deterioration (odds ratio: 4.1, 95%, confidence Interval: 1.06 to 15.7). Patients with cardiovascular risk factors and male patients tended to develop cardiotoxicity at higher rates, whereas age, white blood cell counts at diagnosis and AML type (*de novo vs.* secondary) had no impact on cardiotoxicity. The decrease in cardiac function was temporary in 10.9% of the patients (N=6) with subsequent normalization of left ventricular EF in those patients.

Table 1.

Table1. Patient characteristics

Demographics	Total		N=55
	Age, years		59 (range 18-37)
	Gender	Male	N=28 (51%)
		Female	N=27 (49%)
Cardiovascular risk factors	Any		36 (69.2%)
	HTN		21 (38.2%)
	DM		12(21.8%)
	Hyperlipidemia		24(43.6%)
	Smoker		12 (21.8%)
Cardiovascular disease	Any		10 (19.2%)
	CVA/TIA		1 (1.8%)
	PVD		2 (3.6%)
	AFib		2 (3.6%)
	s/p MI		3 (5.5%)
	s/p CABG or PCI		4 (7.3%)
	Valvular disease		2 (3.6%)
	s/p valcular surgery		1 (1.8%)
	Arrhythmia		2 (3.6%)
Cardiac parameters at baseline	LVEF (%)		60 (range 45-67)
	LVEDD (mm)		45 (range 37-55)
	LVESD (mm)		28 (range 19-42)
hematological parameters	WBC count		10K (range 0.95-169.9)
	De-novo leukemia		29 (52.7%)
	Secondary leukemia		26 (47.3%)
Anthracycline dose	90mg/m2/day		15 (27.3%)
	60 mg/m2/day		34 (61.8%)
	45 mg/m2/day		2 (3.6%)
	salvage chemotherapy		27 (49.1%)
EF change post induction	No change		38 (69.1%)
	Temporary decrease		6 (10.9%)
	Permanent decrease		8 (14.5%)

Summary/Conclusions: The use of daunorubicin at a dose of 60mg/m^2/day or less is associated with significantly lower rates of acute cardiotoxicity. Our findings should be taken into consideration when choosing the anthracycline dose, particularly in male patients with cardiovascular risk factors who are candidates for HSCT.

E950

AN INTEGER WEIGHTED GENOMIC MUTATION SCORING (IWGMS) USING THE TRUSIGHT MYELOID SEQUENCING PANEL SHOWS HIGHER MORTALITY IN PATIENTS WITH INTERMEDIATE RISK ACUTE MYELOID LEUKEMIA- A RETROSPECTIVE STUDY

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Background: AML is currently classified by European LeukemiaNet into favorable, unfavorable, and intermediate prognosis based on cytogenetic aberrations. Although favorable and unfavorable categories have good prognostic values, the intermediate category encompasses the majority of patients and offers unclear prognosis. The development of Cancer Genome Atlas (TCGA) opens new windows for the incorporation of next generation sequencing (NGS) into cytogenetics to enhance prognostic risk stratification. However, few studies explore the combination of cytogenetics and NGS in prognostic predictions.
Aims: Here we have developed a system of Integer Weights for the Genomic Mutation Score (IWGMS) for a quantifiable stratification of the prognostic risks associated with a combination of cytogenetic aberrations and genomic mutations. Our next step is validating the scoring system through its application to data obtained from other institutions.
Methods: Patient data at Houston Methodist Hospital was queried from Methodist Environment for Translational Enhancement and Outcomes Research (METEOR), a clinical data warehouse that integrates research databases and national registries. The diagnosis of AML was queried along with patient demographics, cytogenetics, NGS and OS. The resultant patients were divided into three categories based on their MRC cytogenetic risks- favorable, intermediate, and poor. Using the TruSight Myeloid Sequencing Panel (Illumina), mutations in 54 genes associated with myeloid disorders were tested in NGS. A scoring system was developed that assigned each of the nine TCGA mutation categories (Transcription- Factor Fusion, Nucleophosmin (NPM1), Tumor Suppressor Genes, DNA-Methylation related genes, Signaling Genes, Chromatin Modifying Genes, Myeloid Transcription Factor Genes, Cohesion complex Genes and Spliceosome-complex genes) a score between -2 (good risk) and +2 (poor risk). The IWGMS for each patient was calculated by the sum of the individual mutation scores. A IWGMS score greater than 3 was considered significant as a poor prognostic factor. Statistical analysis was done using Chi-Square, Mann Whitney U test and multivariate logistic regression analysis. Data from other institutions will be analyzed in a similar fashion for the confirmatory portion of the project.
Results: A hundred of the 1200 AML patients met the criteria for having both cytogenetic and NGS data availability. The two-year mortality rates were 43%, 52%, and 51% respectively for the favorable, intermediate, and poor cytogenetic groups. In the intermediate cytogenetic group, high IWGMS score (>3) was associated with higher mortality when compared to low IWGMS score (80% *vs* 44%, p=0.045, Fig 1). A look at the gene mutation distribution in the intermediate risk cytogenetic group also showed a general correlation between known favorable gene mutations with low IWGMS scores and unfavorable ones with high IWGMS scores. We thus hypothesize the IWGMS scoring system can be utilized to divide the intermediate cytogenetic group into higher and lower mortality subgroups based on a combination of cytogenetic and genetic mutations. We expect similar results with data from other institutions.

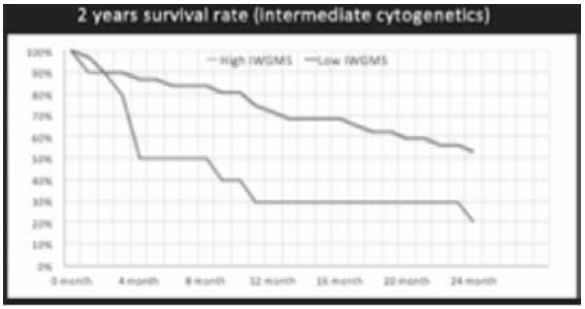

Figure 1.

Summary/Conclusions: Most studies in current literature focuses on the individual contributions of cytogenetic aberrations or genetic mutations to risk stratification and treatments risk stratification and treatment response. However, prognosis varies widely in the heterogeneous, intermediate cytogenetic class, where 60% of the AML patients belongs. We propose a systematic approach that integrates cytogenetics with genetic mutations in stratifying prognostic outcomes with a focus on the intermediate cytogenetics group. The ability to differentiate in this specific group opens great potentials for targeted therapies and improving outcomes.

Aggressive Non-Hodgkin lymphoma - Clinical

E951

SUCCESSFUL IDENTIFICATION OF SPECIFIC AMINO ACID-DEPENDENCE IN ADULT T-CELL LEUKEMIA / LYMPHOMA (ATL) AND PRECLINICAL APPLICATION FOR NEW THERAPY

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Background: Adult T-cell leukemia / lymphoma (ATL) is highly aggressive malignancy caused by human T-cell leukemia virus type 1 (HTLV-1). As leukemia/lymphoma cells are often resistant to combination chemotherapy and recent antibody therapy, new strategies should be developed. Our laboratory recently found that proliferation and survival of hematopoietic stem cells are critically dependent on the amino acid valine (Science, 2016).
Aims: We here aimed to assess amino acid-dependence of lymphoma and leukemic stem cells, and tried to establish a novel therapy by utilizing the differences in amino acid-dependence between normal and leukemic stem cells.
Methods: First, primary ATL cells were sorted from samples of 7 typical acute-type ATL patients by 12-color flow cytometry, and serially passaged on stromal cells. Then passageable ATL cells from 3 patients were transduced with GFP-expressing lentivirus for tracking and counting by image cytometry. Using complete medium and twenty different culture media each lacking a single amino acid, we examined amino acid dependency of ATL cells. Amino acids vital for ATL cells were screened by co-culture with stromal cells. Effects of these media on normal lymphocytes of healthy volunteers were also examined. Finally, the effectiveness of amino acid restriction was evaluated *in vivo* by xeno-transplantation of ATL cells into NOG mice. Mice were fed with different diets lacking specific amino acids at 6 weeks after transplantation, and sacrificed at 10 weeks for analysis of peripheral blood, organs, and lymphoma size.
Results: *In vitro* studies revealed that ATL cells have dependency on specific amino acids: cysteine, methionine, and valine. As 2-weeks restriction of the former two amino acids damaged stromal cells or normal lymphocytes, valine was picked up for further analysis. Proliferation of ATL cells was dramatically inhibited by valine restriction while the influence on normal cells was limited. Interestingly, valine restriction did not effect a significant change in the proportion of normal CD4+ populations, such as Treg, naïve, central memory, effector memory, and effector T-cells. Moreover, 4-week restriction of valine succeeded in eradicating ATL cells *in vitro* and no recurrence was observed after refilling valine although 2-weeks restriction was insufficient for extermination. In-vivo model also showed that 4-weeks restriction of valine could dramatically reduce ATL tumor size. Valine-depleted diet did not significantly reduce hemoglobin or platelet count, and there were no significant organ damages as far as examined macroscopically.
Summary/Conclusions: We discovered that proliferation and survival of adult T-cell leukemia / lymphoma cells were dependent on valine. ATL cells could be eradicated by 4-weeks of valine in vitro. In-vivo model also showed that the growth of ATL cells was significantly inhibited by dietary restriction of valine. Massive lymphoma cells, which are known to be resistant to antibody therapy, were also vulnerable to the valine restriction. There were no severe complications such as anemia, thrombocytopenia, and organ damages which are often seen in chemotherapy recipients. These data demonstrate that valine restriction may potentially provide a new option for leukemia/lymphoma therapy.

E952

VEGF AND VEGFR2 POLYMORPHISMS ARE INVOLVED IN AGGRESSIVENESS AND PROGNOSIS OF DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Angiogenesis (AG), with participation of the vascular endothelial growth factor (VEGF) and its receptor (VEGFR2), plays a key role in clinical features and outcome of patients with diffuse large B cell lymphoma (DLBCL). The ability to induce AG is variable in humans, once that *VEGF* and *VEGFR2* genes have several single nucleotide polymorphisms (SNPs) described with distinct proteins production. The wild-type alleles of *VEGF* -2578 C/A (rs699947), -2489C/T (rs1005230), -1154G/A (rs1570360), -634G/C (rs2010963), -460C/T (rs833061), 936C/T (rs3025039), and *VEGFR2* -271G/A (7667298) and -604T/C (rs2071559) SNPs determine higher production, transcriptional activity or binding efficiency of VEGF/VEGFR2.

Aims: Since the roles of these SNPs in clinical aspects, response to therapy and prognosis of DLBCL treated with R-CHOP- are still unknown, these were the aims of the present study.
Methods: Our analysis included 168 consecutive DLBCL patients at diagnosis seen at University Hospital from July 2009 to September 2014. Genotypes were identified in DNA of peripheral blood by real-time polymerase chain reaction, using a Taqman SNP Genotyping Assay. Replicates were performed in 10% of the reactions, achieving 100% of concordance. Chi-Square test, Fisher's Exact test, and multivariate analysis, using the logistic regression model, served to assess associations between genotypes and clinical aspects. Kaplan-Meier analysis was used to evaluate the effect of clinical features and genotypes on the cumulative probability of event free survival (EFS) and overall survival (OS). EFS and OS were calculated from the date of diagnosis to first event date (relapse, progression or death by disease) or last seen date and death by any cause or last seen date, respectively. The Cox proportional hazards regression model was used to evaluate the effects of clinical features and genotypes of the above mentioned SNPs on PFS and OS, and the results of analysis were presented as hazard ratios (HRs) with their corresponding 95% confidence intervals (CIs). First, these associations were examined using univariate Cox proportional hazards regression. In a second step, all variables with $P<0.10$ were included in a multivariate Cox regression. All reported P values were two-sided, and $P<0.05$ was considered to indicate statistical significance.
Results: Concerning clinical features, the frequency of the wild-types *VEGF* -1154G allele and *VEGF* -634GG genotype were more common in stage III or IV patients. The wild-type *VEGFR2* -604TT genotype was more common in high intermediate and high international prognostic index (IPI) patients. Concerning response rate, patients with the wild-type *VEGF* 936CC genotype was associated with higher complete response (CR). These patients had 2.65 more chances of achieving CR to therapy than others. The median follow-up time of 168 DLBCL patients enrolled in the study was 43 months (range: 1-105). The estimated probabilities of 60-months EFS and OS were 58.8% and 66.0%, respectively. At 60 months of follow-up, patients with the variant *VEGF* 1154 A and 936 T alleles had 1.52 and 1.82 more chances of presenting disease relapse or progression, and 1.47 and 1.60 more chances of evolving to death in univariate analysis, respectively. After correction with other classical prognostic factors in DLBCL (IPI and GCB subtype), only the *VEGF* 1154 G/A SNP was associated with PFS and OS: patients with the variant *VEGF* 1154 A allele had 1.88 and 1.83 more chances of having an event.
Summary/Conclusions: Our data present, for the first time, preliminary evidence that inherited abnormalities in AG pathway, related to the *VEGF* -1154G/A, -634GG and 936C/T, and *VEGFR2* -604T/C, influence clinical features, response to R-CHOP and outcome of DLBCL patients.

E953

BONE MARROW BIOPSY SUPERIORITY OVER PET/CT IN PREDICTING PROGRESSION FREE SURVIVAL IN A HOMOGENOUSLY-TREATED COHORT OF DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Recently, several studies have reported uneven results when evaluating the role of bone marrow biopsy (BMB) and PET/CT in the staging of diffuse large B cell lymphoma (DLBCL). The retrospective nature of the studies and the heterogeneity of the inclusion criteria might explain part of these discrepancies. Given the lack of a "gold-standard" to examine the most accurate test to assess BM involvement (BMI) at diagnosis, the prognostic value of each technique emerges as a key factor to determine the correct management at baseline.
Aims: The main aim of this study was to evaluate the prognostic impact on progression free survival (PFS) of BMI at baseline, assessed by means of BMB or PET/CT, in DLBCL with a priority in narrowing the heterogeneous inclusion criteria used in most reports to date.
Methods: This is a retrospective multicenter study including patients older than 17 years, with a BMB and a PET/CT performed simultaneously as part of the routine pre-therapy staging for newly diagnosed DLCBL. Patients had not received either chemotherapy or corticosteroids and no concomitant malignancy was known to be present at the time of both procedures. Only patients treated with R-CHOP as first line therapeutic strategy were included. Only variables with a $p<0.150$ in univariate analysis were included in the multivariate Cox regression for outcome predictors.
Results: A total of 271 DLCBL patients were initially identified; we excluded: 31 patients who received low intensity chemotherapy regimens (R-COP, Mini-CHOP-R, monotherapy with steroids) due to advanced age, comorbidities or fragility, and 35 patients enrolled in clinical trials including standard regimens plus new agents (Bortezomib, Lenalidomide, Ibrutinib) or non-standard regimens (R-CHOP/14, Da-EPOCH-R, MACOP-B, Mega-CHOP, Hyper-CVAD)
In the homogeneously treated (R-CHOP/21) 205 DLBCL patients subset, the median age at diagnosis was 61 y.o. (range 18-85), with a balanced gender distribution (103 females/ 102 males). Twenty-six of these patients (12.7%) had BMI on BMB, whereas 43 (21%) had BMI according to PET/CT finding. Fifty-three patients (25.9%) had BMI according to either BMB or PET/CT. Concordant BMI by means of both techniques was present in 16 (7.8%) patients.
With a median follow-up of 25 months (15-47 months, p25-p75), 50 patients (24.4%) progressed or relapsed and 41 (20%) died. The 3-year estimated progression-free survival (PFS) and overall survival (OS) were 70%, and 78%, respectively. By univariate analysis, factors associated with a shorter PFS, with a $p<0.150$, were: female gender, IPI≥3, abnormally elevated B2-microglobulin levels, PET/CT-BMI(+) and BMB-BMI(+). In multivariate analysis only two factors, BMB-BMI(+) (HR: 3.27, 95%CI 1.58-6.78; p=0.001) and IPI≥3 (HR: 1.96, 95%CI 1-3.76; p=0.04) were independently associated with a shorter PFS. By univariate analysis, factors predictive of a shorter OS, with a $p<0.150$, included: IPI≥3, abnormally elevated B2-microglobulin levels, PET/CT-BMI(+). In multivariate analysis only IPI ≥3 (HR: 2.6, 95%CI 1.13-5.14; p=0.006) was independently associated with a shorter OS.
Summary/Conclusions: In our DLBCL cohort, treated with a uniform first-line chemotherapy regimen, BMI by BMB complemented IPI in predicting those patients with a higher risk for relapse or progression, while IPI defined a subset of patients with a worse survival. In this cohort, BMI by PET/CT could not independently predict a shorter PFS and/or OS.

E954

THE PROGNOSTIC SIGNIFICANCE OF CD11B+CX3CR1+ MONOCYTES IN PATIENTS WITH NEWLY DIAGNOSED DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Interest in the role of myeloid-lineage cells, including monocytes and their precursors, has been increasing in prognosis of lymphoma. It has been shown that the circulating monocyte count at the time of diagnosis shows prognostic significance in diffuse large B-cell lymphoma (DLBCL), suggesting the role of specific subset of monocyte in prognosis of DLBCL. Recent studies suggest $CD11b^+$ monocytes expressing CX3CR1 promote angiogenesis and suppress anti-tumor immunity through the interaction with fractalkine (CX3CL1), the only ligand for CX3CR1. However, limited data is available regarding the prognostic significance of $CD11b^+CX3CR1^+$ monocytes in DLBCL patients.
Aims: The study investigates the prognostic significance of peripheral blood (PB)- and bone marrow (BM)- $CD11b^+CX3CR1^+$ monocytes on progression-free survival (PFS) and overall survival (OS) in newly diagnosed DLBCL patients treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) therapy.
Methods: The prospective cohort study was conducted in two Korean institutions from May 2011 to August 2015. Patients were eligible if they were newly diagnosed DLBCL, treated with R-CHOP, and provided informed consents. Percentages of $CD11b^+CX3CR1^+$ cells in total mononuclear cells (>50,000 cells) were measured by flow cytometric analysis using fresh PB and BM aspirate samples before treatment.
Results: Eighty-nine patients (male, 52) were enrolled. The median age was 65 years (range, 19-88). 37 patients (41.6%) were classified as high-intermediate (HI) or high risk according to National Comprehensive Cancer Network International Prognostic Index (NCCN-IPI). $CD11b^+CX3CR1^+$ monocytes were detectable in all PB and BM samples analyzed. The median percentage of $CD11b^+CX3CR1^+$ cells was 3.31% (range, 0.21 to 21.66%) in PB and 3.09% (range, 0.20-20.01%) in BM. Patients were categorized into high (PB- or BM-$CD11b^+CX3CR1^+$ cells >median) and low (<= median) groups. High PB-$CD11b^+CX3CR1^+$ cell group was significantly associated with unfavorable parameters including age >=60 years, advanced stage, elevated serum LDH level, and extranodal involvement ($P<0.05$), which were clinical factors associated with higher risk NCCN-IPI ($P=0.004$). However, BM-$CD11b^+CX3CR1^+$ cells were not associated with clinical variables. With a median follow-up of

27.7 months (IQR, 14.6-46.1), low PB-CD11b$^+$CX3CR1$^+$ cell group had significantly better PFS (3-year, 77.1% *vs* 58.7%; *P*=0.006) and OS (3-year, 86.6% *vs* 58.4%; *P*=0.004) than high PB group. No significant survival differences were observed between high and low BM-CD11b$^+$CX3CR1$^+$ cell groups. Univariate analyses demonstrated that age, ECOG performance status, B symptoms, extranodal involvement, NCCN-IPI, and PB-CD11b$^+$CX3CR1$^+$ cell group were significantly associated with OS. However, HI or high risk NCCN-IPI was an only independent prognostic factor for reduced OS (hazard ratio, 4.41; 95% confidence interval, 1.17-16.59) in the multivariate analysis. In subgroup analysis according to the NCCN-IPI, 3-year OS of high PB-CD11b$^+$CX3CR1$^+$ monocytes was significantly inferior to that of low group (34.0% *vs* 77.9%; *P*=0.026) in DLBCL with HI to high risk NCCN-IPI. In contrast, PB-CD11b$^+$CX3CR1$^+$ monocytes failed to predict OS (3-year, 91.7% *vs* 96.7%; *P*=0.878) in the low to low-intermediate risk NCCN-IPI subgroup.

Summary/Conclusions: Our study represents PB-CD11b$^+$CX3CR1$^+$ monocytes can be used in differentiating patients with high risk for early death and are associated with risk stratification by the NCCN-IPI, possibility of potential therapeutic target in DLBCL.

E955

RARE NON-HODGKIN LYMPHOMAS (R-NHLS) IN CHILDREN: THE AIEOP EXPERIENCE

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Background: Clinical management of pediatric rare non-Hodgkin lymphomas (r-NHL) (<1case/1million) is unclear.

Aims: To characterize children with r-NHLs in AIEOP centers. Performing a retrospective analysis of r-NHLs AIEOP case records, describing main epidemiologic, clinical and histopathological parameters. To review the histopathological case records according to WHO 2008 classification. Evaluation of treatment response - chemotherapy or *wait and see* (W&S) in terms of overall survival (OS) and of complete remission (CR), relapse and resistance cases, secondary neoplasias and deaths.

Methods: Data from the AIEOP database were collected between 1997 and 2015.

Results: The incidence of r-NHL in AIEOP registry was 6.5% (67 pts). Forty-eight were male and 19 female, median age was 11 years (0.3-21 years). Classification according to St.Jude stage was: stage I n=36; II n=13; III n=11; IV n=7. Bone marrow (BM) involvement was diagnosed in 7 cases; central nervous system (CNS) in one case. Patients who presented LDH >500 UI were 18. B-rNHLs accounted for approximately 49% (33 pts) of the entire population analysed, T-rNHLs for another 40% (27 pts), the remaining 11% (7 pts) of the population under study being categorized as "*others*" (other than those deriving from B or T/NK-cells). The most common histological subtypes were: follicular lymphoma (FL) amongst B-rNHLs; peripheral T-cell lymphoma (PTCL) n.o.s., mycosis fungoides (MF), subcutaneous panniculitis T-cell lymphoma (SPTCL) and lymphomatoid papulosis (LP) amongst T-rNHLs; histiocytic sarcoma (HS) amongst "*others*" category. A similar proportion for both B and T/NK NHL underwent either W&S approach only or active treatment (AT): 45% and 55% were W&S and AT approach, respectively. Patients in "*others*" category were almost actively treated (71%). Therapy was based on AIEOP B-, T/NK-NHLs and ALCL protocols +/- RT, and/or immunotherapy. Surgical resection has been performed in case of localized disease B-rNHLs only, followed by a W&S strategy, with 100% 3-yr OS. It has been seen that B-rNHLs have a more favorable prognosis and very few events (development of resistance to therapy, relapse, secondary malignancy, death). Amongst T/NK rNHLs-related events, death remained the most likely to occur, differently than B-rNHLs which showed a slight prevalence of relapses; as for the category "*others*", no relative preponderance has been registered for any of the above-mentioned events. The 3-year OS has shown to be significantly higher for B-rNHLs than for T/NK-NHL (94% *vs* 69%, p-value 0.024), as illustrated in *Figure 1*. Regarding the treatment, the 3-year OS was 100% for the patients underwent a W&S approach whereas 75% for treated patients (p-value 0.037). FLs show favourable clinical course and outcome, limited stage at diagnosis. Differently from adults, pFLs have a higher 3-years OS with respect to that of other histological pediatric NHLs subtypes (100% *vs* 75%, p-value 0.049).

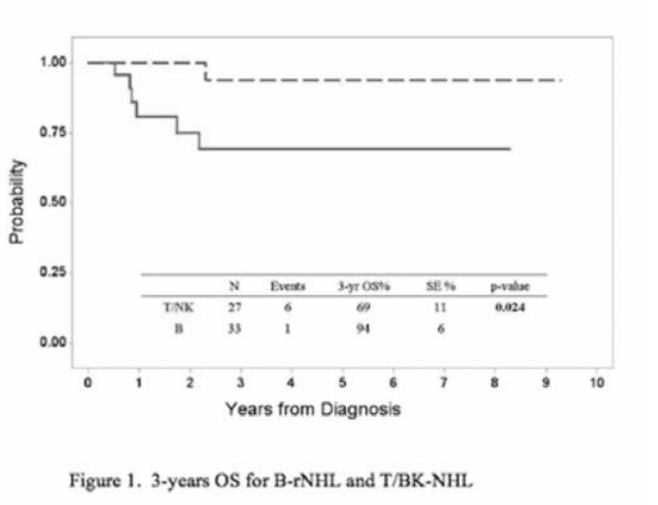

Figure 1. 3-years OS for B-rNHL and T/BK-NHL

Figure 1.

Summary/Conclusions: The incidence of AIEOP pediatric rNHLs is in line with the literature. In case of localized disease, a W&S approach was successfully applied; of these, the T/NK rNHLs being most often registered and with best prognosis are the cutaneous lymphomas (*i.e.* LyP, MF). Patients' prognosis varies greatly depending on the histological subtype. The better survival was observed in the B-rNHLs compared to other categories. An international collaboration is warranted, in order to create new guidelines or protocols for an appropriate management of pediatric rNHLs.

E956

PRIMARY ANALYSIS OF THE EFFECT OF HEMATOPOIETIC STEM CELL TRANSPLANTATION IN THE TREATMENT OF 110 CASES OF T CELL LYMPHOMA

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Background: T cell lymphoma(T-NHL) is a rare and heterogeneous group of lymphoid malignancies with mostly poor outcome with conventional treatment.Recent studies have suggested that Hematopoietic stem cell transplantation(HSCT) has a better curative effect and is superior to traditional chemotherapy.

Aims: To investigate the effect of HSCT in the treatment of T cell lymphoma.

Methods: The clinical data of 110 patients with T cell lymphoma treated by HSCT from January 2006 to August 2016 in our center were retrospectively analyzed.

Results: (1)110 T-NHL patients, 70 males and 40 females, aged 7-64 years (median age 26 years). Disease subtypes: 35 cases of T-cell lymphoblastic lymphoma(T-LBL), 23 cases of NK / T cell lymphoma(NK/TCL), 24 cases of peripheral T-cell lymphoma (PTCL, NOS), 24 cases of variable large cell lymphoma (ALCL), 3 cases of subcutaneous panniculitic T cell lymphoma(SPTCL) and 1case of hepatosplenic T cell lymphoma(HSTCL).Transplantation type: 56 cases of autologous hematopoietic stem cell transplantation (auto-HSCT), 54 cases of allogeneic hematopoietic stem cell transplantation (allo-HSCT). The follow-up was ended in December 2016, the duration of following-up ranged from 2 to 130 months (median follow-up time was 22 months). (2)56/110 patients with auto-HSCT, 3 year overall survival (OS) and disease-free survival (EFS) were 76.5% and 60.9%, respectively. (3)54/110 patients with allo-HSCT, 3 year EFS and OS of allo-HSCT were 61.7% and 58.9%, respectively. (4)36/56 patients with CR1 status before auto-HSCT, 3 year OS and EFS were 87.3% and 68.7% respectively. 20/56 patients with non-CR1 status before auto-HSCT, 3 year OS and EFS were 60.6% and 40.2%. The OS and EFS of the two groups were significantly different (P=0.001).(5)45/110 cases were young and high-risk patients (age<60 years, IPI score ≥3).25/54 cases treated with allo-HSCT, the 3 year OS and EFS were 62.8% and 60.8%. 20/56 cases treated with auto-HSCT, the 3 year OS and EFS were 47.6% and 36.9%. The OS and EFS of the two groups were also significantly different (P=0.001).

Summary/Conclusions: HSCT can improve the efficacy of T cell lymphoma. Auto-HSCT in first complete remission (CR1) enables T-NHL patients with

greater benefit. Allo-HSCT can cure some T-NHL patients, which can be considered for the treatment of young and high-risk T-NHL patients.

E957

SHORT COURSE OF R-HYPERCVAD/MTX/ARA-C FOLLOWED BY ASCT AS FIRST-LINE THERAPY IN MANTLE CELL LYMPHOMA PATIENTS PROLONGS PROGRESSION FREE SURVIVAL TO MORE THAN 9 YEARS. SINGLE CENTER EXPERIENCE

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Background: Mantle cell lymphoma (MCL) is considered an incurable disease with an historical median overall survival around 3-4 years with short progression free survival (PFS) periods. Regimens that include high dose cytarabine and consolidation with autologous stem cell transplant (ASCT) have become standard therapy for fit patients. The median PFS reported after 4-6 cycles HyperCVAD followed by ASCT consolidation is 4.5 years (Ahmadi et al, BMT 2012). Nevertheless, toxicity is high and many patients cannot obtain stem cells for transplant. In this setting, some groups use 6-8 cycles R-HyperCVAD without ASCT consolidation, achieving the same median PFS of 4.6 years (Romaguera et al, Br J Hematol 2010). Based on this we have review our experience using a short course of HyperCVAD followed by transplant consolidation.
Aims: To analyze our experience treating fit patients with MCL in first line with a short course of 2 cycles of R-HyperCVAD followed by consolidation with ASCT.
Methods: from January 2002 to August 2016, the patients diagnosed with MCL treated in first line with a short course of 2 cycles of R-HyperCVAD and ASCT were included in this retrospective analysis. International working group response assessment criteria were used, PFS was calculated from the date of start therapy until date of relapse/progression or last contact.
Results: During the study period 85 MCL patients were registered: 7 (8.2%) did not receive immediate therapy, 44 (52.4%) were not eligible for intensive therapy due to comorbidities or age and 33 (39.3%) were treated with R-HyperCVAD. Clinical characteristics at diagnosis of these 33 patients were: M/F ratio: 26/7 (78.8%/21.2%), median age: 63 y.o (limits: 40-73), ECOG 0-1: 26 (86.7%), Ann Arbor stage III-IV 28/31 (90.3%), MIPI score: low risk: 5 (16.7%), intermediate risk: 17 (56.7%), high risk: 8 (26.7%). Thirty (90.9%) patients completed the 2 cycles of R-HyperCVAD. Reasons for discontinuation were: 2 deaths for sepsis and 1 CNS progression. Intention to treat response rate was: CR 26 (78.8%), PR 2, (6.0%), progressive disease 3 (9.0%), not evaluable 2 (6.0%). Among the 28 patients in CR / PR considered eligible for consolidation with ASCT, 8 patients were not transplanted: 4 (14.3%) had harvest failure (all before plerixafor availability), 2 had persistent toxicity (prolonged neutropenia and severe mucositis) and were not longer considered for ASCT, 1 rejected, 1 unknown cause. Conditioning regimen was BEAM/LACE in 18 (90%) patients and cyclophosphamide-TBI in 2 (10%). One patient died 10 days after infusion for sepsis. With a median follow-up of 35 (1-131) months, the median PFS was 73.0 (95%IC 38.2-107.8) months (6.08 years) for the whole group, 114 (47.3-180.7) months (9.4 years) for the transplanted patients *vs* 21 (3.1-38.9) months (1.8 years) for the not transplanted group. The median OS was 123 (31.9-214.1) months, median OS was not reached for transplanted group *vs* 31.0 (7.5-54.6) months for not transplanted.

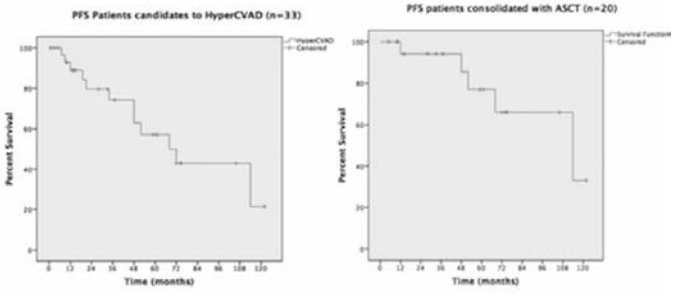

Figure 1.

Summary/Conclusions: A short course of R-HyperCVAD achieves a very high remission rate in fit patients with MCL. Stem cells could not be obtained in a small proportion of patients, all of them before the use of plerixafor. Two thirds of the patients could complete the planned therapy with ASCT consolidation, and those patients have an excellent outcome, with a PFS of more than 9 years.

E958

THE FREQUENCY OF INCIDENTAL MALIGNANCIES DETECTED BY PET/CT SCANS IN PATIENTS WITH LYMPHOMA AND THE ASSOCIATED CLINICAL IMPLICATIONS

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Background: PET/CT imaging has a well-established role in the investigation of malignant lymphoma. Given the widespread clinical applications, unexpected findings are occasionally identified. Whilst there is substantial information pertaining to additional primary cancers identified on PET/CT in patients with solid organ malignancy, there is a relative paucity of data in patients with lymphoma.
Aims: The primary aim was to identify the frequency of incidental second malignancies detected by PET/CT scans in patients with lymphoma. Qualitative data related to histological diagnosis and staging, interruptions or obstacles to lymphoma therapy, therapy for the second malignancy and the overall impact upon prognosis were also reviewed.
Methods: A total of 550 PET/CT images were performed in 298 patients at The Prince of Wales Hospital, Sydney Australia between January 2013 – March 2016. Patients with both Hodgkin's and Non-Hodgkin's lymphoma, with PET/CT imaging performed for all medicare-approved indications were included. All PET/CT reports suggestive of an incidental second malignancy prompted further review of electronic medical records, MOSAIC cancer database and paper medical records. Where a clear diagnosis of second malignancy was confirmed, information regarding histological findings and staging, as well as the implications of this diagnosis related to treatment of the underlying lymphoma and impact on overall prognosis was collected.
Results: 510 PET/CT scans in 259 patients had confirmed diagnoses of lymphoma. Patients aged 17 to 96 were included in the study, with a median age of 62 years. Of the 259 patients included (M=155; F =104), 55 patients had a diagnosis of Hodgkin's lymphoma and 204 patients a diagnosis of Non-Hodgkin's lymphoma. A total of 33 out of 259 patients with a diagnosis of malignant lymphoma had PET/CT findings suspicious for an underlying second malignancy (12.7%). Of the 33 patients, 19 underwent further invasive investigation, with a total of 8 patients having a biopsy proven histological diagnosis of a second malignancy (3.1%). Qualitative information was gathered regarding the patients who did not have further investigation.
Summary/Conclusions: The frequency of incidental malignancies detected by PET/CT imaging in patients with lymphoma was found to be comparable to other similar international retrospective studies. The majority of incidental second malignancies were early stage and gastrointestinal in origin. Further retrospective as well as prospective data may assist in the establishment of guidelines, to address a standardized diagnostic approach to investigating incidental lesions discovered on PET/CT imaging that are suggestive of a second malignancy.

E959

CLINICAL IMPACT OF KARYOTYPIC EVOLUTION ON THE PROGNOSIS OF DIFFUSE LARGE B CELL LYMPHOMA

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Background: The acquisitions of additional chromosomal abnormalities are generally accompanied by the emergence of therapeutic resistance and eventually lead to poor treatment outcome in cancers. However, the actual clinical impact of karyotypic evolution on prognosis differs depending on the type of hematologic malignancy. Although several prognostic indexes, including the International Prognostic Index (IPI), revised IPI (R-IPI), National Comprehensive Cancer Network (NCCN)-IPI, and Kyoto Prognostic Index (KPI) which we have developed (Kobayashi T. Blood Cancer J 2016), have the determinants for prognosis, little is known concerning the prognostic impact of karyotypic evolution in diffuse large B cell lymphoma (DLBCL), the most prevalent subtype of non-Hodgkin lymphoma.
Aims: We in this study investigated the clinical impact of karyotypic evolution on the treatment outcome of DLBCL.
Methods: We retrospectively examined the medical records of 465 DLBCL patients who were diagnosed and treated with either rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP) or with a R-CHOP-like regimen at three independent institutes in Kyoto, Japan, between January 2006 and April 2014. We analyzed the relationship between the number of subclones and prognosis utilizing the Kaplan-Meier curve and Cox proportional hazards regression analysis. We also utilized Fisher's exact test to investigate the correlation between the number of subclones and the conventional prognostic indexes, *i.e.* R-IPI, NCCN-IPI, and KPI. This study was conducted in accordance with the ethical principles of the Declaration of Helsinki

and was approved by the institutional review boards of all participating institutes.
Results: Among the 465 DLBCL cases, karyotypic analyses by G-banding were performed on biopsied tumor specimens before the start of treatment in 181 patients. Among the 181 patients, metaphase spreads were available for G-banding in 120 patients. Neither overall survival (OS) nor progression free survival (PFS) was statistically significantly different between the patients with available metaphase and no available metaphase spreads. Based on the result of G-banding, we next divided the 120 patients with available metaphase spreads into two groups, *i.e.*, patients with karyotypic abnormalities accompanied by ≥2 subclones and patients with 0-1 subclones. We found that the presence of ≥2 subclones was significantly associated with poor OS (3 year OS rates of patients with ≥2 subclones and 0-1 subclones were 67.6% and 82.8%, respectively (p=0.035), and tended to associate with a shorter PFS. Among the 120 patients with available metaphase spreads, the R-IPI-defined high-risk patients and KPI-defined high-risk patients were significantly more frequent in the group of patients with ≥2 subclones. Ages and genders were not significantly different between patients with ≥2 and with 1-2 subclones.
Summary/Conclusions: DLBCL is a cytogenetically and molecularly heterogenous disease entity. No specific chromosomal abnormality has been associated with the shorter survival, except double or triple hit lymphomas. However, in this study, it was possible to divide DLBCLs into two groups based on karyotypic evolution, *i.e.*, DLBCLs with 0-1 subclones and with ≥2 subclones, because the OS was the most markedly different between these two groups. In our study, more subclones were associated with poor prognosis, suggesting the significance of karyotypic evolution in DLBCL. In conclusion, our study suggests that more advanced cytogenetic clonal evolution underlies the development of high-risk disease feature in DLBCL.

E960

REGIMEN INTENSIFICATION MAY IMPROVE OUTCOMES IN PATIENTS WITH HIGHER RISK HUMAN IMMUNODEFICIENCY VIRUS (HIV) RELATED AGGRESSIVE B-CELL LYMPHOMAS

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Background: Despite effective combination antiretroviral therapy for HIV, there remains an increased incidence of HIV related B-cell Non-Hodgkin lymphomas (NHL). The introduction of early antiviral therapy and effective chemotherapy have led to improved outcomes overall. Regimen intensification (RI) in HIV associated B-cell NHLs has shown improved survival, especially in the rituximab era (Barta et al, Blood 2013).
Aims: To examine the effect of RI on the overall survival (OS) and progression free survival (PFS) compared to CHOP based chemotherapy according standard risk stratification.
Methods: Patients with HIV associated aggressive B-cell NHL were identified between 2001- 2015 at Moffitt Cancer Center. Patients with primary central nervous system lymphoma, T-cell NHL and indolent NHLs were excluded. Patients received R-CHOP or intensive chemotherapy (IC) including DA-EPOCH, hyperCVAD or CODOX/IVAC as initial treatment. Data collected included patient demographics, disease baseline characteristics, CD4 count, HIV viral load, treatment regimen, response, and outcomes including relapse and OS. The IPI score was calculated, and patients were divided into two groups: lower risk group (low and low-intermediate IPI risk) and higher risk group (high-intermediate and high). Descriptive statistics were used for baseline characteristics. Kaplan Meier method was used to estimate PFS and OS, and the log-rank test was used to compare OS and PFS between lower and higher risk groups.
Results: A total of 83 patients were included. The M:F ratio was 9.4. Median age was 45 years (y) (range 25 – 65). Two thirds of patients were Caucasian. The median time from HIV to NHL diagnosis was 29 months (range 0 – 284). Eighty two percent presented with stage III/IV disease. Bulky disease was present in 27%, elevated LDH in 66%, and CD4 count<100/mL at diagnosis in 22% patients. Fifty percent of patients were on HAART therapy at time of lymphoma diagnosis. Chemotherapy regimens included: RCHOP (n=30, 36%), CHOP (n=12, 15%), DA-EPOCH-R (n=27, 33%), DA-EPOCH (n=1, 1%), hyperCVAD (n=11, 13%) and CODOX/IVAC (n=2, 2%). The median follow up was 2.7 y (95% CI 2.0-3.4). The median OS and PFS for the whole cohort was 5.9 and 4 y, respectively. The median OS was 4 y (95% CI 1.5-6.5) for patients who received CHOP based regimen and was not reached (NR) for patients who had IC (p=0.44). Based on the IPI, the median OS was NR for the lower risk group compared to 1.8 y in higher risk group (p=0.025). Among patients who received CHOP, the median OS for those with lower risk disease was NR compared to 1.8 y in patients with higher risk disease (p=0.07). For patients who received IC, the median OS was NR among both lower and higher risk groups (p=0.2). Among patients who received CHOP, the median PFS for those with lower risk disease was NR compared to 1 y in patients with higher risk disease (p=0.05). For patients who received IC, the median PFS was NR among lower and 1.4 y higher risk groups (p=0.34).
Summary/Conclusions: The IPI score remains prognostic in HIV related B-cell NHLs. There was a trend for improved OS and PFS using IC regimens. CHOP treatment remained associated with worse outcome among higher risk patients while IC regimens may overcome the higher risk features based on the IPI.

E961

EPSTEIN-BARR VIRUS LATENT MEMBRANE PROTEIN 1-MEDIATED OVEREXPRESSION OF MYC AND BCL2 CAN PREDICT POOR PROGNOSIS IN PATIENTS WITH EXTRANODAL NK/T-CELL LYMPHOMA, NASAL TYPE

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Background: Recently double-hit lymphoma or double protein expressor lymphoma has been identified as a distinct group of diffuse large B cell lymphoma with poor prognosis. However, the expression status, clinical and prognostic effect of combined overexpression of MYC and BCL2 in extranodal NK/T-cell lymphoma, nasal type (ENKTL) are not known.
Aims: This study aims to explore the clinical and prognostic effect of combined overexpression of MYC and BCL2 in ENKTL.

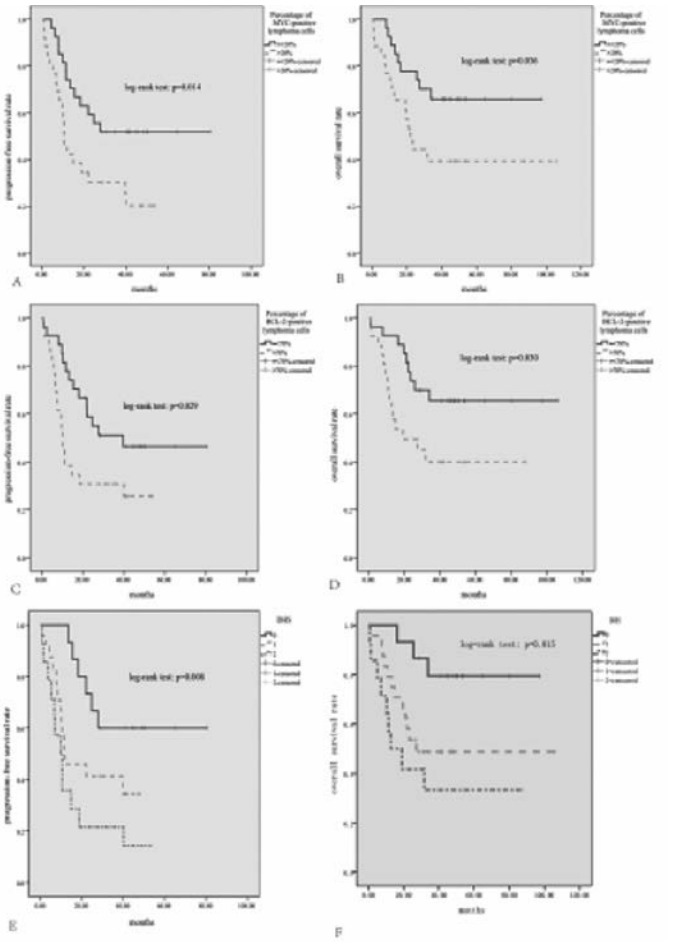

Figure 1.

Methods: Paraffin-embedded lymphoma samples from 53 patients with newly diagnosed ENKTL were studied using immunohistochemistry for MYC and BCL2, and fluorescent *in situ* hybridization (FISH) for MYC and BCL2 were done on 5 tissue sections with highest percentages of both MYC and BCL2 positive lymphoma cells.
Results: The median percentage of MYC-positive lymphoma cells and BCL2-positive lymphoma cells were 20% (range, 5% >45%) and 70% (10% >95%), respectively. Using median scores as cutoffs, we assigned each patient an IHC double-hit score (DHS) that ranged from 0 to 2. Using this DHS, 15 patients (28.3%) had a DHS of 0, 24 patients (45.3%) had a DHS of 1, and the remaining 14 patients (26.4%) had a DHS of 2. FISH analysis was performed on 5 tissue sections with DHS of 2, and none of them had MYC or BCL2 rearrangement. The DHS was not associated with patients' age, gender, disease stage, LDH level, B symptoms, performance status, or local tumor invasiveness. However,

patients with tumor localized in extranasal sites seemed to have higher expression of BCL2 and higher DHS than nasal lesions (p=0.014 and 0.042, respectively). In univariate survival analysis, either high expression of MYC or BCL2 was significantly correlated with inferior PFS and OS (p<0.05). According to the DHS, patients with ENKTL could be divided into three significantly different risk groups for PFS and OS (3-year PFS rate for DHS of 0, 1, and 2 was 60%, 41%, and 21%, respectively, p=0.008; 3-year OS rate for DHS of 0, 1, and 2 was 79%, 49%, and 33%, respectively, p=0.015). In multivariate survival analysis, it was found that DHS was an independent prognostic factor for both PFS and OS (p=0.006 and 0.011, respectively).
Summary/Conclusions: Our study demonstrated that DHS can help identify patients with newly diagnosed ENKTL who are at a high risk for a poor clinical outcome, which needs to be validated in prospective clinical trials with patients treated uniformly.

E962

SOLUBLE INTERLEUKIN-2 RECEPTOR AS A PREDICTIVE MARKER FOR SPONTANEOUS REGRESSION OF OTHER IATROGENIC IMMUNODEFICIENCY-ASSOCIATED LYMPHOPROLIFERATIVE DISORDERS; A RETROSPECTIVE STUDY

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Background: Patients treated with immunosuppressive drugs (ISD) for autoimmune diseases are at an increased risk of developing other iatrogenic immunodeficiency-associated lymphoproliferative disorders (OI-LPD). Some patients with OI-LPD shows spontaneous regression after withdrawal of ISD, but some requires chemotherapy. The factors that are associated with spontaneous regression and outcomes of chemotherapy remain uncertain.
Aims: The aims of our retrospective study are to assess the clinical factors that predict spontaneous regression of lymphoma after ISD withdrawal in patients with OI-LPD and to evaluate the outcomes of patients who underwent chemotherapy without spontaneous regression.
Methods: We collected data from all patients with autoimmune disease who were pathologically diagnosed with OI-LPD between January 2002 to October 2016 at Yokohama City University Hospital, and Yokohama City University Medical Center.

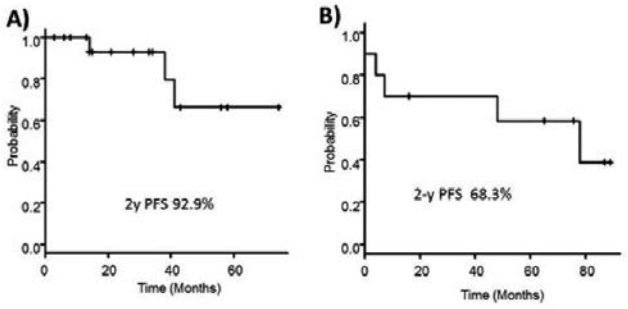

Figure 1. Kaplan–Meier curves for progression-free survival (PFS) (A) in patients with spontaneous regression, and (B) in patients with DLBCL without spontaneous regression treated with CHOP/CHOP-like ± rituximab. (B), PFS is defined as the time from the first day of chemotherapy to progression.

Figure 1.

Results: The patients included 12 males and 28 females, with a median age at diagnosis of 65 years (range 30-81). Methotrexate (MTX) was administered to all patients at any point of the clinical course before OI-LPD. The median time from diagnosis of autoimmune disease to OI-LPD development, and the median duration of MTX administration were 129 months (range 11-564), and 89 months (range 4-297), respectively. The histological findings of OI-LPD were diffuse large B-cell lymphoma (DLBCL) in 26 patients, follicular lymphoma in 1, MALT in 2, peripheral T cell lymphoma, not otherwise specified in 3, Hodgkin lymphoma in 4, and LPD in 4. EBER *in situ* was examined in 30 patients:17 positive, and 13 negative. The median observation period in the surviving patients was 34 months (range 3-119). The 2-year progression-free survival (PFS) and overall survival (OS) rates for all 40 patients were 69% and 79%, respectively. A total of 18 patients (45%) had spontaneous regression after ISD withdrawal. The median time from ISD withdrawal to spontaneous regression was 4 months (range 1-13). Among the 18 patients with spontaneous regression, 3 relapsed. Of the 22 patients without spontaneous regression, 20 subsequently underwent chemotherapy: 18 underwent CHOP/CHOP-like ± rituximab, and 2 underwent other regimens. In total, 7 patients died: all died from lymphoma progression. Compared to those without spontaneous regression, patients with spontaneous regression had clinical stages I-II (*P*=0.021), performance status of 1-2 (*P*=0.028), normal levels of LDH (*P*=0.026), and lower levels of sIL-2R (*P*=0.005). The ROC curve analysis showed the appropriate cut-off of sIL-2R levels to be 2400 U/mL for predicting spontaneous regression (AUC, 0.74; sensitivity, 0.81; specificity, 0.67). On multivariate analysis, only an sIL-2R level of <2400 U/mL was associated with spontaneous regression (odds ratio, 0.03; 95% CI, 0.002-0.39; *P*=0.007). Thirteen patients with DLBCL who did not have spontaneous regression received CHOP/ CHOP like ± R therapy. The CR rates, and 2-year PFS and OS of these 13 patients were 38%, 68.3%, and 82.1%, respectively.
Summary/Conclusions: Our study revealed that an sIL-2R level of <2,400 U/mL was significantly associated with spontaneous regression in patients with OI-LPD. Because CR rates with chemotherapy in patients without spontaneous regression are low, evaluation of sIL-2R in patients with OI-LPD may be useful for an early withdrawal of ISD, resulting in a higher chance of spontaneous regression.

E963

PROGRAMMED DEATH-1 PROTEIN EXPRESSION AND ITS RELATION WITH HISTOLOGIC AND CLINICAL VARIABLES IN MYCOSIS FUNGOIDES

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Background: Mycosis fungoides (MF) is a T-cell malignancy with affinity for the skin. In early stages,treatment directed to the skin can induce long-lasting remissions. However, advanced stages are characterized by short-duration remissions and progressive disease. The programmed death cell surface protein-1 (PD-1) is expressed on activated T cells. Interactions between PD-1 and its ligands control the induction and maintenance of peripheral T-cell tolerance during the normal immune response. These interactions may also play a role in the immune evasion of tumors in which PD-1 ligand is overexpressed.
Aims: To described histologic characteristics and the proportion and intensity of PD1 expression by tumor cells, as well as the presence of PD1 positive lymphocytes in the epidermis in patients with MF. To identify histologic variables that might have an impact in clinical outcome.

Table 1.

Characteristics of Patients	Frequency	Percentage
Age (<60 vs. ≥60 years old)	< 60 years old: n= 37	57 %
Gender	Male n= 39	60 %
Stage	Early Stage n=52; Advanced Stage n= 13	80 %/20%
Intensity of PD1 in positive cells	No intensity n= 5	8 %
	+1 n= 30; +2 n=19; +3 n=11	46 %/29%/17%
Tumoral cell expressing PD1	<60% n= 40; ≥60% n= 25	62%/39%
Presence of mitosis in PD1+ cells	Positive n= 4	6%
	Negative n=61	94%
Intraepidermal PD1+ cells	No cells n= 32	49%
	+1 n=25; +2 n =8	39%/12%
Intensity of tumoral infiltrate	+1 n=18; +2 n=33;+3 n=13 ; +4 n=1	28%/51%/20 %/1%
Epidermotropism of tumoral cells	+1 n= 24; +2 n=22; +3 n=19	37%/34%/29 %
Degree of atypia	+1 n= 38; +2 n= 23; +3 n= 4	59 %/35%/6%
Accompanying non tumoral cellularity	+1 n=42; +2 n=19 ; +3 n= 4	65%/29%/6%
Loss of CD7 expression	Normal expression n=27	42%
	< 75% n= 10 ; ≥75% n= 28	15%/43%

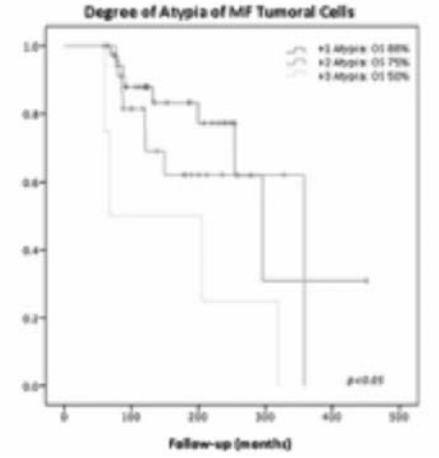

Figure 1.

Methods: Histological preparations of 85 patients diagnosed with with MF were evaluated. Survival analysis was performed with the Kaplan-Meier method. A univariate analysis was performed with clinical variables (stage and age) and anatomopathological variables (*i.e.* intensity of the inflammatory infil-

trate, epidermotropism, cellular atypia, tumor density, presence of folliculotropism and phenotypic alterations) and the proportion and intensity of PD1 expression by tumor cells, the presence of PD-1 positive lymphocytes in the epidermis. Likewise, a Pearson correlation analysis was performed between the degree of atypia and the ratio of PD-1 expression, PD-1 intensity, and loss of CD7 expression in tumor cells. Statistical analysis was performed using the IBM SPSS Statistics version 21.0.
Results: The median follow-up was 125 months (range 6-450 months). Characteristics of patients are in **table 1.** The overall survival (OS) at 10 years was 81%. OS in the early stages was 85% vs.64% in advanced stages (p<0.05). The OS for patients <60 years was 85%, and 75% for patients ≥60 years (p<0.05). Regarding istologic findings, the degree of atypia was the only variable that had an impact in OS.(see Figure 1) The presence of atypia grade +1 had an OS of 88%, grade +2 of 75%, and grade+3 of 50% (p<0.05). We performed a correlation analysis between degree of atypia and the ratio of PD-1 expression, PD-1 intensity, and loss of CD7 expression. A positive correlation was detected, however it was weak (r<0.5).
Summary/Conclusions: MF tumoral cells express PD-1 protein in a high proportion of cases being a potential therapeutic target. Advanced disease, age ≥60 years and the degree of atypia of the tumoral infiltrate had an impact on survival.

E964

CIRCULATING MICRORNAS AS BIOMARKERS IN DIFFUSE LARGE B-CELL LYMPHOMA: A PILOT PROSPECTIVE LONGITUDINAL CLINICAL STUDY

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Background: Diffuse large B-cell lymphoma (DLBCL) is highly heterogeneous in terms of phenotype and treatment response in patients. These characteristics make the prognosis difficult to establish and hinder the use of new personalized treatments in clinical practice. In this context, there is currently a necessity to define new biomarkers enabling a better definition of DLBCL subtypes, prognosis evaluation and an overview of the resistance to chemotherapeutics. We decided here to focus on circulating microRNAs that are found in all biological fluids. This accessibility makes them good candidates for biomarkers studies.
Aims: This research aims at studying microRNAs found in plasma from DLBCL patients and at investigating their potential as biomarkers of survival in these patients. For this purpose, a plasma biobank was created with samples from DLBCL patients at different times of their treatment. This follow-up of microRNAs level during the course of treatment is particularly innovative in this study.
Methods: A plasma biobank from DLBCL patients was set up at the Centre Hospitalier Universitaire (CHU) UCL Namur Yvoir, Belgium (ethical agreement number B039201419613). Informed consents of all patients were obtained. In this way, blood samples from patients were taken before any treatment (C_0), at the administration of the second and the fourth chemotherapeutic cure (C_2 and C_4) and at the remission review (C_f). In the case of an autograft, a sample was taken at the post-graft review (C_{pg}). The first step of this study was the selection of the microRNAs that will be quantified in all the samples of the biobank and that could potentially be used as biomarkers. To this end, a quantification of 377 microRNAs was performed by TaqMan® Low Density Array on the plasma samples of two selected DLBCL patients and one healthy donor with no history of cancer. These DLBCL patients were selected based on their highly different response to treatment. One of them obtained a complete remission after a R-CHOP treatment, while the other presented a refractory disease to the same treatment. Thereafter, we determined some criteria to use in a scoring system to evaluate their potential as biomarkers. In this way, one point was given to a microRNA each time it meets the criteria enabling it to be defined as a potential diagnostic, prognostic and/or remission biomarker, biomarker of a disease progression, biomarker of an inherent resistance to treatment, and/or biomarker of an acquired resistance to treatment.
Results: On the 377 microRNAs quantified into the plasma of the 3 selected donors (2 DLBCL patients and 1 healthy donor), 81 microRNAs were detected. Three microRNAs obtained the highest score of 5 points: miR-197, miR-20a and miR-451. Four points were attributed to miR-122, miR-19b and miR-19a. Two additional microRNAs were also selected: let-7e, for its prognostic value at C0, C2 and C4 and miR-21, for its numerous citations in the literature.
Summary/Conclusions: miR-197, miR-20a, miR-451, miR-122, miR-19a, miR-19b, let-7e and miR-21 have been selected in this study and are currently quantified into the plasma of the entire biobank. Since then, 19 patients have been included in the study and the potential of these microRNAs as biomarker are statistically evaluated.

E965

COMBINED CHEMOTHERAPY PLUS RADIATION THERAPY IS MORE EFFECTIVE IN LIMITED-STAGE DIFFUSE LARGE B-CELL LYMPHOMA OF THE TONSIL

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Background: Primary extranodal non-Hodgkin's lymphomas of the head and neck account for 10-20% of all non-Hodgkin's lymphomas. Primary tonsillar lymphoma accounts for less than 1% of head and neck malignancies, although the tonsil is the most common primary extranodal site of head and neck non-Hodgkin's lymphomas.
Aims: The purpose was to evaluate the prognostic factors and treatment outcome of patients with diffuse large B-cell lymphoma (DLBCL) of the tonsil.
Methods: In all, 114 patients with DLBCL of the tonsil with stage I or stage II, treated at multicenter in Korea, from September 1995 to April 2011, were included. The median age was 59 years and the majority of patients (61%) were male. Systemic symptoms were present in 6% of patients. International prognostic index (IPI) score was 0 in 54 patients (48%), 1 in 40 (35%), 2 in 14 (12%), and 3 (3%). Ten patients (8%) showed elevated level of lactate dehydrogenase (LDH). Treatment consisted of a combination of chemotherapy (CTx) and radiotherapy (RTx) for 38 patients (34%) and 72 patients (65%) received CTx only. Among those receiving RTx, the median RTx dose was 39 Gy.
Results: After median follow-up of 32 months (range,0.4-106 months), event free survival (EFS) and overall survival (OS) were 25.9% and 42.5%, respectively. Significant prognostic factors included: age (≥ 60 year-old *vs* <60 year-old), LDH level (> upper normal limit and ≤upper normal limit), IPI score (0-1 *vs* 2-3), and treatment (CTx plus RTx *vs* CTx only). On multivariate analysis; LDH level (hazard ratio [HR], 10.522; 95% confidence interval [CI], 2.548-43.449, p=0.001) and treatment (HR, 12.393; 95% CI 2.151-71.410) were independent prognostic factor of EFS and age (HR, 8.920; 95% CI 1.089-73.053, p=0.043), LDH (HR, 8.316; 95% CI 1.914-36.127, p=0.005), and treatment (HR, 8.943; 95% CI 1.089-73.425) retained statistical significance in OS.

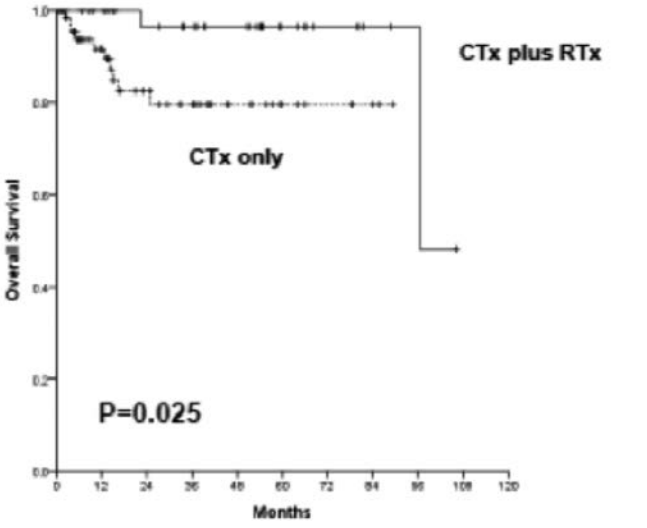

Figure 1.

Summary/Conclusions: LDH level and age significantly influence outcome. A combined modality treatment, consisting of CTx and RTx, results in a satisfactory outcome in patients with stage I or II DLBCL of the tonsil.

E966

Abstract withdrawn.

E967

SEQUENTIAL TREATMENT WITH BENDAMUSTINE, RITUXIMAB AND DEXAMETHASONE FOLLOWED BY RITUXIMAB CONSOLIDATION AND LENALIDOMIDE MAINTENANCE FOR FRAIL ELDERLY PATIENTS WITH AGGRESSIVE B-NON HODGKIN LYMPHOMA

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Background: Frail elderly patients with aggressive B non-Hodgkin Lymphoma (a-B-NHL) in most cases show comorbidities such as to preclude the use of antracycline-based standard regimen. Although significant advances have recently been achieved in the therapy of older patients with a-B-NHL, there is still need for treatment strategies able to overcome the impact of drug toxicity in elderly frail patients.
Aims: The safety and efficacy of bendamustine and rituximab plus dexamethasone (RD-Benda) regimen were prospectively investigated in 14 elderly and frail patients with newly diagnosed a-B-NHL.

Methods: Fourteen (4 female, 10 male) consecutive frail elderly patients (median age: 79 years; range 68-86 years) with a-B-NHL (11 DLBCL, 1 Burkitt NHL, 1 Burkitt-like NHL and 1 Mantle cell lymphoma) were enrolled in a phase II study with bendamustine 70mg/m^2 i.v. on days 1 and 2, rituximab 375mg/m^2 *i.v.* on day 1and oral dexamethasone 20mg total dose on days 1-4 for four cycles. Frailty criteria were age > or =80 years, or age > or =70 years associated with three or more grade 3 comorbidities or at least one grade 4 comorbidity according to the cumulative illness rating scale (CIRS), as well as not self-sufficient or the presence of geriatric syndromes.
Results: P*atients* who showed complete (CR) or partial response (PR) after the fourth induction cycle of RD-BENDA started a consolidation course with four weekly doses of rituximab (375mg/m^2 *i.v.*) followed, in the case of persistence of CR or PR, by a maintenance treatment with monthly courses of lenalidomide (10mg/m^2, days 1-21). All patients performed G-CSF prophylaxis to avoid febrile neutropenia. Patients with progressive disease after RD-BENDA started maintenance therapy with monthly courses of full dose lenalinomide. PET-CT scans were performed for the assessment of therapy response after RD-BENDA induction course and after rituximab consolidation. After a median follow-up of 6 months (range 2-18), the overall response rate was 81%, with CR and PR of partial response rates of 63 (n=7) and 21% (n=2) respectively. Two patients died due to multiple organ failure and disease progression after 1 and 8 months from diagnosis, respectively. In our frail and elderly patient cohort, the sequential treatment strategy was well-tolerated. After R-BENDA cycles, grade II infectious disease was observed in 2/11 patients (18%) and DNA-CMV reactivation was detected in other 2 additional patients (18%). However, 2 out of five patients who started maintenance lenalidomide treatment discontinued therapy for renal and hematological grade 3 toxicity. At the time of analysis, the estimated median *18-month* progression free survival (PFS) and overall survival (OS) were 75 and 66%, respectively.
Summary/Conclusions: Our preliminary data show that sequential treatment with RD-BENDA followed by four weekly doses of rituximab and finally by lenalidomide maintenance is a feasible and safe therapy option in frail elderly a-B-NHL patients, but needs to be assessed in a larger subsequent trial.

E968

CLINICAL RELEVANCE OF SARCOPENIA IN DIFFUSE LARGE B-CELL LYMPHOMA - TWO ARE BETTER THAN ONE

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Background: Sarcopenia is known to be associated with poor clinical outcome in patients with diffuse large B-cell lymphoma (DLBCL). There is no consensus concerning the optimal method to define sarcopenia in DLBCL.
Aims: In this study, given the uncertainty about the optimal SMI to define clinically meaningful sarcopenia in DLBCL, we compared the characteristics and clinical outcome between sarcopenic patients determined by L3 skeletal muscle index (L3-SMI) and those determined by pectoralis muscle SMI (PM-SMI) who were treated with standard front-line R-CHOP therapy. Furthermore, the synergistic role of L3- and PM-SMIs as prognostic markers was also investigated.
Methods: We retrospectively reviewed 193 DLBCL patients treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) therapy. Sarcopenia was classified by the region where the pretreatment skeletal muscle index (SMI) was measured.
Results: Both the sarcopenia-L3 and sarcopenia-pectoralis muscle (PM) groups had increased incidences of severe treatment-related toxicities and treatment discontinuation compared with the non-sarcopenia-L3 and non-sarcopenia-PM groups, respectively. The sarcopenia-L3 and non-sarcopenia-L3 groups had 5-year overall survival (OS) rates of 40.5% and 67.8% (p<0.001), respectively. The sarcopenia-PM and non-sarcopenia-PM groups had 5-year OS rates of 35.9% and 69.0% (p<0.001), respectively. When the sarcopenia-L3 alone and sarcopenia-PM alone groups were compared, there were no differences in baseline characteristics, treatment toxicity, or survival. In multivariate analysis, when compared with the non-sarcopenia-both group, OS was significantly worse in the sarcopenia-both group (HR, 2.480; 95% CI, 1.284-4.792; p=0.007), but not in patients with either sarcopenia-L3 alone or sarcopenia-PM alone (p=0.151).
Summary/Conclusions: L3- and PM-SMIs are equally useful to define sarcopenia, which is related to intolerance to R-CHOP therapy and to worse survival in patients with DLBCL. More prognostic information can be obtained when these two SMIs are combined to define sarcopenia.

E969

INTENSIFIED TREATMENT REGIMENS IMPROVE EVENT-FREE AND OVERALL SURVIVAL IN YOUNGER NEWLY DIAGNOSED HIGH-RISK PATIENTS WITH B-LARGE CELL LYMPHOMA; A RETROSPECTIVE OBSERVATIONAL STUDY OF KROHEM

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Background: Standard therapy for newly diagnosed B-large cell lymphoma (B-LCL) is R-CHOP. Patients with high-risk disease have unsatisfactory outcomes. Non-randomized trials have suggested that intensified regimens, such as R-CHOEP14 and DA-R-EPOCH, improve treatment results in younger patients.
Aims: We performed this analysis to compare response rates, event-free (EFS) and overall survival (OS) rates of newly diagnosed patients with high-risk disease treated with R-CHOP21 and more intensive regimens (R-CHOEP14 and DA-R-EPOCH).
Methods: Outcomes of B-LCL patients younger than 60 with aaIPI ≥2 treated at two different centres with R-CHOEP14 and DA-R-EPOCH were collected retrospectively from patient files and compared to outcomes of patients with same characteristics treated with R-CHOP21 from the registry of KroHem, the Croatian Cooperative Group for Hematologic Diseases. All three regimens were administered according to standard guidelines for 6-8 cycles. Patients in PR or with initial bulky disease were irradiated after the end of systemic treatment. Twelve patients treated with DA-R-EPOCH were autografted in 1st remission.
Results: 54 patients were treated with R-CHOP21, 40 with R-CHOEP14 and 22 with DA-R-EPOCH. R-CHOEP14 and DA-R-EPOCH treated patients did not differ in response rates, EFS and OS and were grouped together for further analysis. R-CHOP treated patients had less frequently bulky disease (25% *vs* 49%, P=0.007) than more intensively treated patients; there was no difference in age, gender, stage, elevated LDH or PS ≥2. Patients receiving R-CHOP had similar response rates as those receiving more intensive regimens (80% *vs* 85%, P=0.405), but inferior EFS (HR 2.12, 95% C.I. [1.09-4.12], P=0.028) and OS (HR 2.15, 95% C.I. [1.07-4.3], P=0.034) (Figure 1). 5-year EFS rates were 58% and 78% and 5-year OS rates 60% and 80% for R-CHOP21- and more intensively treated patients, respectively. Differences in outcomes between R-CHOP and intensified regimens remained significant in a multivariate Cox regression model adjusted for age, gender and presence of bulky disease (HR 2.45, 95% C.I. [1.11-5.4], P=0.026 for OS and HR 2.46, 95% C.I. [1.16-5.24], P=0.019 for EFS).

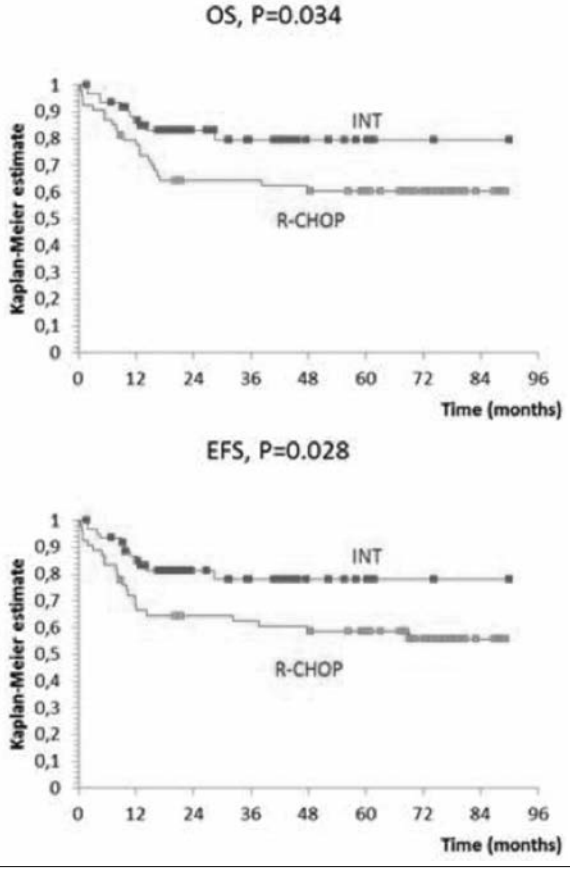

Figure 1.

Summary/Conclusions: Our data suggests that the addition of etoposide to R-CHOP and increase in dose-intensity improve EFS and OS of younger patients with newly diagnosed high-risk B-LCL. R-CHOEP14 and DA-R-EPOCH seem to be similarly effective in this setting.

E970

HIGH COMORBIDITY INDEX ALONG WITH HIGH NCCN-IPI STRONGLY INFLUENCE SURVIVAL OF DIFFUSE LARGE B CELL LYMPHOMA PATIENTS: SERBIAN LYMPHOMA GROUP EXPERIENCE

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Background: A few studies have validated the prognostic significance of the NCCN International Prognostic Index (NCCN-IPI) so far. However, some patients with low risk according to NCCN-IPI have poor survival, and thus clinical parameters, that might better characterized patients within risk groups, need to be analyzed.
Aims: The aim of this study was to evaluate prognostic significance of current indexes such as International Prognostic Index (IPI), NCCN-IPI, and the influence of comorbidities on the overall survival (OS) of patients with newly diagnosed diffuse large B cell lymphoma (DLBCL).
Methods: A total of 708 patients (363 males/345 females) with the median age of 58 years (range 18-89) were included in the study. Majority of patients received R-CHOP (Rituximab, Cyclophosphamide, Doxorubicine, Vincristine, Prednisone) protocol, 652 (92.1%), while 29 (4.1%) received R-EPOCH (Rituximab, Etoposide, Cyclophosphamide, Doxorubicine, Vincristine, Prednisone), and 27 (3.8%) received R-CVP (Rituximab, Cyclophosphamide, Vincristine, Prednisone).
Results: According to the Ann Arbor classification, stage I and II had 332 patients (46.9%), while stage III and IV had 376 patients (53.1%). Bulky disease was present in 201 patients (28.4%), and B symptoms in 437 patients (61.7%). Poor European Cooperative Oncology Group (ECOG) performance status (≥2) had 145 patients (20.5%). Bone marrow involvement was present in 97 patients (13.7%). At least one comorbid condition had 309 patients (43.6%), while high Charlson Comorbidity Index (CCI) had 44 patients (6.2%). Majority of patients had cardiovascular disorders (223, 31.5%), endocrinological (63, 8.9%), neurological (20, 2.8%), reumatological (19, 2.7%), previous malignancy (19, 2.7%), pulmonary (18, 2.5%), psychiatric (13, 1.8%), nephrotic (8, 1.1%), autoimmune (6, 0.8%), and other (13, 1.8%). According to IPI, low, low intermediate, high intermediate and high risk had 332 patients (46.9%), 174 (24.6%), 132 (18.6%), and 70 (9.9%), respectively, while according to NCCN-IPI, 139 (19.6%) patients had low risk, 335 (47.3%) low intermediate, 198 (28.0%) high intermediate, and 36 (5.1%) high risk. Overall treatment response (ORR) was achieved in 615 patients (86.9%). Disease relapse was confirmed in 116/615 patients (18.9%). The patients with B symptoms (Log Rank=18.50, p<0.0001), and bulky disease (Log Rank=14.79, p<0.0001) had inferior OS compared to those without B symptoms or bulky disease. All parameters incorporated in IPI, as well as in NCCN-IPI, were significantly associated with OS (p<0.01). Moreover, the patients with at least one comorbidity had inferior OS (Log Rank =5.41, p=0.20), as well as those with high CCI ≥2 (Log Rank =7.59, p=0.006). Regarding OS, IPI (Log Rank=97.36, p<0.0001), and NCCN-IPI (Log Rank=102.29, p<0.0001) confirmed its prognostic significance. Furthermore, the patients with high CCI had significantly inferior median OS in the high risk group according to IPI (19 months *vs* 37 months), and NCCN-IPI (12 months *vs* 19 months).
Summary/Conclusions: NCCN-IPI represents useful prognostic index in DLBCL patients, and can better describe patients within risk groups, compared to IPI. Moreover, comorbidities contribute to inferior survival through frailty, drug dose reduction and poorer tolerability.

E971

SUBSTITUTING DOXORUBICIN WITH ETOPOSIDE IN R-CHOP RESULTS IN A REGIMEN WITH SIMILAR EFFICACY FOR TREATMENT OF NEWLY DIAGNOSED ELDERLY PATIENTS WITH B-LARGE CELL LYMPHOMA (B-LCL)

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Background: R-CHOP is standard front-line treatment for B-LCL. However, anthracycline-induced cardiac toxicity limits its use in elderly and patients with preexisting heart disease. R-CEOP, in which doxorubicin is substituted with etoposide, has been suggested as a potential solution of this problem, but reports on the efficacy of this regimen vary substantially, especially in patients with non-GC DLBCL. We have been using this regimen regularly for front-line treatment of patients with B-LCL and preexisting heart disease and present here our experience.
Aims: To compare outcomes of newly diagnosed patients with B-LCL treated at a single institution with R-CEOP and R-CHOP.
Methods: We performed a retrospective analysis of all newly diagnosed B-LCL patients treated with R-CEOP at our centre from 2011 to 2016 and compared them to patients 60 years or older treated during the same period with R-CHOP, the standard regimen used at our centre for non-frail elderly without significant cardiac comorbidities. The dose of etoposide in R-CEOP was 50mg/m^2 iv or 100mg/m^2 orally daily for 3 days. Both regimens were given every 3 weeks for 6-8 cycles. Patients with initial bulky disease or in PR after systemic treatment were irradiated.
Results: 31 patients, 15 male and 16 female, received R-CEOP and 48, 25 male and 23 female, R-CHOP. Patients in the former group were older (median age 77 y, range 58-87 *vs* median age 66 y, range 60-83), had more often low performance status (81% *vs* 31%) and advanced disease (84% *vs* 54% stage 3 and 4) resulting in a significantly higher proportion of patients with IPI 3-5 (74% *vs* 40%, p=0.019). Proportions of patients with increased LDH were similar between the groups. There were no significant differences in frequency of grade 3-4 toxicity between the regimens; 48% of patients in both groups required emergency hospitalization; thrombocytopenia or anemia occurred in 16% of R-CEOP and 23% R-CHOP treated patients, infections in 32% and 31% and cardiovascular events in 16% and 21%. However, 7 patients (23%) in the R-CEOP group died during treatment due to adverse effects in comparison to 4 (8%) in the R-CHOP group. Efficacy was similar, 65% responded to R-CEOP and 79% to R-CHOP. After a median follow-up of survivors of 27 mo, 3y-OS was 55% in the R-CEOP group and 52% in the R-CHOP group; 3-y EFS was 50% and 50%, respectively (figure). Outcomes of patients with GC and non-GC DLBCL categorized according to Hans's algorithm were similar irrespective of treatment.

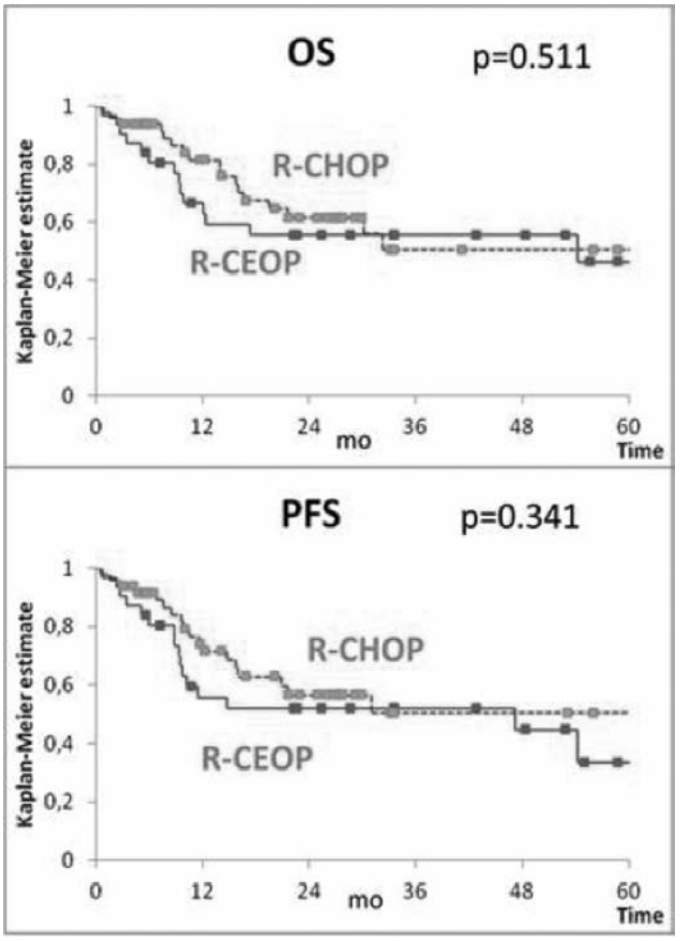

Figure 1.

Summary/Conclusions: Long-term outcomes of newly diagnosed B-LCL patients treated with R-CEOP seem as good as those achieved with R-CHOP irrespective of cell of origin. Observed differences in treatment-related mortality were most probably caused by differences in age, comorbidities and performance status. R-CEOP should be considered as a regimen of choice for B-LCL patients with cardiac contraindications for anthracycline treatment.

E972

POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS: A SINGLE-CENTER CASE SERIES

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Background: Post-transplantation lymphoproliferative disease (PTLD) is a complication of both solid organ transplant (SOT) and haematopoietic cell transplant (HCT) and represent a very heterogeneous group.
Aims: The objective of this study is to evaluate the epidemiology, clinical features, characterization and therapeutic management of this disease.
Methods: We evaluated a total of 52 patients diagnosed between May 1995 and February 2017. We analyzed the following data: type of transplantation, immunosuppression time from transplantation to lymphoma diagnosis, clinical stage, response to treatment and survival. We classified them morphologically according to the WHO criteria (2016 revision), using immunohistochemical and molecular techniques and their relationship with the Epstein-Barr virus (EBV) by studying the expression of EBERs. We correlated clinical data with morphological, immunohistochemical and molecular results.
Results: Among the 52 patients, 31 were men (59.6%) and 21 women. PTLD after SOT were 45 (86.5%), of which 16 were after liver transplant (35.6%), 14 cardiac (31.1%), 9 pulmonary (20%), 4 renal (8.9%) and 2 double (cardiac-pulmonary and cardiac-renal) (4.4%). There were 7 PTLD after HCT, 2 identical HLA family donor, 2 unrelated donor, 2 dual umbilical cord blood and 1 autologous. Of the 52 PTLD, 48 were B lymphomas (92.3%), of which 26 were diffuse large B-cell lymphomas (DLBCL) (54.2%), 7 polymorphic (14.6%), 7 low-grade (14.6%), 4 Burkitt lymphomas (8.3%), 1 Hodgkin's lymphoma (2.1%) and 3 were non-classifiable. Other 4 PTLD were T lymphomas (6.7%), 2 anaplastic, 1 T/NK lymphoma, and 1 gamma/delta T lymphocytosis. 35/52 PTLD were EBV + (67.3%). The median time of immunosuppression was 123 months in renal transplant, 93 months in liver, 85.5 months in cardiac, 51 months in lung and 3 months in HCT. Histologically, it was 96 months in T lymphomas and 80 months in B lymphomas, being 51 months in EBV + and 124 months in EBV-. Fifty percent of Burkitt lymphomas were diagnosed after lung transplant, while 85% of low-grade lymphomas were diagnosed after liver transplant. Clinical stage was III/IV in 73% of the patients (38). Among the 52, 45 received treatment (86.5%), 37 with immunochemotherapy (82.2%) and 8 with Rituximab (17.8%). Three patients responded to reduction of immunosuppression (5.8%) and 3 did not receive any treatment for early death (5.8%). At the time of writing, 19 patients remain alive (36.5%) and 33 have died. The median survival of these patients was 19.5 months (0-198).
Summary/Conclusions: PTLD constitute a very heterogeneous group. Its appearance is much earlier in the HCT than in the SOT and, within this latter group, it is earlier after lung transplant and later after renal transplant. The most common type in our series is DLBCL. The majority are related to EBV, so post-transplant monitoring is essential, and its diagnosis is earlier than in EBV-. Most low-grade lymphomas appear post-liver transplant, either in relation to virus C or with autoimmune diseases. Survival is significantly lower than in other primary LPS. :AR-SA'>We analyzed the following data: type of transplantation, immunosuppression used in both induction and maintenance, immunosuppression time from transplantation to lymphoma diagnosis, clinical stage, response to treatment and survival. We classified them morphologically according to the WHO criteria (2016 revision), using immunohistochemical and molecular techniques and their relationship with the Epstein-Barr virus (EBV) by studying the expression of EBERs. We correlated clinical data with morphological, immunohistochemical and molecular results.

E973

SURVIVAL OUTCOMES AFTER FIRST-LINE THERAPY IN DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) USING A UNITED STATES (US) ELECTRONIC MEDICAL RECORD (EMR)-BASED COHORT

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Background: In the rituximab era, the recommended first-line therapy (1LT) in DLBCL patients who can tolerate combination therapy is rituximab combined with chemotherapy. For refractory/relapsed disease, high-dose chemotherapy with stem cell transplant, combination chemotherapy, or single-agent rituximab are considered. While the efficacy of rituximab has been shown in clinical trials, few studies have evaluated survival outcomes in patients seen in routine clinical care.
Aims: We evaluated survival outcomes in a US population of newly diagnosed DLBCL patients seen in routine clinical care.
Methods: In this retrospective study, adult patients ≥18 years old with newly diagnosed DLBCL were identified from the Humedica, a large US electronic medical record database, between 01/01/08 and 07/31/15. DLBCL diagnosis was determined by the presence of ≥1 inpatient record or ≥2 outpatient records with DLBCL diagnosis codes; the first DLBCL record served as the index date. Following the index date, initiation of 1LT for DLBCL was required. For the assessment of the survival outcomes, patients were evaluated from the date of treatment initiation until death, loss to follow-up, or end of study (09/30/15). Median progression-free survival (PFS) (defined as initiation of second-line therapy, evidence of supportive care >30 days after the end of a line of therapy, or death), median overall survival (OS), and PFS and OS rates at 2 years following initiation of 1LT were evaluated using unadjusted Kaplan-Meier analyses.
Results: 1,436 newly diagnosed DLBCL patients who initiated 1LT met the patient selection criteria. 54.0% were male, and the mean age was 66.4 years (SD: 13.7). At baseline, 27.4% of patients had a Charlson Comorbidity Index of ≥2, and the most common comorbidities were diabetes (20.3%), chronic pulmonary disease (15.5%), and moderate to severe renal disease (9.5%). In 1LT, 92.1% of patients received combination therapy, with R-CHOP (63.5%) being the most common combination therapy. 7.9% of patients received monotherapy upfront, with rituximab (77.2%) being the most commonly used single agent. At 2 years following initiation of 1LT, the Kaplan-Meier OS and PFS were 79.2% and 67.3%, respectively. Median OS was not reached, and median PFS was 53.9 months (95% confidence interval: 45.2, 61.5). OS and PFS were also compared among patients receiving monotherapy *vs* combination therapy in unadjusted analysis. At 2 years, OS was 80.2% for patients receiving combination therapy *vs* 67.4% (P=0.0093) for patients receiving monotherapy. Also at 2 years, PFS was 68.3% for patients receiving combination therapy *vs* 55.1% (P=0.0051) for patients receiving monotherapy.
Summary/Conclusions: In this population of patients with newly diagnosed DLBCL receiving 1LT, survival outcomes at 2 years were significantly improved for patients treated with combination therapy *vs* monotherapy. Future analysis will explore the differences in clinical characteristics of patients treated with monotherapy *vs* combination therapy in the 1LT setting.

Bleeding disorders (congenital and acquired)

E974

AN EXPERIENCE WITH LONG ACTING FACTOR VIII PROPHYLAXIS IN PAEDIATRIC AND YOUNG ADULT PATIENTS WITH HAEMOPHILIA A

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Background: Hemophilia is an X linked inherited bleeding disorder. Recurrent Joint bleeds and muscle bleeds are the commonest manifestations leading to long term comorbidities in hemophilia. High dose factor prophylaxis has been proven to be very effective in preventing joint problems in western world. We look for a cost effective and feasible way for Indian patients in terms of reduced dose and frequency of factor infusion. Data on prophylaxis with low dose long acting factor infusion on twice weekly dosing schedule is limited.
Aims: To study the efficacy and safety of long acting factor VIII (Eloctate) for tertiary prophylaxis in pediatric and young adult patients with moderate and severe haemophilia A.
Methods: Thirty eight patients with moderate and severe haemophilia A without inhibitors and age range from 1 to 25 years were included in this study. Patients were initially observed for 4 months during which they received therapeutic doses of long acting factor VIII, ELOCTATE (Factor VIII with Fc Fusion Protein) on episodic basis after clinical bleed. In next 4 months they received prophylactic ELOCTATE, given intravenously at doses of 20 unit/kg body weight on twice weekly schedule. Annual bleeding rates, school absenteeism, emergency visits, aspects of quality of life and joint scores were compared during observation and prophylaxis period.
Results: Total number of bleeds during observation and prophylaxis period was 607 and 90 respectively. Annual bleeding rate was 47.9 during observation period and 7.1 during prophylaxis. There was 85.1% reduction in bleeding rates on prophylaxis. School/college absenteeism was 3.1 days/ month and 0.84 days/month during observation and prophylaxis respectively. Emergency visits were significantly more during observation. None of the patients developed inhibitors and two patients had superficial thrombophlebitis during prophylaxis. Quality of life assessment using KIDSSCREEN QOL questionnaire showed moderate to marked improvement in quality of life domains during prophylaxis.
Summary/Conclusions: Low dose, twice a week, long acting factor VIII prophylaxis can be a reasonable option for patients with haemophilia A in developing countries. It significantly reduces joint bleeds, school absenteeism, Joint scores significantly without risk of inhibitor formation and also improves all domains of quality of life.

E975

NOVEL MUTATIONS IN THAI CHILDREN WITH CONGENITAL FACTOR VII DEFICIENCY

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Background: Congenital factor VII (FVII) deficiency is a rare autosomal recessive coagulation disorder resulted from mutations in the FVII gene (*F7*). The disease severity is not correlated with FVII levels but might be determined by molecular defects in *F7*.
Aims: To delineate the phenotypic and genotypic characteristics of patients with congenital FVII deficiency
Methods: We described demographic data, clinical manifestations, and outcome of patients with congenital FVII deficiency. *F7* mutation analysis was performed by PCR-direct sequencing.
Results: Of the ten patients diagnosed with FVII deficiency, five (50%) were males. The median age (range) at diagnosis was 19 days old (1-730). Consanguinity was found in 50% of the patients. Of the nine patients (90%) classified as severe, six patients presented with intracerebral hemorrhage within the first month of life, two presented with gastrointestinal bleeding and one presented with hemarthrosis. There were eight different alterations identified. Four have been previously reported (c.1091G>A (p.R364Q), c.1238G>A (p.R413Q), c.1256C>T (p.T419M), and c.681G>T (IVS6+1T)). Four were novel (c.1192G>T (p.D398Y), c.1313G>T (p.G420V), c.291+2T>C (IVS3+2T>C), and IVS6-2A>G) and associated with major bleeding especially during infancy.
Summary/Conclusions: This study reported Thai children with congenital FVII deficiency presented with life-threatening bleeding especially in the first year of life. Pathogenic including newly identified variants in the *F7* gene were detected in all cases. Genetic counseling can be appropriately provided to reduce the risk of disease recurrence in the families at risk.

E976

RETROSPECTIVE EVALUATION OF PHENOTYPE AND MANAGEMENT OF A-HYPO-FIBRINOGENEMIA IN A COHORT OF ITALIAN PATIENTS

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Background: Afibrinogenemia (AF) and hypo-fibrinogenemia (HF) patients (pts) experience hemorrhages or thromboses, and the clinical management can be difficult.
Aims: To obtain information on AF/HF clinical phenotype and management.
Methods: This is a spontaneous, retrospective, multicenter national study. Data are collected from clinical records.
Results: 2 AF and 12 HF pts have been enrolled (6M, 8F). Median follow-up: 39 months (1-553). Median fibrinogen activity/antigen level: 78mg/dL (0-150)/73mg/dL (0-140). Five pts experienced epistaxis, hematomas, ecchimoses, menometrorrhagia, intra-abdominal bleeding, gum bleeding, hemarthrosis. Fresh frozen plasma, fibrinogen concentrate (FC), cryoprecipitate, whole blood, tranexamic acid were administered in the majority of these events. One ischemic stroke, 1 lower limb arterial and 1 cerebral sinus thrombosis, 1concomitant aortic and inferior vena cava thrombosis occurred: 3 events during FC therapy, 1 during puerperium. Heparin, low molecular weight heparin (LMWH), anti-platelet agents, fibrinolytic agents, warfarin were then administered. One gastrectomy, 1 lower limb amputation, 5 gynecological, 1 otorhinolaryngological and 1 plastic surgery were performed in 2AF and 3 HF pts: in AF pts, FC or FFP prohylaxis was administered while in HF pts was not. Bleeding was observed after 2 surgeries performed without prophylaxis. Eight pregnancies were initiated in 3 HF women. Two spontaneous deliveries (SD) and 2 cesarian sections (CS) were performed; 4 abortions occurred. FC prophylaxis and LMWH were administered during pregnancy in 3 and 4 cases, respectively. One venous thrombosis, 2 hemorrhages, 1 DIC and 4 complicated pregnancies were recorded. FC was administered at delivery and LMWH during puerperium, for the 2 CS. No complications at delivery occurred.
Summary/Conclusions: AF and severe HF pts experience severe hemorrhagic/thrombotic events. The intervening clinical situations are difficult to manage. Further large scale data collections are necessary in order to provide useful information to better characterize and manage patients suffering from these rare diseases.

E977

RETROSPECTIVE REVIEW OF FOUR DAYS OF VON WILLEBRAND'S FACTOR AS SURGICAL PROPHYLAXIS IN VON WILLEBRAND'S DISEASE

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Background: Von Willebrand disease (vWD) is the most common inherited bleeding disorder that manifests as easy bruising, mucocutaneous bleeding and excessive hemorrhage with invasive procedures. In 2007, Humate-P, a lyophilized concentrate of purified VWF and FVIII, was approved in the United States for treatment and prophylaxis. Current guidelines per National Heart, Lung, and Blood Institute (NHLBI) Expert Panel recommend 40-60 U/kg preoperatively, followed by maintenance dose of 20-40 U/kg for 7-14 days for major surgery and 5 days for minor surgery. Here, we report a single-institution experience of a short course of Humate-P as surgical prophylaxis.
Aims: To assess if an abbreviated schedule of Humate-P given as perioperative dose of 40 U/Kg for 2 days (one dose pre-op and one post op) for extensive dental procedures and for 4 days (one dose pre-op and for 3 days post-op) for minor and major surgical procedures as surgical prophylaxis would result in equi-efficacious hemostasis without compromising patient outcomes.
Methods: We retrospectively identified 202 patients with known diagnosis of VWD at our institution that underwent surgical procedures requiring prophylaxis between 2002-2017. Ninety elective surgical events occurred among these patients that required peri-operative prophylaxis with Humate-P. These patients were treated with peri-operative dose of 40 U/Kg on D0-1 for extensive dental procedures and for D0-3 for minor and major surgery. The definition of bleeding risk was based on NHLBI guidelines.
Results: Eighteen (20%) were males and 72 (80%) were females. Type I VWD constituted 94.4% (85/90). 84.4% Caucasian patients, 8.8% African American, 1.1% South Asian. Twenty-nine (32.2%) procedures were categorized as major surgeries, 51 (56.6%) were minor and 2 (2%) uncomplicated dental procedures. Eighty-four (93.3%) achieved excellent hemostatic efficacy defined as clinical hemostasis within normal limits. Six (6.6%) surgeries had good hemostatic efficacy defined as slight oozing. Five (6%) patients required blood transfusions

(1-3 units packed red blood cells). 100% excellent-good outcomes similar to longer factor replacement schedules were noted. No deaths or thromboembolic events were noted. One patient required re-admission for post-op hematoma following gynecologic procedure. Thirty-four (37.7%) patients were discharged either same day or after overnight observation. Von Willebrand factor (VWF) is necessary to form a platelet plug by recruiting Factor VIII and platelets to damaged vessels walls. We believe that four days is sufficient time for the formation a stable platelet plug and further downstream hemostasis is presumably VWF-independent. Here, we demonstrate safety and efficacy of a two to four day regimen of Humate-P, with only one person requiring re-admission for a post-operative hematoma. Current guidelines for surgical prophylaxis of VWD are based on expert opinion and lack level 1 evidence. Prolonged exposure may place patients at risk for unnecessary side effects, including thromboembolism and protracted hospitalization, and also causes financial toxicity. One unit of Humate-P costs 1.2 USD, which translates to enormous cost savings. Cumulatively, we were able to save ~1.5million USD in these 90 surgical events using our abbreviated schedule.
Summary/Conclusions: Perioperative dose of 40U/Kg for 2 days (one dose pre-op and one post op) for extensive dental procedures and for 4 days (one dose pre-op and for 3 days post-op) for minor and major surgical procedures are associated with excellent hematologic outcomes and significant cost savings. However, this is single institutional data with limited number of patients and there remains a need for further studies to better define the exact dosing and duration of surgical prophylaxis.

E978

AUDIT ON MANAGEMENT OF HIGH INTERNATIONAL NORMALIZED RATIO (INR) IN WARFARINISED INPATIENTS
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Background: Warfarin is the commonest used oral anticoagulant with an effective antidote. The British Committee for Standards in Haematology guidelines recommend administration of 25-50µg of four factor Prothrombin Complex Concentrate (PCC) and intravenous (IV) Vitamin K 5mg for patients with major bleeding, 1-3mg of Vitamin K intravenously for those with minor bleeding and 1-5mg of Vitamin K orally for patients with INR >8 and who have no signs of bleeding.
Aims: The aim of this audit was to compare our hospital's performance against the above guidelines.
Methods: A total of 76 patients admitted between 01/08/2015-31/01/2016 were analysed retrospectively.
Results: There were 103 incidents with INR level 5-8 and 24 with INR >8 in these 76 inpatients. Bleeding was documented in 18/127 cases, which included 6 incidents of major and 12 incidents of minor bleeding. In major bleeding, warfarin was withheld and Vitamin K administered. However, 4/6 (66.7%) of these patients got a dose different to 5mg advocated. Also, PCC was prescribed in only 50% of these patients. While 9/12 (75%) patients with minor bleeding received Vitamin K, only 3 of these 9 patients received the recommended dose of 1-3mg IV. Vitamin K was unnecessarily given to 9/83 (10.8%) non-bleeders with an INR between 5-8. Additionally, the recommended dose and route of administration of Vitamin K 1-5mg PO was followed only in 7/16 (44%) of non-bleeders with INR >8.
Summary/Conclusions: Our audit highlighted that there is less than 100% compliance in the recommended dose and route of vitamin K administration. A flowchart containing the guidelines will be designed to improve the management of high INR. To increase the awareness of this issue, teaching sessions for junior doctors and nursing staff are planned. A re-audit will be conducted once these steps are in place.

E979

NOVEL AND RECURRENT F7 MUTATIONS IN KOREAN PATIENTS WITH COAGULATION FACTOR VII DEFICIENCY
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Background: Coagulation factor VII deficiency is one of rare hereditary bleeding disorders with relatively limited clinical and genetic data.
Aims: This study aimed to characterize *F7* gene mutational patterns of Korean patients with coagulation Factor VII deficiency including their clinical and laboratory variability.
Methods: *F7* gene mutations of total 16 unrelated Korean patients with Factor VII deficiency were identified by direct sequencing analyses of all exons and flanking intronic sequences. Variants were assigned according to the recently released criteria of 2015 ACMG standards and guidelines.
Results: A total of 14 mutations (pathogenic or likely pathogenic) were detected including four novel mutations (Glu66Lys, c.681+3A >T, Glu66Alafs, Ile290del). Six (38%) patients have 2 mutant alleles and three mutations were recurrently identified. The most frequent mutation detected in this study was Cys389Gly detected in 37% (11/30) patients, validating the data of our previous patient cohort.
Summary/Conclusions: Correlation of genetic data with coagulation laboratory and clinical findings suggested the presence of modifiers, which warrants further investigation in a larger cohort of patient for better clinical prediction and management in this rare bleeding disorder.

Bone marrow failure syndromes incl. PNH - Clinical

E980

Abstract withdrawn.

E981

UTILITY OF CD157 IN A FLAER BASED SINGLE TUBE FIVE COLOR COMBINATION FOR SCREENING OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA CLONE

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Background: Fluorescent Aerolysin (FLAER) based flow cytometric analysis of polymorphs and monocytes is the gold standard for the screening of paroxysmal nocturnal hemoglobinuria (PNH) clone. In recent years CD157 has been identified as a PNH marker which targets both polymorphs and monocytes. It can be used in a single tube five color combination to screen polymorphs and monocytes simultaneously.
Aims: The objective of this study was to analyse the utility and advantage of CD157 in the PNH screening along with its ability to replace CD24 and CD14.
Methods: Our routine protocol for PNH screening included single tube six color antibody cocktail in following combination: FLAER-AF488, CD24-PE, CD15-PerCPCy5.5, CD14-PeCy7, CD64-APC, CD45-APC H7. We assessed the utility of single tube 5 color combination of FLAER-AF488, CD157-PE, CD15-PerCPCy5.5, CD64-APC, CD45-APCH7 for PNH screening and compared the results with the routinely used 6 color panel. Laboratory cutoff for CD157 was defined by running 10 samples from healthy individuals. Sensitivity analysis was assessed in spiking experiments by diluting a PNH positive sample with large clone size in a serial 10 fold dilution. Inter assay and intra assay precision analysis was done by running samples in triplicates across different clone size range and calculating the coefficeint of variance (CV). Correlation of PNH clone size obtained from CD24/CD14 and CD157 was assessed by analysing a total of 30 samples across a wide range of PNH clone size (0.06-97.3%).
Results: CD157 was sensitive at the level of 10^{-4} and better. Frequency of cells with PNH phenotype in normal samples were found to be <0.002%. The CVs of intra-/interassay precision analysis ranged from 0.9/2.6% to 3.2/4.64% for granulocytes and 1.9/2.5 to 5.3/8.9% for monocytes. The PNH clone size as obtained by CD157 based analysis was highly comparable to those obtained by CD24/CD14 based assay (R^2>0.993). CD157 was found much better than CD24/CD14 in identifying the type II PNH clones. There was no false positive or false negative result. The cost of analysis was found to be approximately 15% lesser than the routinely used 6 color assay.

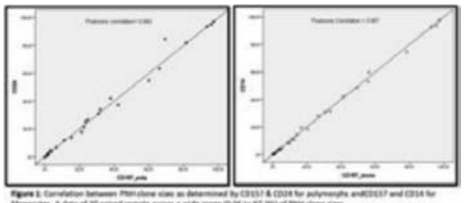

Figure 1.

Summary/Conclusions: CD157 is a robust, reliable and potentially useful universal marker for PNH screening. Its inclusion in a single tube five color FLAER based panel is a cost effective approach which is ready to replace CD24/CD14 from routine PNH screening.

E982

IMMUNOPHENOTYPIC DYSPLASTIC FEATURES IN PATIENTS WITH APLASTIC ANEMIA

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Background: Multicolor flow cytometry (MFC) of bone marrow (BM) is a promising additional approach to the diagnosis of myelodysplastic syndromes (MDS). Aplastic anemia (AA) as MDS characterizes by cytopenias and dysplastic features in BM by morphology are absent. It is well known that up to 15% of AA transformed in MDS over time. It is possible to suggest that in some cases of AA immunophenotypic abnormalities can also be identified.
Aims: To study and compare the presence of dysplastic features by MFC in AA and MDS without excess of blasts.
Methods: The study included 14 patients with AA (8m, 6f, median age 33), 28 patients with MDS *de novo* without excess of blasts by morphology (13m, 15f, median age 59). MDS group included 3 patients with 5q-syndrome, 4 - RCUD, 3 - RARS, 18 - RCMD. 20 patients with cytopenias constituted the control group (4m, 16f, median age 42) due to B-12 deficiency anemia, iron-deficiency anemia, Fanconi anemia, hemolytic anemia, β-thalassemia, ITP, hepatitis C, multiple myeloma, Burkitt's lymphoma. BM of 33 healthy donors were analyzed for the reference values. MFC was performed according to International LeukemiaNet by 6-color cytometer BD FACSCanto II. We enumerated the proportion of CD34+ myeloid cells from CD45+ cells (normally <2%), the proportion of CD19+ (B-cell progenitors) from CD34+ cells (normally >5%), the expression of CD34, CD45, CD117, CD7, CD56 on CD34+ myeloblasts. Among granulocytes we analyzed: their proportion, granularity, CD14, CD64, CD10, CD56 expression and patterns CD16vsCD13, CD16vsCD11b, CD13vsCD11b. Among of monocytes we measured: their proportion, CD64, CD56 expression and analyze patterns CD14vsCD36, CD11bvsCD13, CD11bvsHLA-DR. The final MFC conclusion was done by scale Ogata/Wells (van de Loosdrecht, 2013): A - does not correspond to MDS; B - reveales some features which commonly appears in MDS; C - results are consistent with MDS.
Results: Among MDS patients without excess of blasts assessment "B" and "C" scores were obtained in 78.6% (sensitivity). Increased proportion of CD34+ myeloblasts was in 35.7% of cases, increased CD56 and CD7 - in 42.9%. The most common abnormalities were: increased CD56 (53.6%), abnormal patterns (39.3%), low granularity (35.7%) in granulocytes; increased proportion (21.4%) and abnormal patterns (28.6%) in monocytes. 64.3% (n=9) patients with AA were assessed as "A", 21.4% (n=3) - "B" and 14.3% (n=2) - "C". In cases with "C" abnormal expression of CD117 and expression of CD56 on CD34+ cells were seen. AA patients with "B" and "C" showed increase expression of CD56, CD64 and decrease CD10 expression on granulocytes. Abnormal patterns were less common than in MDS patients. The increased proportion and CD56 expression in monocytes were more frequent than in MDS patients.
All patients not diagnosed with AA, MDS were assessed as "A" (specificity-100%). But some MFC abnormalities were found in them: abnormal expression of CD34 (35%) and CD45 (30%) on CD34+ myeloblasts, and increased CD64 expression (20%) on granulocytes (pic.).

Table 1.

Diagnosis	MDS		not AA, not MDS		AA	
number	28		20		14	
A by Ogata/Wells	6	(21.4%)	20	(100.0%)	9	(64.3%)
B by Ogata/Wells	5	(17.9%)	0	(0.0%)	3	(21.4%)
C by Ogata/Wells	17	(60.7%)	0	(0.0%)	2	(14.3%)
CD34+ cells						
%CD34+ myeloblasts ≥2%	10	(35.7%)	1	(5.0%)	0	(0.0%)
%CD34+/19+ <5%	13	(46.4%)	3	(15.0%)	5	(35.7%)
abnormal expression CD45	15	(53.6%)	6	(30.0%)	4	(28.9%)
abnormal expression CD34	7	(25.0%)	7	(35.0%)	1	(7.1%)
abnormal expression CD117	21	(75.0%)	3	(15.0%)	1	(7.1%)
increased CD56	12	(42.9%)	2	(10.0%)	2	(14.3%)
increased CD7	12	(42.9%)	1	(5.0%)	1	(7.1%)
Granulocytes						
decreased %	15	(53.6%)	6	(30.0%)	4	(28.6%)
low granularity	10	(35.7%)	0	(0%)	1	(7.1%)
increased CD64	6	(21.4%)	4	(20.0%)	9	(64.3%)
increased CD56	15	(53.6%)	2	(10.0%)	5	(35.7%)
decreased CD10	3	(10.7%)	0	(0.0%)	1	(7.1%)
abnormal CD13 vs CD16	11	(39.3%)	1	(5.0%)	4	(28.6%)
abnormal CD13 vs CD11b	11	(39.3%)	1	(5.0%)	2	(14.3%)
abnormal CD16 vs CD11b	6	(21.4%)	0	(0%)	3	(21.4%)
Monocytes						
decreased %	1	(3.6%)	1	(5.0%)	0	(0.0%)
increased %	6	(21.4%)	0	(0.0%)	7	(50.0%)
increased CD64	3	(10.7%)	1	(5.0%)	4	(28.6%)
increased CD56	6	(21.4%)	0	(0.0%)	6	(42.3%)
abnormal HLA-DR vs CD11b	8	(28.6%)	2	(10.0%)	1	(7.1%)
abnormal CD13 vs CD11b	8	(28.6%)	1	(5.0%)	1	(7.1%)
abnormal CD36 vs CD14	5	(17.9%)	0	(0.0%)	2	(14.3%)

Summary/Conclusions: Flow cytometry MDS study with Ogata/Wells scale has a high sensitivity and specificity. Immunophenotypic abnormalities characterizing dysplastic features can also be found in AA patients up to 35% of cases. Increased expression of CD56 on CD34+ myeloblasts, granulocytes and monocytes is commonly found in AA patients. Perhaps the appearance of MFC dysplastic features foreshadows the MDS-transformation of AA, but requires further prospective studies.

E983

SURGICAL MANAGEMENT OF PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) – DATA FROM THE SPANISH PNH REGISTRY

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Background: Patients with PNH often need surgery due to disease-related complications. However, surgery, anesthesia, and possible surgical complications, including inflammation and acidosis, can trigger complement activation, making surgery an important risk factor for hemolysis (Ando K, *et al.* Ann Hematol 2012;91:1987-8; van Bijnen ST, *et al.* Eur J Haematol 2011;87:376-8).
Aims: Here we report data on the clinical management and treatment results of patients with PNH undergoing surgery.
Methods: We collected data on 14 surgical interventions of 11 patients (8 males; age, 25-76 years). All patients had a high prevalence of PNH clone cells (55-99% in PMN) and were receiving eculizumab (ECU). Types of surgery were: 6 laparoscopic cholecystectomies, a transjugular intrahepatic portosystemic shunt, a distal splenorenal shunt, a laparoscopic Achilles allograft ligamentoplasty, a gastrectomy, an emergency appendectomy, and 3 urologic interventions. Ten patients received ECU 900mg, while one (patient E, surgery 6) received 1200mg since he had developed hemolysis at a previous surgical intervention (surgery 5). In two cases (patient G, surgery 8; patient H surgery 11), an additional dose of ECU was administered before surgery. Patient H (surgery 11) had developed hemolysis at previous surgical interventions (surgeries 9 and 10). In most cases, either the date of the ECU dose was taken into account when scheduling surgery or the ECU dose was moved forward to coincide with the date of surgery. The time between the last ECU dose and surgery was normally one day (range, 1-8).
Results: In nine cases, transfusions were required due to hemorrhagic complications. Patient I (surgery 12) had a thrombotic event leading to acute myocardial infarction one week after surgery. Increased hemolysis was observed (increased LDH and/or presence of hemoglobinuria) in five cases (patients E, H, I and K; surgeries 5, 9, 10, 12 and 14) during the week following surgery. Two of these patients (patients E and H) later underwent additional surgery (surgery 6, and surgeries 10 and 11, respectively). The pre-surgical ECU dose was increased in surgery 6 (patient E) and an extra dose was administrated in surgery 11 (patient H) and no hemolysis was observed. (See Table 1).

Table 1.

Surgery	Patient	Patient Age at Surgery	Surgical Procedure	Pre-surgical ECU Dose	Days between last ECU Dose and Surgery	Transfusion	Post-surgical Increase in Hemolysis
1	A	56	laparoscopic cholecystectomy	900mg	2	2 Packed red blood cells (PRBCs)	no
2	B	58	laparoscopic cholecystectomy	900mg	1	2 PRBCs	no
3	C	38	portosystemic shunt	900mg	7	4 PRBCs	no
4	D	46	distal splenorenal shunt	900mg	1	4 PRBCs + 1 platelet pool	no
5	E	49	ligamentoplasty	900mg	1	no	yes (1 week after surgery)
6	E	50	laparoscopic cholecystectomy	1200mg	1	no	no
7	F	76	laparoscopic cholecystectomy	900mg	7	no	no
8	G	67	gastrectomy	900mg (additional dose)	1	no	no
9	H	69	left ureterorrenoscopy and lithotripsy with placement of double J stent	900mg	4	3 PRBCs	yes
10	H	69	replacement of double J stent	900mg	8	3 PRBCs	yes
11	H	69	Holmium laser lithotripsy with placement of double J stent	900mg (additional dose)	1	1 PRBCs	no
12	I	63	emergency appendectomy	900mg	2	4 PRBCs	yes
13	J	60	laparoscopic cholecystectomy	900mg	5	no	no
14	K	25	laparoscopic cholecystectomy	900mg	1	2 PRBCs	yes

Summary/Conclusions: Our findings lead us to recommend to perform the intervention within 24 hours of the administration of Ecu in programmed surgery for which it is necessary to program the dose. While in urgent surgical interventions put a new dose on the day of the intervention independently of the previous dose. Also the normal ECU dose could be increased or an extra dose be administered in order to minimize the risk of hemolysis in high-risk patients or in those with a previous history of surgery-related hemolysis.

E984

EFFICACY OF ECULIZUMAB IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) PATIENTS WITH OR WITHOUT APLASTIC ANEMIA; PROSPECTIVE STUDY OF KOREAN PNH COHORT

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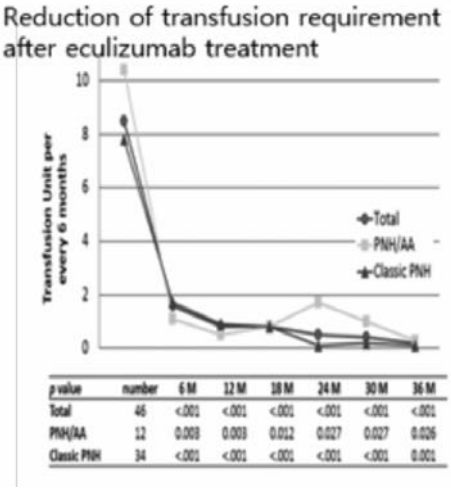
Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a hematopoietic stem cell disease characterized by the intravascular lysis of red blood cells. PNH patients often have underlying bone marrow failure (BMF), with aplastic anemia (AA) as the most frequently associated type. Eculizumab, a humanized monoclonal antibody that binds specifically to human complement protein C5, has been used in Korea since 2012.
Aims: The purpose of this study was to determine whether eculizumab-treated patients show clinical benefit and reduced risk of complications regardless of concomitant AA in a Korean population.
Methods: Forty-six PNH patients ≥18 years of age diagnosed by flow cytometry and treated with eculizumab for more than 6 months were analyzed in the prospective Korean PNH registry. Patients were categorized into two groups: PNH patients with concurrent AA (PNH/AA) and without (classic PNH). Patients with severe AA/PNH were excluded. Biochemical indicators of intravascular hemolysis, hematological laboratory values, transfusion requirement, and PNH-associated complications assessed by the treating physician were reported every 6 months after enrollment.



p value	number	6 M	12 M	18 M	24 M	30 M	36 M
Total	46	<.001	<.001	<.001	<.001	<.001	<.001
PNH/AA	12	0.008	0.008	0.012	0.027	0.027	0.026
Classic PNH	34	<.001	<.001	<.001	<.001	<.001	0.001

Figure 1.

Results: The median age of the study population was 49 years (range, 18-73 years) at eculizumab initiation and the median duration of eculizumab treatment was 34 months (range, 6-44 months). Median LDH fold x upper limit of normal was 7.29 (range 2.4-23.7) and GPI-deficient granulocytes was 92.8% (range, 15.7-100%) at the time of eculizumab treatment. PNH-related signs and symptoms were thromboembolism (TE, n=19), renal failure (n=20), pulmonary hypertension (n=5), and severe/recurrent abdominal pain requiring opioids (n=17). Of 46 total patients, 12 (26%) were classified as having PNH/AA and 34 with classic PNH. There were no substantial differences in laboratory findings, transfusion requirement, or clinical signs and symptoms at baseline between the two groups. Treatment with eculizumab induced a rapid inhibition of hemolysis. At the time of 6 month follow-up, LDH level decreased to near normal levels in all patients and this effect was maintained until 36 months follow-up regardless of concomitant AA. Mean hemoglobin level significantly increased from the first 6 months of eculizumab treatment, and the effect (hemoglobin above 10 g/dL) was sustained throughout 36 months in both groups. Transfusion-independence was achieved by 54.3% within the first 6 months of treatment and 86.4% by the last 36 months (83.3% in PNH/AA *vs* 87.5% in classic PNH). The mean number of RBC units transfused was significantly reduced from 8.5 units during the previous 6 months to 1.6 units for the first 6 months in total PNH patients (Fig). There were no significant differences in clinical outcomes (ie, LDH and transfusion unit per every 6 months) with eculizumab between the two groups. All TE (n=19) patients in whom 6 received concomitant anticoagulation therapy were resolved on the eculizumab; one classic PNH patient had recurrence of TE at the same site after discontinuation of anticoagulation therapy while on eculizumab. Among 9 patients who had baseline eGFR less than 60 ml/min/1.73m^2, 5 patients (56%) showed improvement of eGFR during the eculizumab treatment and 4 patients stabilized eGFR.

Summary/Conclusions: Clinical outcomes with eculizumab were significantly improved compared with the baseline in patients with both PNH/AA and classic PNH. This study demonstrated that eculizumab has a beneficial role in the management of patients with PNH/AA, similar to that of classic PNH, by inhibiting hemolysis and reducing transfusion requirements, thus resulting in the improvement of clinical signs and symptoms.

E985

DIAGNOSIS AND FOLLOW-UP OF THE CLONES OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA BY FLOW CYTOMETRY

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a very rare chronic disease associated with a clonal expansion of one or several hematopoietic stem cells carrying acquired somatic mutations of PIG-A gene resulting in GPI-AP deficient blood cells and great susceptibility to complement mediated cell lysis. Diagnostis of PNH is of importance and flow cytometry (FC) is a required tool for this. We report 33 cases of PNH diagnosed and monitored by FC.
Aims: To show the interest of flow cytometry for the diagnosis and follow-up of PNH clones in some risky haemopathies.
Methods: A PNH clone has been researched in 234 patients since August 2009 to January 2017. The PNH clone was investigated for bone marrow aplasia with or without haemolysis, regenerative hemolytic anemia with negative direct coombs test (DCT), myelodysplasia (MDS), unexplained cytopenia and thrombosis. The search for the PNH clone by FC is based on the analysis of the following monoclonal antibodies: Flear and CD59 with gating on CD45 for neutrophils, Flear and CD14 with gating on CD64 for monocytes and CD59 with gating on Glycophorin A for red blood cells. We judged that the patient has an PNH clone when the deficiency is >05% on at least two markers highlighted on two different lines. FC surveillance is provided in the absence of a deficit or in the case of a very moderate or single-line deficit.
Results: Out of 234 cases analyzed, 201 cases (85%) showed absence of PNH clone and 33 cases (14%) had a PNH clone. There are 14 women and 19 men; Sex ratio (M/F) = 1.35, mean age = 42.27 years (17-73). Among patients that should be screened for positive PNH clone we have bone marrow failure: 25 positive (21,9%) of 114 cases screened, hemolytic anemia with negative direct coombs test: 4 positive/63 cases (6.34%), thrombosis: 2 positive/28 (7.14%), one negative case of AML2, myelodysplasia with 02 (11.2%) positive/18 cases and cytopenias: 0 positive/13 cases. The types of PNH was type II in 3 cases (9%), type III in 24 cases (72.8%) and mixed deficits in 6 cases (18.2%). The mean degree of CD59 deficiency was 29.4% (5-82) on red blood cells 48.21% (5-95) on neutrophil (N); the mean degree of Flear was 55.33% (6-99) on N in 22 cases ; the mean degree of CD14 deficiency on monocytes was 44% (7-97) in 17 cases, the mean degree of Flear (8 cases) was 51.8% (12.9-92). During surveillance, PNH clone appeared in 02 cases and clone size increase in 08 cases (initially less than 2%) and in one case the clone doubled in size from 54% to 98% and 6 months after the patient developed extensive thrombosis. In 44 cases, a moderate deficiency <5% was noted on a single line either N or red blood cells, thus monitoring by FC.
Summary/Conclusions: In our study the clone PNH was noted in 11.2% of cases of MDS which joins the literature were the rate is 10 to 23%. (1). In the group where a moderate deficit was observed, there are no biological signs of haemolysis ; in these cases, the indication of a follow-up of the size of the PNH clone is then necessary and must include a haemolysis report repeated with LDH assay; this is verified in our study since in 8 cases of increase in the size of the clone a thrombosis developed in one case. The follow-up of clone sizes is important given that there are 8 cases of increase in clone size, and in 01 case the increase was accompanied by thrombosis. This interest was confirmed by the study of B. Höchsmann who demonstrated in 155 cases of followed PNH, a significant increase of the clone in 28% of the cases and in 9% of the cases a new clone appeared. (2). The application of flow cytometry enabled us to make the diagnosis and to determine the phenotypic profile of PNH clones and to specify their sizes as well as to follow up the patients.

E986

ASSOCIATION OF T-, B-, NK AND NKT CELLS WITH THE DURATION, COMPLETENESS AND OTHER CHARACTERISTICS OF REMISSION IN PATIENTS WITH APLASTIC ANEMIA

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Background: Immune-mediated dysregulation of hemopoiesis is the basis for pathogenesis of aplastic anemia (AA). Dysbalance of T cell subsets, especially Th1 and Th2 and produced by them cytokines serves as a possible mechanism of this phenomenon. It is suggested, that NK-T cells play an important role in regulation of Th1:Th2 balance. The role of NK-T cells in development of aplasia of hemoiesis in AA now is broadly studied. Nevertheless, up to this moment, the features of balance of T lymphocyte subsets, and, especially NK-T cells during stable and prolonged remission are not characterized yet.
Aims: To evaluate the association of T-, B-, NK и NK-T cell levels of AA patients with the duration of remission, its completeness, duration of period free of immunosuppressive therapy (IST) and the size of PNH-clone.
Methods: The studied group included 36 patients with AA in remission, reference group – 20 patients with primary diagnosed AA. Level of T-, B-, NK and NK-T cells in peripheral blood (PB) and bone marrow (BM) was evaluated using 5-color flow cytometry (Beckman Coulter, FC-500).
Results: Group of AA patients in remission was divided into subgroups in four variants: 1) according to the remission duration (<12 months, 12-24 months, 24-36 months, >36 months); 2) completeness of remission: partial (PR) and complete (CR); 3) duration of IST-free period (<1 year, ≥1 year); 4) PNH-clone size (0,1-1%, 1-10%, >10%). Levels of T-, B- and NK cells in AA patients with remission varied broadly in different subgroups, but there were not revealed any clear tendency of their dynamics in all assigned subgroups, except for NK-T cells. In primary AA patients the level of NK-T cells in PB and BM exceeded normal level 1,8- and 2,2-fold, respectively. In remission lasting less then 12 months NK-T cell level decreased significantly; then, along with increase of remission duration (24-36 months), it normalised, and in patients with remission ≥36 months it significantly decreased both in PB and BM (data presented in **table 1**). In patients with PR, as compared with primary AA patients, NK-T cells decreased 2,8- and 1,9-fold, respectively, and further in CR this tendency persisted. Duration of IST-free period less than 1 year and ≥1 year was also accompanied by a significant and stable decrease of NK-T cells in PB and BM. It is known that PNH-clone presence is a favourable factor for treatment response of AA patients. Therefore, it was of interest to study the association of NK-T cell level in AA remission with the size of PNH-clone. In subgroup with small PNH-clone (0,1-1%) NK-T cell level was decreased as compared to primary AA patients, and it significantly decreased further along with growth of PNH-clone size.

Table 1. NK-T cell level (%) in patients with AA in remission according to subgroups.

Group	Primary AA	Remission duration (months) (1)				Remission completeness (2)		IST-free period (years) (3)		Size of PNH-clone (4)		
		<12	12-24	24-36	>36	PR	CR	< 1	≥1 y.	0,1-1%	1-10%	>10%
PB	8,6	2,4	3,9	4,9	1,8	3,1	2,9	2,7	1,7	3,8	1,1	1,7
BM	10,5	4,9	4,3	n.d.	3,4	5,6	4,3	6,4	4,0	5,7	5,4	2,9

Summary/Conclusions: Thus, in patients with AA the decrease of NK-T cell level was observed along with recovery of hemopoiesis in all the subgroup variants. Previously we have shown that the decrease of NK-T cells accompanies the decrease of TNFα and increase of IL-4. This pattern, taking into consideration our earlier obtained data, may be the evidence of the role of NK-T-cells in regulation of balance Th1:Th2 and produced by them cytokines.

E987

A NOVEL DUAL-REAGENT SINGLE TUBE FLOW CYTOMETRIC ASSAY TO SCREEN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired hematopoietic stem cell disorder resulting from loss of membrane-bound glycosyl phosphatidy linositol (GPI) anchor protein. The disease is characterized by heterogeneous clinical phenotypes including intravascular hemolysis, cytopenia(s), bone marrow hypoplasia and atypical site thrombosis. Screening guidelines recommend documentation of the lack of at least two GPI-liked antigens on at least two cell lineages. Alexa fluor 488 conjugated fluorescent Aerolysin (FLAER-AF488) has become a mandatory component in FCM based PNH assays.
Aims: We have analyzed the feasibility of a novel dual-reagent assay for a simplified, single tube, cost-effective approach for PNH screening.
Methods: EDTA anti-coagulated peripheral blood of patients referred to department of Hematology with clinical suspicion of classical-PNH/ aplastic anemia, was tested with a single tube panel of FLAER-AF488/CD33APC. Simultaneously, the routine two tube flow cytometry assay (established sensitivity of 0.1%) used in our lab for PNH screening (FLAER/ CD15/ CD24 for granulocytes and FLAER/CD33/CD14 for monocytes) was performed in the same sample. Each tube was run till a minimum of 50,000 granulocytes were acquired or till the tube ran dry. A cluster of at least 20 FLAER negative events was considered for reporting PNH-clone positivity in both granulocytes and monocytes. The granulocyte and monocyte PNH positivity and the respective clone sizes detected by both the strategies were compared.
Results: A total of 33 patients and 7 healthy controls were analyzed by both dual-reagent and conventional strategies. Among the thirty-three patients, twelve patients concurrently showed the presence of PNH clones by both methods (36%). The rest twenty-one patients were negative for PNH clones in granulocytes and monocytes by both strategies, indicating complete concordance at a sensitivity of 0.2% (Chi Square p=0.000). Of the PNH positive cases, the mean PNH clone sizes among the granulocytes by dual-reagent and conven-

tional methods were 3.79% (range, 0.2-18.2) and 3.60% (range, 0.1-18.6), respectively. The mean PNH clone sizes among the monocytes by dual-reagent and conventional methods were 7.30% (range 0.2-29.4) and 7.32% (range 0.1-28.8), respectively. There was no significant difference in the granulocyte and monocyte PNH clone sizes determined by both the methodologies (p=0.000). There were significant correlations between the granulocyte PNH clone sizes (Pearson's r=0.993, p=0.000) and the monocyte PNH clone sizes (Pearson's r=0.991, p=0.000) detected by both the analysis strategies.
Summary/Conclusions: This pilot study demonstrates the practical feasibility of a simple, cost-effective and widely applicable dual-reagent, single tube PNH-screening assay at a sensitivity of 0.2%.The study needs to recruit patients of various hematological disorders besides healthy controls, and although seems effective for analyzing classic and subclinical PNH, the strategy has to be further standardized to achieve a sensitivity of 0.01%.

E988

TREATMENT OF REFRACTORY APLASTIC ANEMIA WITH ELTROMBOPAG: EXPERIENCE OF A CENTER
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Background: Eltrombopag, a thrombopoietin receptor agonist, was approved in 2008 for the treatment of immune thrombocytopenic purpura. More recently, benefits demonstrated in the proliferation and maintenance of hematopoietic stem and progenitor cells (HSTC) led to its use and approval in the treatment of severe aplastic anemia (AA) refractory to immunossupressive therapy.
Aims: In this report, we evaluated response to eltrombopag in patients with refractory AA and associated side effects.
Methods: Retrospective analysis of six patients with a diagnosis of aplastic anemia and thrombocytopenia (platelet count ≤30.000/uL), refractory to imunossupressive therapy and ineligible for allotransplant, treated with eltrombopag. Patients characteristics, response, clinical evolution and adverse effects were evaluated.
Results: Four patients were female and median age at diagnosis was 66 years (36-76). Previous treatments included horse antithymocyte globulin (1), cyclosporine (4), intravenous immunoglobulin (1), corticosteroids (4) and danazol (1). Treatment with eltrombopag was associated to cyclosporine in four patients; two cases had chronic renal failure and consequent contraindication to cyclosporine. The median duration of treatment with eltrombopag at the time of this analysis was 7 months (3-12). At 3 months, all patients had platelet counts >30.000/uL (median increase, 16.500/uL). Five patients improved hemoglobin levels (median increase, 2.2g/dL); 3 of them were previously dependent on red cell transfusions, and no longer needed transfusions. Four patients increased neutrophil counts (median increase, 1110/uL). All but one patient received a maximum dose of 150mg per day. Only one patient needed temporary discontinuation due to hepatic abnormalities, that were rapidly resolved. One other patient had mild elevation of liver enzyme levels. No other relevant side effects occurred.
Summary/Conclusions: Treatment with eltrombopag was associated with hematologic response of one or more hematopoietic lineage, independence of blood transfusions and improved quality of life of patients with refractory severe AA. Except for infrequent and reversible hepatic abnormalities, tolerability was excellent. Thus, eltrombopag might be used in situations where other measures have failed in patients who have no indication for allogeneic stem cell transplant. A caution, however, should be taken on the risk for clonal evolution that should be carefully screened and surveilled.

Chronic lymphocytic leukemia and related disorders - Biology

E989

DECREASED EXPRESSION OF ADHESION MOLECULES IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) CELLS OF PATIENTS TREATED WITH IBRUTINIB
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Background: The B-cell receptor (BCR) pathway in CLL plays a well recognized role in the onset and progression of the disease and the resulting development of mechanism-driven drugs is revolutionizing the therapeutic management. Ibrutinib is a BTK inhibitor that plays an important role in the BCR pathway and induces several alterations in CLL cells.
Aims: The modulation of the expression of adhesion molecules on the surface of CLL cells from patients treated with ibrutinib has been evaluated to analyze the effect of treatment on the relationship between the microenvironment, that promotes cell survival and proliferation, and the leukemic cells with the consequent cell mobilization and increased drug exposure.
Methods: In a cohort of 101 CLL patients treated with ibrutinib (420mg/die) and rituximab (375mg/m^2/week) in the GIMEMA LLC1114 trial, we evaluated, before and after 15 days of therapy, the surface expression on leukemic cells of several adhesion molecules. In detail, using 8 color antibody combinations (all from Becton Dickinson, BD, San José, CA) we evaluated the MIF expression (using the FACSCanto II, BD) of CD11a, CD18, CD38, CD40, CD43, CD44, CD49d, CD62L, CD69, CD80, CD81, CD86, CD154, CD184, CD185 on CD5/CD19+ leukemic cells.
Results: The number of CD5/CD19+ did not increase after 15 days of treatment (52.8±58.8 *vs* 53.4±51.5 x 10^9/L; p=0.36) probably because of the concomitant rituximab administration, which 'masks' the mobilization effect induced by ibrutinib. We observed a significant down-modulation of CD62L (461±435 *vs* 171±146; p<0.0001), a molecule (L-selectin) that has been reported as the key factor controlling the binding of CLL cells to the endothelial walls in vivo. CD69 expression resulted also significantly decreased (744±784 *vs* 438±716; p<0.0041), is expressed on CLL cells in the tissue microenvironment, both in the bone marrow and in lymph nodes. We observed the significant down-modulation of the expression of CD43 (3285±2282 *vs* 2515±1826; p<0.0063); this antigen is utilized in CLL for the detection of minimal residual disease (MRD) and does therefore not seem a reliable marker in patients treated with ibrutinib. On the contrary, CD81 expression, another antigen utilized for MRD detection, resulted unchanged after 15 days of treatment. CD185 expression was significantly decreased (1502±1327 *vs* 804±687; p<0.001), while we unexpectedly observed the up-modulation of CD184 (2244±2022 *vs* 3182±1877; p<0.003); both antigens participate in the BTK signaling pathway. CD40, that interacts with activated CD4+ T cells, resulted down-modulated (722±467 *vs* 395±262; p<0.0001). CD38 and CD49d, when expressed in >20% of the leukemic cells, resulted significantly (p<0.028 and p<0.021) down-modulated: both molecules have a role in the crosstalk between the leukemic cells and the microenvironment. No significant changes were detected in the expression of CD11a, CD18, CD44, CD80, CD86 and CD154.
Summary/Conclusions: Within an ancillary biologic study of the GIMEMA LLC1114 protocol we observed a significant down-modulation in the expression of several adhesion molecules on the surface of CLL cells of patients treated with ibrutinib. Since these molecules promote the binding of the leukemic cells with the microenvironment, these results help to elucidate the mobilization process of CLL cells from the different compartments observed with ibrutinib and support its progressive efficacy over time in controlling the disease. A follow-up clinical analysis will define a possible correlation between these findings and response to treatment.

E990

Abstract withdrawn.

E991

CLL CELLS UNDERGO METABOLIC REPROGRAMMING AND UTILIZE FREE FATTY ACIDS AS THEIR PRIMARY ENERGY SOURCE
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Background: The gene expression profile of chronic lymphocytic leukemia (CLL) cells revealed a homogeneous phenotype related to memory B cells accompanied by an aberrant expression of several proteins. For example, lipoprotein lipase (LPL), typically expressed in adipocytes, is readily detected in CLL cells. However, unlike their normal counterparts which are resting cells, CLL cells do proliferate. What energy source CLL cells use and which metabolic pathway they recruit is currently unknown. Because the gene expression profile of CLL cells is skewed towards that of adipocytes, and because they proliferate at similar rates, we hypothesized that like adipocytes CLL cells utilize free fatty acids (FFA).
Aims: (A) Determine whether CLL cells are capable of utilizing FFA for energy production. (B) Determine whether lipid metabolism in CLL is LPL dependent. (C) Determine why LPL is aberrantly expressed in CLL cells.
Methods: Peripheral blood (PB) and bone-marrow derived lymphocytes were obtained from previously untreated patients with CLL. Imaging of CLL cells was done by electron microscopy, and PB lymphocytes were stained for Oil red O. Confocal microscopy studies helped in determining the cellular localization of LPL. To study the capacity of CLL cells to utilize FFA we developed an assay that measured the oxygen concentration in the sera of cultured CLL cells prior to and after adding FFA. In addition we measured the oxygen consumption of CLL cells derived from ibrutinib-treated patients. We used chromatin immunoprecipitation (CHIIP) and luciferase assays to study the binding of STAT3 to the *LPL* promoter.
Results: To study whether CLL cells are capable of utilizing FFA we cultured freshly isolated CLL cells from 20 patients and measured the concentration of cultured media-dissolved O_2 (dO_2) prior to and after adding FFA, assuming that if the cells oxidize the acid, dO_2 levels will drop. Indeed, after 48 hours incubation with FFA dO_2 levels were markedly reduced as compared with the dO_2 media levels of CLL cell incubated without FFA. Remarkably, unlike cultured CLL cells, dO_2 media levels of cultured normal B cells did not changed. Intriguingly, the levels of dO_2 remained unchanged if CLL cells were incubated in the presence of FFA and ibrutinib. Similarly, the dO_2 levels of CLL cells obtained from ibrutinib-treated patients remained constant, suggesting that ibrutinib disrupts the capacity of CLL cells to utilize FFA. Oil Red O staining of CLL bone marrow smears detected lipid deposits and electron microscopy confirmed the presence of lipid vacuoles in the cytoplasm of peripheral blood CLL cells but not in normal B cells, suggesting that like adipocytes, CLL cells store lipids in intracytoplasmic lipid vacuoles. Similar to adipocytes CLL cells express LPL which mediates the uptake of lipid particles into the cells and catalyze the hydrolysis of triglycerides into FFAs. Indeed, we detected LPL in the cell membrane and in the cytoplasm of CLL cells. Furthermore, using small interfering RNA (siRNA) we knocked-down LPL mRNA levels and found that LPL-siRNA reduced the capacity of CLL cells to utilize FFA, suggesting that the lipid metabolism in CLL is LPL-dependent. Because STAT3 is constitutively active in CLL cells, and because the LPL gene harbors STAT3 binding sites, we sought to determine whether STAT3 activates the LPL gene. Indeed, transfection of luciferase reporter gene constructs driven by LPL promoter fragments into MM1 cells revealed that STAT3 activates the LPL promoter. In addition, ChIP confirmed that STAT3 binds to the LPL promoter. Furthermore, transfection of CLL cells with STAT3-shRNA downregulated LPL transcripts and protein levels, confirming that STAT3 activates the LPL gene.
Summary/Conclusions: Our data suggest that CLL cells undergo metabolic reprograming and use strategies normally utilized by adipocytes. This process is driven by constitutively activated STAT3 and is inhibited by ibrutinib.

E992

INHIBITION OF ARGININE UPTAKE VIA HUMAN CATIONIC AMINO ACID TRANSPORTER-1 (CAT-1): A NOVEL APPROACH FOR CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) THERAPY

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Background: Interference with cancer metabolism by specifically restricting extracellular nutrients is a rapidly emerging field of research. A variety of tumor entities depend on the availability of the amino acid arginine since they have lost the ability to synthesize it endogenously. The systemic depletion of arginine, induced by the arginine-metabolizing enzymes arginine deiminase or arginase is currently explored clinically in phase I-II studies. An alternative, largely unexplored strategy to restrict nutrient availability for cancer cells would be to target the specialized cell membrane transporter proteins. Arginine uptake into cells is mediated by members of different solute carrier families (hCAT-1, hCAT-2A, hCAT-2B, hCAT-3; y^+LAT1, y^+LAT2, $ATB^{0,+}$and $b^{0,+}$AT), which differ in expression and regulation between individual cell and tissue types.
Aims: We wanted to clarify (i) if CLL cells depend on exogenous arginine, (ii) which transporter is responsible for arginine transport in human CLL cells and (iii) if the reduction of arginine availability via knockdown of this transporter inhibits CLL cell growth and viability.
Methods: Experiments were performed with both, primary human CLL cells, isolated from highly leukemic peripheral blood, and immortalized CLL cell lines. Primary CLL cells were left unstimulated or were stimulated via Toll-like receptor 9. The expression levels of arginine transporters were determined by quantitative RT-PCR and Western Blot. Arginine uptake was measured by [^{3}H]-arginine import, cell proliferation by [^{3}H]-thymidine DNA incorporation and cell viability by Annexin-V + propidium iodide staining in flow cytometry. The expression of hCAT-1 was downregulated in HG3 CLL cells using lentiviral shRNA technology. HG3_hCAT-1_knockdown cells were injected s.c. in NOD/SCID/gc^{null} mice and tumor growth was monitored.
Results: We show that primary CLL and immortalized CLL cell proliferation depends on the availability of extracellular arginine. Screening a large panel of individual CLL patient samples and different immortalized CLL cell lines demonstrated that hCAT-1, y^+LAT1 and y^+LAT2 are the predominantly expressed arginine transporters. Upon activation the expression level of hCAT-1 further increased significantly. Arginine uptake both, in primary CLL cells and in the CLL cell lines, was inhibited by the CAT inhibitor N-ethylmaleimide. Lentiviral downregulation of the hCAT-1 transporter in HG3 CLL cells resulted in a significant reduction of arginine uptake, associated with an inhibition of cell proliferation and viability *in vitro*. The corresponding *in vivo* data of tumor growth upon hCAT-1 knockdown in a murine xenograft model will be presented at the conference.
Summary/Conclusions: Our results demonstrate that the hCAT-1 transporter is a potential pharmacological target structure in CLL cells. Development of small molecule- or antibody-based inhibitors of hCAT-1 might lead to a novel therapeutic approach for CLL.

E993

FCMR IS A NEGATIVE REGULATOR OF B-CELL RECEPTOR SIGNALING IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

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Background: Chronic lymphocytic leukemia (CLL) cells frequently display features of anergic B cells, including reduced B-cell receptor (BCR) signaling capacity and downregulation of membrane IgM (mIgM). These features are particularly evident in freshly isolated peripheral blood (PB) CLL cells belonging to the indolent, M-CLL subset (Lanham S et al, Blood. 2003). The mechanism responsible for this phenomenon is still unclear, but chronic stimulation with autoantigens has been considered as a possible explanation because of the observation that BCR signalling capacity and mIgM expression can spontaneously recover in CLL cells following prolonged *in vitro* culture (Mockridge CI et al, Blood. 2007). An alternative explanation for this phenomenon is that these anergic features are induced by soluble IgM molecules, which are absent from standard cell culture media, and could interact *in vivo* with the leukemic BCRs through recently described intermolecular immunoglobulin interactions or by binding to the Fcμ receptor (FcμR). The latter is highly overexpressed in CLL cells, particularly those belonging to the M-CLL subset (Li FJ et al, Blood. 2011).
Aims: Investigate the role of the FcμR in regulating BCR signaling in CLL cells.
Methods: CLL cells were isolated from PB or lymph nodes (LNs) using standard procedures. FcμR stimulation was done using pentameric human Fcμ fragment, whereas BCR stimulation was done using goat anti-human IgM or anti-human Ig light chain F(ab')2 fragments. FcμR knockdown was done by RNA interference using the Nucleofector system and solution V/C-009 program. Surface FcμR and IgM levels were measured by flow cytometry on gated CD19+/CD5+ cells.
Results: We recently reported that FcμR stimulation results in activation of certain downstream BCR signaling pathways and increased CLL cell survival *in vitro* (Gobessi S et al, ASH 2016, abstract 2015). To investigate whether FcμR regulates BCR signaling capacity, we analyzed activation of downstream signaling molecules in CLL cells that had been pretreated for one hour with Fcμ and then stimulated with an anti-Ig light chain antibody. Decreased phosphorylation of SYK, AKT and ERK was detected in the Fcμ-treated samples, suggesting that FcμR negatively regulates BCR signaling in CLL cells. Consistent with this finding, we also observed that FcμR knockdown by RNA interference resulted in greater activation of SYK, AKT and ERK in anti-IgM stimulated primary CLL cells. Because IL-4 was recently shown to increase BCR signaling capacity and surface IgM expression on CLL cells (Aguilar-Hernandez MM et al, Blood. 2016; Guo B et al, Blood 2016), we next investigated whether it will have an opposite effect on FcμR expression. Stimulation of CLL cells (n=7) for 48 hours with IL-4 resulted in a mean 2.4 fold reduction in surface FcμR expression and a 3.9 fold increase in surface IgM expression compared to unstimulated cells ($P<0.001$ and $P=0.016$, respectively). Since IL-4 is produced by T cells, which typically interact with CLL cells in LNs, we next compared surface FcμR and IgM expression in two paired LN and PB CLL samples. Interestingly, in both cases the levels of surface FcμR were lower on LN than PB CLL cells, whereas no difference was detected in the expression of surface IgM. To further understand the mechanisms through which IL-4 regulates BCR signaling, we compared BCR signaling capacity of CLL cells cultured for 48 hours in the pres-

ence or absence of IL-4. Most of the investigated samples in this series showed reduced surface FcμR expression and increased surface IgM expression after IL-4 treatment, but a few cases showed only reduced FcμR expression and no change in IgM expression. Interestingly, these samples also showed greater anti-IgM induced phosphorylation of SYK, PLCγ2, AKT and ERK, suggesting that downregulation of FcμR is the primary mechanism through which IL-4 regulates the BCR signaling capacity of CLL cells.
Summary/Conclusions: These data show that FcμR is a negative regulator of BCR signaling in CLL cells. Overexpression of FcμR could be at least in part responsible for the reduced BCR signaling capacity of PB CLL cells. FcμR is downregulated by IL-4 and shows reduced expression in LN CLL cells, which could represent a mechanism to allow CLL cells to respond more effectively to stimulation with antigen encountered in the appropriate context.

E994

TRANSCRIPTION FACTORS AND CHECKPOINT INHIBITORS EXPRESSION WITH AGE: MARKERS OF IMMUNOSENESCENCE?
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Background: Aging is characterized by a progressive decline in immune surveillance that favors tumor development in older patients. One mechanism used by malignant cells to escape immune surveillance is the upregulation of immune checkpoint inhibitors. Another process associated with aging is genetic or epigenetic modifications of tumor suppressor genes (TSGs).
Aims: We previously reported a correlation between 6q deletion and progression into a T cell lymphoproliferative disease, identifying the *BACH2* gene as a candidate TSG. We thus examined the expression of specific transcription factors (*BACH2 and PRDM1)* and checkpoint inhibitors (*PD-1* and *PD-L1*) in the major lymphocytes subsets for their potential role in immunosenescence.
Methods: Peripheral blood mononuclear cells were isolated from whole blood using Lymphoprep (Stemcell Technologies) density gradient centrifugation. Lymphocyte subsets (CD19+, CD3+CD4+; CD3+CD8+) were isolated for subsequent molecular analyses using the MACS Technology (Miltenyi), with the purity of each lymphocyte subpopulation between 95%>99%. PD-1 (*PDCD1*), PD-L1 (*CD274*), *IL4*, *IFNG*, *BACH2* and *PRDM1* mRNA transcripts were quantified using qRT-PCR. *BACH2* and *BLIMP1 (PRDM1)* protein expression were examined by Western blotting.
Results: Blood samples were obtained from 60 healthy volunteers and 41 untreated B-cell chronic lymphocytic leukemia (B-CLL) patients (median: 67yo). Healthy donors (HD) between the ages of 20 to 90 years subdivided into <50 yrs (median: 36yo) and ³50 yrs (median: 65yo). *BACH2* mRNA expression in the HD groups is significantly down-regulated in CD4+, CD8+ T cells and CD19+ B cells from the older HD group (p=0.0012; 0.0045 and 0.0367, respectively). *BACH2* expression was further reduced in CD4+, CD8+ T cells and CD19+ B cells from CLL patients compared to HD well balanced for age (p=0.001; <0.0001 and 0.0043). *PRDM1* mRNA expression was inversely correlated with *BACH2* in $CD4^+$, $CD8^+$ T cells and $CD19^+$ B cells (r=0.61; 0.71 and 0.65, respectively). Curiously, *PRDM1* was – as expected - significantly up-regulated in CD4+ and CD8+ T cells (p=0.0034; p=0.0017) from B-CLL patients but not in their leukemic B cells. Western blotting analysis demonstrated that *BACH2* and *BLIMP1* (*PRDM1*) protein expressions in the T and B cell subpopulations were significantly correlated with transcript expression. *BACH2*-deficient mice have been shown to have an increased numbers of *IL4*-producing $CD4^+$ T cells. We also observed that *BACH2* down-regulation is correlated with increased *IL-4* mRNA expression (r=0.67) but not *IFNγ* in $CD4^+$ T cells. These observations suggest that *BACH2* down-regulation in $CD4^+$ T cells could enhance the expression of effector memory-related genes, particularly Th2, such as *IL-4* and *PRDM1*. *PD-1* mRNA expression was up-regulated in $CD4^+$, $CD8^+$ T cells (p=0.0153 and 0.0214) in the older HD group and also up-regulated in the T cells from B-CLL patients (p=0.0014 and 0.0023) when compared to age-matched HD population. High *PD-L1* mRNA expression was correlated with increased age in HD B cells (p=0.04) with a further increase detected in leukemic B cells (p=0.001). We also observed an inverse correlation between *BACH2* and *PD-1* in $CD4^+$, $CD8^+$ T cells (r=0.62 and 0.68); and between *BACH2* and *PD-L1* in $CD19^+$ B cells (r=0.66).
Summary/Conclusions: These data suggest that down-regulation of *BACH2/PRDM1* and up-regulation of *PD1/PD-L1* mRNA expression in major lymphocyte subsets from CLL patients and older healthy controls are significantly correlated with the aging immune cells and could be part of the immunosenescence process.

E995

T-CELL EXHAUSTED PHENOTYPE IS ENHANCED DURING DISEASE PROGRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)
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Background: The different biological mechanisms leading the clinical progression of CLL from early stages are currently not fully elucidated. Different prognosis factors that correlate with a higher probability of progression, such as IgHV mutational status, ZAP70 or CD49d expression, or TP53 defects, are still not able to identify an important proportion of patients that eventually progress. Clinical progression from early stages to an advanced CLL is associated with a certainly reduced acquisition of molecular changes that are not able to explain the fifty percent of the CLL cases progressing. CLL cells are dependent on survival and proliferative signals from the microenvironment and are able to evade immune anti-tumoral responses using different mechanisms, which is a crucial feature for cancer development. T-cell dysfunction is one of the main sources of impaired anti-tumor immunity. In CLL, T cells show functional defects and have increased expression of the exhaustion markers PD1, CD244 and CD160 compared to T cells from healthy individuals. Taking this into account, we hypothesize that changes in the microenvironment, and particularly in T-cell exhaustion component, are contributing to the clinical progression of CLL.
Aims: In order to explore the role of the immune system in the progression of CLL we studied the immunophenotype of T cells from CLL patients using paired samples at diagnosis and progression.
Methods: A total of 14 CLL patients (median age, 69 years; median time to progression of 29,5 months) and 6 patients diagnosed with CLL that did not experience clinical progression during a median follow up of 34 months were included in the study. Multicolor flow cytometry was performed in matched samples at two time-points: diagnosis and progression before treatment or diagnosis and follow-up. We studied T-cell differentiation status based on CD45RA and CCR7 expression and the inhibitory receptors PD1, CD244, CD160, LAG3, TIM3 and CTLA4. We also analyzed the expression of the transcription factors T-bet and Eomes.
Results: We observed a significant increase in $CD8^+$ absolute numbers (P=0.0107) and a significant decrease of the CD4:CD8 ratio (P=0.0012) with progression. T cells increased their effector memory (EM) $CD45RA^-$ $CCR7^-$ phenotype during progression (EM $CD4^+$ P=0.0353; EM $CD8^+$ P=0.0023). PD1 expression was significantly increased during progression in absolute numbers on both $CD4^+$ and $CD8^+$ T cell subsets (P=0.0166), as well as in the $PD1^+$ EM subset (EM $PD1^+CD4^+$; EM $PD1^+CD8^+$ P=0.0024). Interestingly, we did not observe these changes in CLL patients that did not progress where the absolute numbers of cells expressing PD1 were either diminished or maintained during the follow-up, pointing out an important role of PD1 in regard to CLL progression. We observed that the percentages of $CD8^+$ cells expressing CD244 and CD160 were higher at the time of progression, especially for CD244 (P=0.0078). Moreover, the co-expression of these markers with PD1 was found on $CD8^+$ T cells and its percentage was increased during progression (P=0.0078). Among the differentiation subsets, the EM and EM $CD45RA^+$ (TEMRA) $CD8^+$ T cells expressed the highest percentages of CD244 and CD160. We did not observe changes in LAG3, TIM3 and CTLA4. T-bet and Eomes are essential to regulate T-cell differentiation and their expression has also been associated with a progenitor ($T\text{-bet}^{hi}$ $PD1^{int}$) or a terminal ($Eomes^{hi}$ $PD1^{hi}$) exhausted phenotype in chronic viral infections. The percentage of $CD8^+$ T cells expressing Eomes and co-expressing Eomes and PD1 were significantly increased during progression (P=0.0186 and P=0.0286, respectively) whereas T-bet expression was more stable.
Summary/Conclusions: T cells from patients with progressed CLL show a more severe exhausted phenotype compared to diagnosis, which is characterized by an effector memory subset with higher expression and co-expression of PD1, CD244 and CD160, as well as higher levels of the transcription factor Eomes, indicating that the terminal exhausted phenotype ($Eomes^{hi}$ $PD1^{hi}$) is predominant. These changes may contribute to the immune evasion that facilitates the progression and to the immunosuppresive scenario that dominates advanced CLL stages. Functional assays to explain why this T cell subset is enhanced during progression are currently ongoing.

E996

EARLY SPECIFIC INCREASED EXPRESSION OF SURFACE IGM BUT NOT OF OTHER ASSOCIATED MOLECULES APPEARS TO REFLECT ANTIGEN DISENGAGEMENT IN CLL PATIENTS ON IBRUTINIB THERAPY
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Background: B cell receptor (BCR) signaling through surface IgM (sIgM) is key to the survival and proliferation of normal and chronic lymphocytic leukemia (CLL) cells, and can be targeted effectively by the BTK inhibitor ibrutinib. Chronic exposure of the BCR to (super)antigen leads to downmodulation of sIgM,

but not of sIgD, levels and signaling capacity. This is evident in the circulating CLL B-cells which are characterized by variably reduced sIgM levels/signaling. The variability influences outcome and cases with relatively higher sIgM levels/signaling capacity, but not sIgD, have more rapid progression, likely due to a larger proliferative component.
Aims: The aim of this study was to investigate the effect of ibrutinib *in vivo* on the dynamics of expression and function of sIgM and of other surface molecules associated with the BCR complex on the circulating CLL cells of patients during the early phases of therapy (first 3 months).
Methods: Peripheral blood mononuclear cells were collected from 12 CLL patients prior to (pre-) and at 1 week, 1 month and 3 months following commencement of single agent ibrutinib therapy. Expression of BCR-complex associated sIgM, sIgD, CD19 and other surface markers was assessed by flow cytometry. Signaling capacity following sIgM stimulation was measured by immunoblotting. Following biotinylation of cell surface proteins, the N-glycosylation pattern of the μ chain was assessed by immunoblotting as a readout of sIgM status. Informed consent was obtained from all patients (REC: H228/02/t).
Results: At week 1 of ibrutinib therapy, there was a dramatic increase in the expression of sIgM on the circulating CLL cells (mean fold increase 1.6, *P*=0.001), while expression of sIgD and CD19 remained constant. At this time point, increased sIgM expression associated with full N-glycan maturation of sIgM heavy-chain, indicative of recovery from antigen engagement at tissue sites. Also, the sIgM levels correlated with increased anti-IgM mediated SYK phosphorylation (r=0.64, *P*=0.03), to indicate functionality upstream of BTK. Sequential assessment at month 1 and 3 revealed that sIgM levels were similar to that observed prior to therapy, with preserved upstream signaling ability. In marked contrast, the other BCR complex associated molecules sIgD, CD19 and CD20 all reduced expression (*P*<0.001). Reduction of these markers was also accompanied by reduction of cell size and of other surface markers while overexpression of autophagy marker LC3B2 was documented.
Summary/Conclusions: Our data point to two major events dissociating sIgM expression and function from other BCR-complex associated molecules. In the initial phase, the increased sIgM expression and maturation, with no changes of other BCR-associated molecules, appears consequent to lack of antigen encounter, likely due to inhibition of chemokine-mediated entry to tissue sites. In the later phases the circulating CLL cells will suffer lack of tissue derived pro-survival stimuli. In their absence, CLL cells will reduce expression of several markers and cell size, possibly explained by autophagocytic mechanisms aiming to protect the circulating CLL cells from death unless ibrutinib therapy is withheld.

E997

TRB REPERTOIRE PROFILING OF TCL-1 TRANSGENIC MICE USING NOVEL NGS TECHNOLOGIES REVEALS OLIGOCLONAL EXPANSIONS: SIMILARITIES WITH CHRONIC LYMPHOCYTIC LEUKEMIA
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Background: Findings from independent studies reported that the BcR pathway and antigenic stimulation occupy a central spot in the development of leukemia in the Eμ-TCL-1 transgenic (tg) mouse, as in the case of chronic lymphocytic leukemia (CLL). Recently, the detailed characterization of the T-cell receptor beta chain (TRB) gene repertoire in patients with CLL revealed gene expression biases and oligoclonality. These characteristics strongly suggested that not only leukemic B cells, but also T cells are selected by antigenic elements. In this context, very little is known regarding the T cell compartment in Eμ-TCL-1 mice.
Aims: Here, we sought to: (i) obtain a comprehensive view of the TRB gene repertoire in TCL-1 mice, and (ii) assess from an immunogenetic standpoint the extent of similarity between TCL-1 mice and CLL patients.
Methods: In total, we analyzed 18 samples from 16 TCL-1 mice that were categorized into 3 distinct groups, based on disease stage: (i) 6 mice with a clone size of <20% (group 1), (ii) 6 mice with a clone size of 30-55% (group 2) and (iii) 6 mice with a clone size of >60% (group 3). Clone size was measured as the percentage of CD5+/CD19+ B cells in the blood. Two different mice were studied longitudinally: one belonged originally to group 1 and progressed to group 2, while the other animal progressed from group 2 to 3. Five C57BL/6 wild-type (wt) mice were also analyzed as controls. TRBV-D-J gene rearrangements were amplified using the immunoSEQ® mouse T-cell receptor beta (mmTCRB) Kit from Adaptive Biotechnologies® and sequencing was performed using the MiSeq® Reagent Kit v3. Data were analyzed using the immunoSEQ® software.
Results: Only productive, in-frame TRBV-D-J rearrangements were included in the analysis that, in total, concerned 383,951 sequences (median 14,239 sequences). The TRB gene repertoire was almost identical in all groups, including the wt mice. In detail, 5 different genes: TRBV13-02, TRBV19-01, TRBV03-01, TRBV13-03, TRBV05-01, TRBV02-01 accounted for almost 50% of the total repertoire. Concerning the TRBJ gene repertoire, the TRBJ02-07 gene was the most frequent gene in all groups. The analysis of the CDR3 length showed the same distribution in all groups with the mean and median CDR3 length being 12 amino acids. Expanded clones were observed in all samples with the average size of the 10 largest clones being: 9.8% for group 1, 18.3% for group 2, 12.9% for group 3 but only 0.4% for wt mice. Comparison of the TRBV repertoire in the expanded clones *versus* the general cohort revealed significant differences with genes TRBV12-01, TRBV12-02, TRBV16-01 and TRBV20-01 being frequent only in the former group. Shared or public clonotypes (identical CDR3 sequences) were only observed in longitudinal samples from the same mice, which also concerned some of the largest clones. Scanning the 10 largest clones of each sample for the existence of highly similar clones led to the identification of 48 clusters that contained 91/180 clonal sequences.
Summary/Conclusions: Overall, the TRB gene repertoires of TCL1 mice were characterized by oligoclonal expansions that could persist over time. The TRB gene repertoire of expanded clones was more restricted than that of the general cohort, whereas comparisons between different samples revealed the existence of identical and highly similar clonotypes. These findings argue that (ongoing) selection by antigenic elements may shape the T-cell compartment in TCL-1 mice, similar to human CLL. These results further support the notion that this mouse model closely resembles CLL, at least from an immunogenetic perspective.

E998

ROLE OF THE COMBINATION MEK1/2 INHIBITOR BINIMETINIB AND AKT INHIBITOR MK2206 IN CLL
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Background: Clinical trials of ibrutinib and idelalisib demonstrate the efficacy of B-cell receptor-targeted therapies for CLL. We sought to investigate the efficacy of targeting both the BCR and the MAPK-ERK1/2 signaling pathways.
Aims: To evaluate the role of targeting the Ras-Raf-MEK1/2-ERK1/2 together with the Pi3K-AKT pathways as a potential novel approach in treating chronic lymphocytic leukemia. In particular, assessing the efficacy of MEK1/2 inhibitor, binimetinib (MEK162), in combination with either a Pi3K inhibitor, idelalisib or an AKT inhibitor, M2206.
Methods: All experiments conducted on primary CLL cells were co-cultured with CD40L-expressing stroma which mimics the support conferred by the tumour environment. Firstly, the effects of MK2206 and idelalisib at doses varying from 1 to 40μM were tested on primary CLL cells. Secondly, binimetinib and MK2206 were tested as single agents and in combination at 20μM against primary CLL cells. Thirdly, binimetinib at 20μM combined with varying doses of idelalisib on primary CLL cells. The mechanisms underlying the effects of binimetinib in combination with MK2206 in primary CLL cells were investigated by western blotting with changes in the expression of phosphorylated and total forms of AKT, MCL-1, and ERK1/2 assessed. Expression of B-actin was used as a loading control.

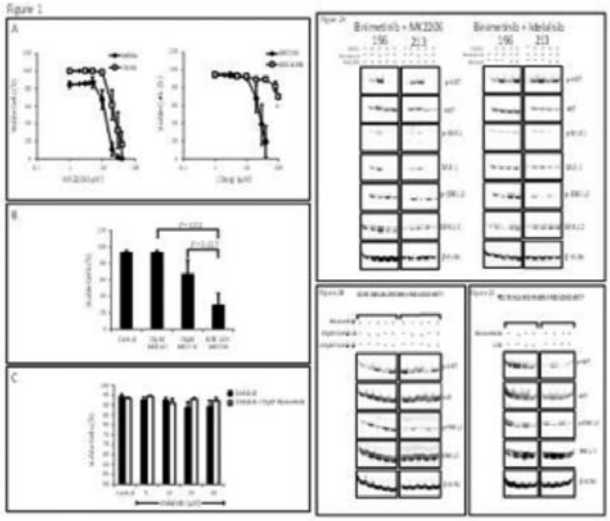

Figure 1.

Results: MK2206 is effective against CLL cells co-cultured with stromal cells in a dose dependent manner. It was also observed that the primary CLL cells co-cultured with the CD40L-expressing stroma were significantly more sensitive to MK2206 than to idelalisib (Figure 1A). No cytotoxic effects of binimetinib

were observed while the combination with MK2206 was significantly more effective than either drug alone, suggestive of synergy between the two drugs (Figure 1B). The analysis of binimetinib at 20μM with idelalisib failed to demonstrate any additive effects or suggestion of synergy between the two drugs (Figure 1C). Binimetinib treatment led to an increase in the activity of AKT and a decrease in ERK1/2 phosphorylation. MK2206 completely abrogated the activity of AKT and MCL-1 phosphorylation when combined with binimetinib (Figure 2A). Although we observed a reduction in AKT phosphorylation following idelalisib alone, it had no effect on the levels of AKT activity induced by binimetinib or the levels of phosphorylated MCL-1 protein. This result was irrespective of the dose of idelalisib used (Figure 2B). We explored the possibility that protein kinase C (PKC) may also be involved in the binimetinib-induced AKT activity. Using the pan-PKC inhibitor GF109203X (GFX), we demonstrated that inhibition of PKC significantly reduces binimetinib-induced phosphorylation of AKT with no effect on the activity of ERK1/2-MAPK (Figure 2C). These data suggest a role for PKC in the regulation of AKT activity in CLL cells.

Summary/Conclusions: The combination of binimetinib and MK2206 *in vitro* has been shown to be effective strategy to treat primary CLL cells. The western blot data reinforce that the increased activity observed in AKT activity in CLL cells following binimetinib treatment is independent of the idelalisib and totally abrogated by MK2206. This PI3-kinase independent regulation may be regulated by protein kinase C and is likely to play a significant role.

Dual inhibition of MAPK-ERK1/2 and AKT signaling may be effective at targeting the proliferative/drug-resistant compartment of CLL that resides in the tumour microenvironment.

E999

TARGETING HIF-1A AND ITS REGULATORY PATHWAYS AS A STRATEGY TO HAMPER LEUKEMIA-MICROENVIRONMENT INTERACTIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: The CXCL12/CXCR4 axis has a fundamental role in the microenvironment-mediated protection of chronic lymphocytic leukemia (CLL) cells from spontaneous and drug-induced cell death. The binding of CXCL12 with CXCR4 activates multiple intracellular pathways, including RhoA- and Ras-dependent signaling. We have previously shown that co-culture with stromal cells (SC) induces in CLL cells the activation of RhoA/RhoA kinase and Ras/ERK1-2 signaling, the upregulation of Akt, and an increased activity of the transcription factor HIF-1α (Rigoni et al., Oncotarget 2015).

Aims: The purpose of this study was to identify new potential pharmacological targets involved in the CXCL12/CXCR4 axis in order to impair the protection exerted by SC towards spontaneous and fludarabine-induced apoptosis in CLL cells.

Methods: Peripheral blood was collected from 62 patients with CLL. In selected experiments, the M2-10B4 murine SC line and the HS-5 human SC line were used. Patient-derived bone marrow SC were generated from 12 patients with CLL. Where indicated, cell cultures were treated with recombinant CXCL12 (100 ng/ml), CXCR4 inhibitor AMD3100 (5 μg/ml), fludarabine (F-ara-A, 10 μM), simvastatin (1 μM), ERK1-2 kinase inhibitor PD98059 (10 μM), HIF-1α inhibitor BAY87-2243 (1 μM), and PI3K inhibitor idelalisib (10 μM). RhoA and Ras activities were evaluated by an ELISA based assay and by pull-down assay, respectively. ERK1-2, HIF-1α amount in whole cell extracts and in nuclear fraction, and HIF-1α phosphorylation were evaluated by Western Blot. RhoA kinase, Akt and HIF-1α activities were measured with specific immunoassay kits. CXCL12 was quantified by ELISA. Cell viability was determined by Annexin-V/propidium Iodide immunostaining and flow cytometry analysis.

Results: The exposure of CLL cells to recombinant CXCL12 led to the activation of RhoA- and Ras-dependent signaling, and to the downstream upregulation of HIF-1α. The CXCR4 antagonist AMD3100 completely abrogated the positive regulation exerted by both CXCL12 and SC, thus unveiling the key role of the CXCL12/CXCR4 axis in the SC-induced modulation of these signaling pathways. The inhibition of Ras and RhoA activity by simvastatin, and the inhibition of ERK1-2 and HIF-1α by PD98059 and BAY87-2243 effectively blocked the SC-induced expression and activity of HIF-1α, significantly impairing the SC-mediated protection of CLL cells, both in absence and presence of fludarabine. Similar effects were observed by targeting the PI3K/Akt pathway with idelalisib. We then investigated whether targeting RhoA- and Ras-dependent signaling could modulate HIF-1α also at the SC level. Simvastatin and BAY87-2243 effectively inhibited HIF-1α expression both in SC lines and in patient-derived SC. Moreover, simvastatin significantly reduced the secretion of CXCL12, which is a known transcriptional target of HIF-1α.

Summary/Conclusions: Our data demonstrate that the targeting of HIF-1α and its regulatory pathways, both at the tumor cell and at the SC level is an appealing strategy to overcome the microenvironment-mediated protection toward spontaneous and fludarabine-induced apoptosis in CLL cells.

E1000

THE ROLE OF GENETIC-BASED PROGNOSTIC FACTORS IN PREDICTING MINIMAL RESIDUAL DISEASE NEGATIVITY IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS TREATED WITH FLUDARABINE, CYCLOPHOSPHAMIDE AND OFATUMUMAB

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Background: Chemoimmunotherapy with fludarabine, cyclophosphamide and rituximab (FCR) is the optimal front-line treatment for fit chronic lymphocytic leukemia (CLL) patients. IGHV mutations and FISH lesions are predictive markers of response and progression-free survival after FCR. Minimal residual disease (MRD) is the single best post-treatment predictor of long-term outcome after FCR, independent of biologic prognostic markers.

Aims: To explore whether conventional biologic markers (*i.e.* IGHV mutations, FISH lesions) and *TP53*, *NOTCH1*, *BIRC3* and *SF3B1* mutations can predict the obtainment of a MRD negativity after first-line treatment of CLL patients with FC and ofatumumab (FCO).

Methods: Eighty young (≤65 yrs) and fit CLL patients from 15 Italian centers were enrolled in the GIMEMA LLC0911 first-line trial and treated with 6 cycles of FCO. CLL diagnosis, treatment requirement and response were defined according to the 2008 iwCLL guidelines. MRD was evaluated in responding patients by 8-color flow cytometry in the peripheral blood (PB) and bone marrow (BM) 2 months after the end of induction (month +8), and every 6 months thereafter; flow negative cases were analyzed by RQ-PCR, according to the guidelines. The association between CLL biologic markers and MRD clearance after FCO was tested by Fisher's exact test; logistic regression models were used to estimate the risk values in univariate and multivariate analyses.

Table 1.

Table 1: Biologic features of the 65 CLL patients evaluated for MRD.

Median age	59 years (36-66)
Morphology (tipycal/atypical)	31/7
IGHV (unmutated/mutated)	37/27
CD38 (pos/neg; cut-off 20%)	35/30 (54%)
CD49d (pos/neg; cut-off 30%)	37/23 (62%)
ZAP70 (pos/neg; cut-off 20%)	9/50 (15%)
FISH: del17p	2/64 (3%)
del11q	11/64 (17%)
trisomy 12	5/64 (8%)
del13q	21/64 (33%)
No FISH lesions	25/64 (39%)
TP53 mutation (one *TP53*mut+del17p+)	2/65 (3%)
NOTCH1 mutation	9/65 (14%)
BIRC3 mutation	3/65 (5%)
SF3B1 mutation	9/65 (14%)

Results: Sixty-five responding patients underwent MRD evaluation at month +8; their biologic features are reported in the Table. By flow cytometry, 25/65 cases (38%) resulted MRD+ in the PB and/or BM, while the remaining 40 (62%) showed no residual CLL cells. The absence of del17p/*TP53*mut/del11q was associated with the achievement of MRD negativity: 37 MRD- (74%)/13 MRD+ among patients with trisomy 12/negative FISH/del13q/*TP53*WT *vs* 2 MRD- (14%)/12 MRD+ among del17p+/*TP53*mut/del11q+ (p=0.0001). Interestingly, when patients were stratified into high (n=6), intermediate (n=22), low (n=21) and very-low risk (n=15) groups by integrating FISH and gene mutations (Rossi

et al, 2013), the high and intermediate risk groups (del17p/*TP53*/*BIRC3*+ or del11q/*NOTCH1*/*SF3B1*+) showed a significantly lower probability of achieving a MRD negativity (36%, 10/28) than the low and very-low risk groups (+12/negative FISH/del13q/WT for 4 genes: 81%, 29/36) (p=0.0003). The 40 flow cytometry MRD- cases were also evaluated by RQ-PCR: 22 (55%) were reclassified as MRD+. By combining the two methods, 47/65 cases (72%) were MRD+ and 18/65 (28%) MDR- at the end of FCO. Mutated (M)-IGHV status was significantly associated to a molecular MRD- (12 MRD-/15 MRD+, 44%) compared to unmutated (UM)-IGHV cases (5 MRD-/32 MRD+, 13%) (p=0.0092). Moreover, when M-IGHV status is reinforced by the absence of del17p/*TP53*mut/del11q, the association with a deep MRD negativity got stronger (12 MRD-/13 MRD+, 48% *vs* 5 MRD-/33 MRD+, 13%; p=0.0036). A multivariate model including FISH lesions, gene and IGHV mutations supports the independent role of FISH and IGHV profile in predicting MRD negativity by flow and RQ-PCR, respectively.

Summary/Conclusions: In CLL patients treated with the FCO combination (LLC0911 Gimema trial), MRD negativity by flow cytometry (62%) can be predicted by the FISH profile: 74% in patients without del17p/del11q *vs* 14% in del17p+/del11q+ cases. A deeper MRD negativity by RQ-PCR (28%) can be anticipated by the IGHV status (44% M *vs* 13% UM) or by combining IGHV and FISH. A longer follow-up will determine whether these parameters can identify patients who maintain over time a good quality of response.

E1001

ISOCHROMOSOME 17Q, UNBALANCED TRANSLOCATIONS AND 8Q GAIN REPRESENT ADVERSE PROGNOSTIC FACTORS IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) WITH 17P DELETION. A GFCH STUDY

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Background: Chromosomal abnormalities are present in about 80% of CLL. Among them, the loss of the short arm of the chromosome 17 (17p-), uncommon at diagnosis (<10%), is frequent in relapsed or refractory patients (up to 50%) and is associated with short survival. Loss of 17p results from various chromosomal abnormalities, including unbalanced translocations, deletions, rings or isochromosomes. All these aberrations lead to the loss of one copy of the *TP53* gene, the remaining allele being generally mutated. In addition, 17p- is frequently accompanied by genomic complexity. Patients with 17p- typically progress quickly and are refractory to most conventional therapies.

Aims: We evaluated if the type of chromosomal abnormality leading to 17p- and the additional aberrations could influence the prognosis.

Methods: We collected data from a multicentric and retrospective cohort of 195 CLL patients harboring a *TP53* deletion detected by FISH, with an informative conventional karyotype (K). All the K were reviewed by the members of the Groupe Francophone de Cytogénétique Hématologique. Overall survival (OS) and time to first treatment (TTFT) were calculated from diagnosis to death or first-line treatment, respectively, or last follow-up. The log-rank test was used to compare Kaplan-Meier curves. Cox regression models were used for multivariate analyses.

Results: The ratio H/F was 1.9. At diagnosis of CLL, the median age was 63 years [33-88], 59% were Binet stage A, 28% B, 12% C. *IGHV* genes were unmutated in 38/47 (81%) patients tested. The median follow-up was 70 months [0-401]. The majority of the patients was treated (87%), with a median TTFT of 13 months and a median of 2 lines of treatment [1-10]. In 28/124(23%) cases, the 17p- was not present at diagnosis and occurred after the first therapy, with a median time of 77.5 months [22-291] from the diagnosis. Karyotype was complex in 141/195(72%) patients, and monosomal in 90/195 (46%). The 17p- was the sole abnormality detected by K in 28/195(14%) cases. A total of 240 17p abnormalities were found in the 195 patients. In the majority of cases, loss of 17p was the consequence of an unbalanced translocation (n=167/240, 70%), with various chromosome partners, the most frequent being the recurrent der/dic(17;18)(q10;q10) (n=32, 13%), followed by translocations involving chromosomes 8, 13, 14, 21, 15. Unbalanced translocations involving 17p and chromosome 8 (n=26, 11%), lead either to del8p (n=17), gain8q (n=6), or del8q (n=3). The other 17p abnormalities were: deletion 17p (n=45, 19%), monosomy 17 (n=15, 6%), isochromosome 17q [i(17q)] (n=9, 4%) and ring of chromosome 17 (n=4, 1%). Among the additional abnormalities accompanying the 17p-, unbalanced translocations were found in 121/195(63%) of patients. Combining FISH and K, del13q was detected in 71/118(60%) of cases, del8p in 40/189(21%), tri12 in 30/195(15%), gain8q in 13/105(12%), and del11q in 20/161(12%). By univariate analysis, the paramaters which were associated with significantly shorter OS were: age ≥65 years, stage B/C, unmutated *IGHV*, i(17q) (69 months *vs* 179, p=.0375), the presence of unbalanced translocations in addition to 17p- (153 *vs* 223 months, p=.03), and gain8q (74 *vs* 123 months, p=.0014). Monosomy 17, a total number of abnormalities ≥6 and gain8q predicted a shorter TTFT. By multivariate analysis, age ≥65 years, stage B/C and gain8q remained significant for OS.

Summary/Conclusions: Among the high risk group of 17p- CLL, i(17q) confers a shorter OS than the other 17p abnormalities. In addition, the gain8q aggravates the outcome as well as the presence of additional unbalanced translocations. These results confirm that patients with 17p- CLL have a variable clinical course and highlight the relevance of conventional karyotyping to identify the alterations that modulate the prognosis within these patients.

E1002

THE MICROENVIRONMENT REGULATES THE EXPRESSION OF MIR-21 AND TUMOR SUPPRESSOR GENES PTEN, PIAS3 AND PDCD4 THROUGH ZAP-70 IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Microenvironment found in bone marrow and lymph nodes supports survival, proliferation and drug resistance in chronic lymphocytic leukemia (CLL). Indeed, CLL cells are highly dependent on interactions with the microenvironment. The BCR is one of the key players involved in the crosstalk between CLL cells and the microenvironment. Furthermore, it has a critical role in pathogenesis and prognosis of CLL. Accordingly, different factors related to increased BCR signaling are adverse prognostic factors in CLL, such as IGHV genes, high expression of ZAP-70 and increased serum levels of CCL3. Expression of ZAP-70 in CLL cells has been related to enhanced response to BCR stimulation, as well as to increased response to diverse migrative and survival stimuli from the microenvironment. MiR-21 is an oncogenic microRNA that has been found to be overexpressed in a wide variety of neoplasms where it participates in oncogenic events such as proliferation, resistance to treatment, and metastasis; its overexpression in CLL has been associated to refractoriness to fludarabine and to shorter overall survival and higher probability of progression.

Aims: In order to further elucidate the molecular mechanisms defining bad prognosis CLL by further elucidation of the role of ZAP-70 in the crosstalk between CLL cells and the microenvironment, we studied the relationship between ZAP-70 protein and miR-21 and how it is influenced by the microenvironment.

Methods: Peripheral blood mononuclear cells (PBMC) from 48 patients diagnosed with CLL were isolated by Ficoll-Paque Plus density gradient centrifugation. Ramos B-cells stably transfected with a vector encoding for ZAP-70 protein fused with Green fluorescent protein (GFP) or GFP only as a control were treated with Akt (LY294002), MAPK (PD98059) and STAT3 (JSI-124) inhibitors for 1 hour. BCR was stimulated with $F(ab)_2$ anti-IgM. PBMC were co-cultured with bone marrow stromal cells with CD40L and CpG to mimick the microenvironment found in proliferation centers. After 48 hours CLL cells were harvested to analyze cell viability, cell proliferation and mRNA expression. Expression levels of primary miR-21, miR-21, PTEN, PDCD4 and PIAS3 were measured by QRT-PCR.

Results: First, we observed that miR-21 expression was significantly higher in patients with high expression of ZAP-70. Subsequently, using stably transfected Ramos B-cells with ZAP-70 protein we found that pri-miR-21 and mature miR-21 expression were significantly increased upon BCR crosslinking, which was enhanced by ectopic expression of ZAP-70. We also observed that inhibition of both MAPK and STAT3 pathways impairs the regulation of miR-21 expression after ZAP-70 activation. Moreover, the induction of miR-21 expression after ZAP-70 activation also induced downregulation of the tumor suppressor genes PTEN, PDCD4 and PIAS3, targeted by miR-21 in malignant cells. The co-culture of primary CLL cells induced ZAP-70 and miR-21 expression, as well as downregulation of the putative miR-21 targets. Interestingly, the increase in miR-21 after co-culture was significantly impaired by ibrutinib, indicating that the BCR signaling pathway is involved in its regulation in primary CLL cells. Finally, co-culture-induced increased CLL survival correlated with miR-21 upregulation.

Summary/Conclusions: In conclusion, stimuli from the microenvironment are capable of regulating expression of miR-21 and tumor suppressor genes

(PTEN, PDCD4 and PIAS3) via a signaling pathway involving ZAP-70, MAPK and STAT3 transcription factor.

E1003

IMPACT OF RECURRENT MUTATIONS ON PROGRESSION-FREE SURVIVAL IN CLL PATIENTS TREATED WITH FRONT LINE RITUXIMAB-BASED REGIMENS

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Background: Regimens consisting of rituximab and DNA-damaging drugs represent an important therapeutic option for patients with chronic lymphocytic leukemia (CLL). Up-to-date studies including clinical trials agreed upon the adverse outcome of *TP53*-defective patients that should be provided alternative treatment approaches. Additionally, mutations in *NOTCH1* gene were connected with a lack of benefit from rituximab added to chemotherapy. A potential impact of other mutations commonly occurring in CLL patients remains less clear, namely regarding a role in relapse development.
Aims: (a) to assess impact of mutations in *ATM*, *SF3B1*, *NOTCH1* and *BIRC3* genes on progression-free survival (PFS) in CLL patients treated with front line rituximab-based regimens, and (b) to analyze clonal evolution of mutations in relapse.
Methods: We analyzed 53 CLL patients administered first line regimens FCR (fludarabine, cyclophosphamide, rituximab) or Q-FCR (FCR with reduced doses) or BR (bendamustine, rituximab); all harbored intact *TP53* gene as assessed by FISH and the yeast functional analysis; 46/53 (87%) had unmutated IGHV. The next generation sequencing using MiSeq (Illumina) was done in 53 pre-therapy samples and 41 relapsed samples using three separate panels: *ATM* (exons 2-63; median coverage (MC) 6100), *SF3B1*/*NOTCH1*/*BIRC3* (exons 14-16, part of 34, and 7-10, respectively, MC 11200), and *TP53* (exons 2-11; MC 31500). Functional impact of *ATM* mutations was verified by SIFT and PolyPhen online tools. Only mutations present in >10% of reads were considered for the PFS analysis (log-rank (Mantel-Cox) test); the interval was calculated from therapy completion to clinical progression (as defined by the iwCLL recommendations).
Results: In the pre-therapy analysis, we identified 23 patients with one disrupted gene and 7 patients with two disrupted genes; the rest of the cohort (n=23) was devoid of the mutations (wt). The most frequently affected gene was *ATM* (15 cases; only mutations with predicted functional impact considered), followed by *SF3B1* (10 cases; hot-spot mutations), *NOTCH1* (7 cases; all deletion c.7541_7542) and *BIRC3* (5 cases; frame-shift mutations). We did not observe significant differences in PFS among the employed regimens: the medians were 16.5 months (m) for FCR (used in 30 patients; median: 4 cycles); 15 m for Q-FCR (15 patients; 4 cycles); and 14 m for BR (8 patients; 3.5 cycles); P=non-significant (ns). In univariate analyses of individual affected genes, we did not observe an adverse impact of mutations on PFS: *ATM*-mut median 19 m *vs* 14 m in wt patients; *SF3B1*-mut 19.5 m *vs* 16 m; *NOTCH1*-mut 32 m *vs* 15.5 m; *BIRC3*-mut 16 m *vs* 16 m (all analyses P=ns). In a more detailed analysis structured according to the type of *ATM* defect, patients with sole 11q- (the other *ATM* allele intact; n=13) manifested the shortest PFS of 11 m, followed by *ATM*-mutated patients (n=15; 19 m; P=0.1) and wt patients (n=24; 24 m; P=0.041). Analysis of clonal evolution in patients who relapsed (41 paired samples) revealed the following significant events: selection of *TP53* mutations in two cases (from 2.3% to 77%, and 5.4% to 64.5%) and *ATM* mutations in two cases (8% to 15% and wt to 18%), as well as more complex changes involving both increase and decrease of mutations in *BIRC3* gene in three patients.
Summary/Conclusions: Our pilot analysis with limited number of samples does not indicate adverse impact of studied mutations in rituximab-based regimens. Some relapsed samples showed quite distinct mutation profile. Supported by projects AZV 16-32743A, MUNI/A/1106/2016 and FNBr, 65269705.

E1004

BCR SIGNALLING PROFICIENT CHRONIC LYMPHOCYTIC LEUKAEMIA B CELLS ARE PRONE TO RITUXIMAB MEDIATED ELIMINATION IN VIVO

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Background: Anti-CD20 monoclonal antibody rituximab (RTX) has improved clinical outcome of patients with CD20-positive B-cell malignancies, including chronic lymphocytic leukaemia (CLL). However, despite the fact that RTX has been clinically used for 20 years, the exact mechanism of its action remains largely unclear.
Aims: The aim of this study was to determine susceptibility of CLL cells' subpopulations to RTX.
Methods: Peripheral blood samples from CLL patients (N=17) were obtained and analysed before (day 0) and 24 hours (day 1) after RTX administration (375mg/m^2, single agent).
Results: It was described that CLL cells that interacted with stromal cells *in vivo* can be characterised by relatively weak cell-surface expression of chemokine receptor CXCR4 and high expression of activation marker CD5 (CXCR4^{dim}CD5^{bright}cells) (Calissano *et al*., 2011). We showed that these cells also have higher CD20 expression than CLL cells circulating in the peripheral blood without contact with immune niches (CXCR4^{bright}CD5^{dim}; Pavlasova *et al*., 2016). We hypothesised that the higher levels of CD20 on CXCR4^{dim}CD5^{bright} cells make them the primary target for RTX *in vivo*, since the cell surface CD20 expression associates with the RTX efficacy (Golay *et al*., 2001). We analysed blood samples obtained from CLL patients treated with RTX as a single agent and indeed, we observed that RTX preferentially and nearly completely eliminates the CXCR4^{dim}CD5^{bright} subpopulation after the first RTX dose (8.3% pre-RTX *vs* 2.1% post-RTX, P<0.0001). We further analysed BCR signalling proficiency of the CXCR4^{dim}CD5^{bright}CD20^{bright} subpopulation, since CD20 was proposed to play a role in BCR signalling. We observed that CXCR4^{dim}CD5^{bright} CLL cells have higher immunoglobulin (IgM) expression (~2-fold, P<0.005) which was coupled with higher responsiveness to BCR crosslinking with anti-IgM (P=0.005). Moreover, CXCR4^{dim}CD5^{bright} cells also have higher levels of CD19 (1.8-fold, P<0.0001), which is an important component of BCR complex that augments signal transduction. Furthermore, we demonstrated that CXCR4^{dim}CD5^{bright} cells have higher phosphorylation of several proteins involved in PI3K/BCR/NFkB signalling pathway (P<0.05) compared to CXCR4^{bright}CD5^{dim} cells obtained from the same patient. This led us to hypothesize that CD20 up-regulation on CXCR4^{dim}CD5^{bright} cells is likely of physiological importance for PI3K/BCR signalling. Indeed, we observed significant reduction in phosphorylation of tyrosine-protein kinases associated with PI3K/BCR signalling after silencing of CD20 by siRNA in B cells.
Summary/Conclusions: We showed that CXCR4^{dim}CD5^{bright} CLL subpopulation in peripheral blood of CLL patients has the highest surface levels of CD20 and is therefore preferentially and effectively eliminated by RTX. These CLL cells likely represent the most "aggressive" subclone of CLL cells since they have relatively high proliferative and BCR signalling capacity.

Supported by: the Ministry of Education, Youth and Sports of the Czech Republic under the project CEITEC 2020 (LQ1601); Czech Science Foundation (project No. 16-13334Y); the Ministry of Health of the Czech Republic, grant No. 16-29622A. All rights reserved. The European Union's Horizon 2020 research and innovation programme under grant agreement No. 692298. This study reflects only the author's view and the Research Executive Agency is not responsible for any use that may be made of the information it contains. MH CZ-DRO (FNBr, 65269705); MUNI/A/1106/2016; GAMU MUNI/H/0865/2016, and G.P. is a city of Ostrava scholarship holder.

E1005

REGULATION OF BCR SIGNALLING IN CHRONIC LYMPHOCITYC LEUKEMIA: ROLE OF E3 UBIQUITIN LIGASE C-CBL

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Background: In normal B cells, the E3 ubiquitin ligase Cbl (c-Casitas B-lineage lymphoma) is involved in the ubiquitin-dependent Lyn degradation and in the down-regulation of BCR signalling. c-Cbl is activated by phosphorylation that releases c-Cbl from its autoinhibited structure by triggering a conformational change that leads to an enhanced transfer of ubiquitin from the E2 enzyme to the substrate proteins. Mutations in this RING/Linker region result in the loss of ubiquitin E3 ligase activity thus prohibiting lysosomal or ubiquitin/proteasome-mediated degradation of tyrosine kinases and thereby unleashing tyrosine kinase signaling. We reported that in Chronic Lymphocityc Leukemia (CLL) Lyn is over-expressed and is in an active conformation as integral component of an aberrant cytosolic multiprotein complex, associated with several proteins, such as Hsp90, HS1 and SHP-1L. In particular, Hsp90 appears tightly bound to cytosolic Lyn, thus stabilizing the aberrant complex and converting individual transient interactions into stable ones.
Aims: The accumulation of clonal B lymphocytes in CLL is mostly due to apoptosis resistance but also to proliferative activity. Abnormalities of molecules involved in signal transduction pathways are connected to CLL pathogenesis and a critical role has already been ascribed to B-cell receptor (BCR)-Lyn axis. Here, we investigated the expression and the role of c-Cbl in CLL B cells since in normal B cells is involved in the ubiquitin-dependent Lyn degradation and in the down-regulation of BCR signaling.
Methods: Blood samples were collected from 30 CLL patients and 15 controls. Untouched peripheral blood B cells were purified using the RosetteSep isolation kit for human B cells. We characterized c-Cbl total protein level and c-Cbl(Y700) by Western blotting. To evaluate the interaction between c-Cbl and Lyn in CLL

B cells we performed a co-immunoprecipitation assay, followed by Western blotting analysis, at steady state and after IgM (10μg/ml) stimulus. We also evaluated the interaction between c-Cbl and Lyn after treatment with 17-DMAG (500nM), a potent HSP90 inhibitor.
Results: We demonstrated that c-Cbl is overexpressed ($p<0.001$, Student's t test in CLL B lymphocytes with respect to normal B cells. We found that in neoplastic B cells c-Cbl did not co-immunoprecipitate with Lyn neither after BCR trigger. We obtained similar results when we treated neoplastic B lymphocytes with 17-DMAG to dissociate the Lyn-Hsp90 complex: after 1h, 2h and 4h of treatment we immunoprecipitated Lyn demonstrating that neither before nor after IgM stimulation c-Cbl interacts with this kinase. These results support the hypothesis that c-Cbl is not involved in Lyn turnover. Data obtained from 10 independent experiments showed that in CLL neoplastic cells the phosphorylation on Y700 increased after 5' and 10' of IgM stimulus, highlighting the involvement of c-Cbl in BCR signaling.
Summary/Conclusions: These preliminary results prompt us to investigate the role of c-Cbl in the development of neoplastic clone. In CLL cells c-Cbl is overexpressed with respect to normal B cells, and upon BCR engagement it undergoes Y700 phosphorylation. However, c-Cbl is unable to stably interact with Lyn suggesting an altered c-Cbl function that contribute to affect cell homeostasis.

E1006

ACTIVATION OF SHP-1/PP2A PATHWAYS TRIGGERS APOPTOSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

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Background: CLL B cells inability to reach programmed cell death is due to intrinsic defect and extrinsic factors. Among the intrinsic fault there is the misregulation of the phosphorylation pattern. Reversible protein phosphorylation is a fundamental post-translational modification by which virtually all cellular events are regulated. The crucial players involved in this dynamic process are protein kinases and protein phosphatases, which are placed at the different levels of cellular signaling. The Src Family Kinase (SFK) Lyn is a key factor in the dysregulation of survival and apoptotic pathways of malignant B cells in CLL. One of the effects of Lyn's action is the spatial and functional segregation of the tyrosine phosphatase SHP-1 into two pools, one beneath the plasma membrane in an active state promoting pro-survival signals, the other in the cytosol in an inhibited conformation and unable to counter the elevated level of cytosolic tyrosine phosphorylation.
Aims: Because CLL is characterized by a high level of Lyn-dependent tyrosine phosphorylation in the cytosol, we focused our attention on compounds capable of directly or indirectly driving the activation of SHP-1 which in turn could counter the action of Lyn and induce cell demise. The goal is to discover new therapeutic strategies to defeat a still incurable disease as CLL.
Methods: B cells were collected from 37 CLL patients. Freshly isolated CLL cells incubated with increasing concentrations of nintedanib (0-24 μM) and MP07-66 *(2,2-diethoxyethyl{[4-(hexyloxy)phenyl]methyl})amine)* for 24 and 48 hours with/without a layer of Mesenchymal Stromal Cells (MSCs). Caspase dependence was demonstrated using the pan-caspase inhibitor z-VADfmk. CLL B cells viability was tested by Flow Cytometer with Annexin V/PI test. SHP-1 and PPP2Ac expression and phosphorylation were evaluated by Western Blotting.
Results: We performed *in vitro* phosphatase activity assays on the cytosolic pool of SHP-1 in the presence of increasing concentrations of nintedanib, a receptor tyrosine kinase inhibitor recently shown to trigger SHP-1 activity. Nintedanib treatment could activate the phosphorylated (at Ser591), and inhibited, form of SHP-1 and to induce apoptosis, depending on the caspase activation, after 24h and 48h at marked level. Interestingly, we recently demonstrated that Ser591 phosphorylation of SHP-1 could be dephosphorylated by PP2A. In this scenario, the restoration of PP2A activity by a fingolimod analogue devoid of immunosuppressive action, called MP07-66, and the subsequent dephosphorylation of PP2A substrates, was shown to trigger apoptosis, like nintedanib, in a caspase-dependent manner. Since our data suggest that the activation of either PP2A or SHP-1 triggered by specific small molecules caused stimulation of each other's activity, we treated CLL cells with nintedanib and MP07-66 together demonstrating an improved effect when used in combination. Similar results, in all the conditions, were obtained in presence of a MSC layer, showing the capability of these treatments to counteract the protective action of tumor microenvironment.
Summary/Conclusions: In conclusion, our findings indicate that phosphatase activators may represent a new weapon against this form of leukemia. Overall, these data corroborate the hypothesis that the inhibition of PP2A is central to CLL cell viability and that its activation is facilitated by the supportive action of SHP-1, as demonstrated by the effect produced by the simultaneous use of the respective activators.

E1007

TARGETING NANOPARTICLES TO CHRONIC LYMPHOCYTIC LEUKAEMIA: EXPLOITING THE PROPERTIES OF CXCR4

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Background: Nanoparticle carriers of therapeutic agents ("drug delivery vehicles") can be used to deliver drugs to specific cells through the incorporation of a "targeting ligand". Targeting provides the therapeutic benefit of achieving high local drug concentrations while reducing off-target effects against other cells; the combined ligand/delivery vehicle system can also be manipulated to determine the uptake pathway or modulate biological effects. The CXCR4 chemokine-receptor is an attractive target for drug delivery vehicles. It is overexpressed in cancers including chronic lymphocytic leukaemia (CLL) (Domanska et al., 2013) and binding to its ligand (CXL12) may induce proliferation, survival or entry into protective cellular niches (Ganju et al., 1998). Targeted nanoparticles that can bind and antagonise CXCR4 could therefore allow specific drug delivery to cancer cells while simultaneously blocking CXCL12-induced chemoprotection.
Aims: A drug-design strategy was developed to synthesise and evaluate a novel CXCR4 targeting motif (BAT1) with structural similarity to Plerixafor, a CXCR4-antagonist in clinical use. A key design principle was to incorporate a polyethylene glycol (PEG) tether with a functional end-group to provide an attachment point for cargoes, particularly liposomes. The evaluation aim was to assess the effectiveness of BAT1 to deliver a chemotherapy cargo to CLL cells within an *ex vivo* culture system.
Methods: A three-step synthesis was used to generate BAT1 (Figure 1A); its structure and purity was confirmed using NMR, MS and HPLC. Bioactivity testing employed primary CLL lymphocytes. Assessments tested: CXCR4 binding-affinity (flow cytometric competition assays), cell-binding characteristics (immunocytofluorescence) and blockade of CXCL12-induced signalling (immunoblot). Initial targeting assessment used a fluorescent label (Cy5) conjugated to the functional PEG tether. Cholesteryl chloroformate was then selected to conjugate BAT1 to PEGylated liposomes.
Results: The binding affinity of BAT1 (Figure 1B) was demonstrated using competition assays (CXCL12, anti-CXCR4 ab, and the bis(cyclam) drug Plerixafor). The studies confirmed BAT1 had high affinity for CXCR4 receptors expressed on primary CLL cells. Immunocytofluorescence comparison with its native ligand confirmed binding of BAT1 to the CLL cell surface, while immunoblotting demonstrated blocking of CXCL12-induced downstream signalling (Figure 1C and 1D). The fluorescent moiety Cy5 was covalently linked to the PEG moiety as a test-cargo, demonstrating that binding affinity was retained in the presence of a cargo and that the drug competed for CXCR4 binding with related bis(cyclam) drugs. This work has been extended to attach BAT1 to liposomes, with present work optimising liposome characteristics for binding and uptake by CLL and the delivery of cytotoxic payload.

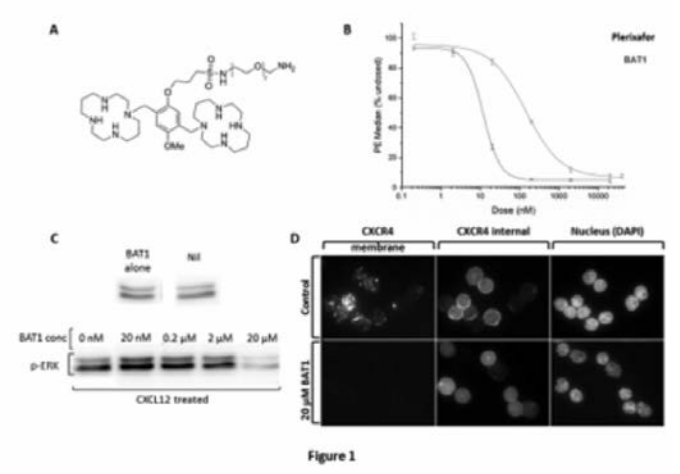

Figure 1

Figure 1.

Summary/Conclusions: A novel bis(cyclam) CXCR4 antagonist and targeting ligand – BAT1 – has been synthesised. BAT1 demonstrates high affinity for the CXCR4 receptor, supporting targeted delivery to CLL cells. Receptor binding is associated with simultaneous blockade of CXCL12-mediated signal initiation and effect, and therefore biological modulation of target cell behaviour. BAT1

is readily attached to liposomes through the PEG moiety, which will allow chemotherapy delivery using stealth-liposomes (Allen and Cullis, 2013). Liposome size and composition will be used to drive pathway-specific uptake to different intracellular compartments. BAT1 therefore offers significant potential to enhance therapy in CLL.

E1008

THE ROLE OF THROMBOPOIETIN AS A TOOL OF IMMUNE MODULATION IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Thrombopoietin (TPO) is the major regulator of platelet production, synthesized mainly by liver cells. The TPO receptor (TPO-R) is known to be expressed on platelets, megakaryocytes and CD34$^+$ cells. It has been reported that patients with immune thrombocytopenic purpura, treated with TPO-R agonists, developed alterations in the T-cell repertoire and pattern of cytokine secretion from B- and T-cells. Thus, clinical activity of these agents could be attributed in part to immune modulation. In chronic lymphocytic leukemia (CLL), characterized by aberrant T-cell responses, high TPO serum levels coexist with low levels of TPO gene transcripts in the malignant cells. These observations could imply that TPO acts as an immune modulator in CLL.
Aims: The aim of the current study was to explore the role of TPO in T-cell modulation in CLL.
Methods: B-cells and CD4$^+$T-cells were isolated from peripheral blood mononuclear cells (PBMCs) of untreated CLL patients (Rai stages 0-IV) and healthy donors. TPO-R (CD110) expression on CD4$^+$ T-cells was estimated by FACS. CD4$^+$ T-cells were incubated with low-dose IL2 and TPO for 5 days and the percentage of CD4$^+$CD25^{hi}FOXP3$^+$ cells (Tregs) was assessed. T-cell proliferation was evaluated using CFSE staining after stimulation with anti-CD3/CD28 antibodies, high-dose IL2 and TPO for 5 days. Percentage of cells retained in G0 (non-proliferating pool) was assessed. CLL B-cells were activated with a TLR9 agonist (ODN) and TPO expression was assessed by Q-PCR.
Results: CD4$^+$ T-cells of CLL patients expressed significantly higher levels of TPO-R (CD110) compared to T-cells of healthy donors, with a mean fluorescent intensity of 764±148 and 498±206, respectively ($p<0.05$; n=6). Stimulation of patient-derived CD4$^+$ T-cells with TPO led to a 12% increase in the number of cells retaining in G0 (from7.5%±5.4 to 8.5%±6.4; $p<0.05$; n=8), whereas proliferation of healthy donor T-cells remained unaffected by TPO (11.5%±5.7 and 11.4%±5.7 of cells in G0; p=NS; n=6). Additionally, TPO stimulation resulted in a 24% increase of Treg levels in patient T-cells (from 2.1%±1.7 to 2.6%±1.7%; $p<0.01$; n=8). However, the Treg levels were not altered in healthy donor T-cells subjected to TPO (0.74%±0.7 and 0.74%±0.8; p=NS; n=5), which is similar to their proliferation response to this growth factor. To determine whether CLL cells could be the TPO source in this disease, TPO mRNA expression in the malignant cells was assessed, demonstrating a baseline ct value of 721±296, which significantly increased to 1033±342 ($p<0.05$; n=6) upon ODN activation.
Summary/Conclusions: In the current study, TPO is found to affect immune properties of CLL patient T-cells, inhibiting their proliferation and increasing Treg levels. These effects have been observed in patient T-cells only, which could be partly explained by higher levels of TPO-R expression revealed on patient T-cells compared to healthy donor cells. The elevated TPO mRNA expression in CLL B-cells could point to them as one of the possible sources of this growth factor in patient serum. Activation of TPO-R may represent a novel mechanism of T-cell inhibition in CLL.

E1009

TREATMENT WITH BCR INHIBITORS INCREASES ROR1 EXPRESSION IN CLL CELLS

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Background: Receptor Tyrosine Kinase-Like Orphan Receptor-1 (ROR1) expression on malignant B-cells is considered a promising target for therapy of CLL and other lymphoproliferative disorders. Recently published data suggest that combination of BCR inhibitor ibrutinib with ROR1 antibody cirmtuzumab can enhance treatment efficacy in CLL. Nevertheless, the variability in ROR1 expression during disease progression, therapy administration and relapse remains unknown.
Aims: In our study we aimed to i) detect ROR1 in CLL cells during different stages of the disease using flow cytometry and qRT-PCR with focus on patients undergoing therapy; ii) analyse changes in ROR1 expression within individual patients during the disease course.
Methods: CLL cohort consisted of 96 CLL patients (152 samples): 23 patients with stable disease, 16 patients with active disease prior first therapy intervention, 6 patients during first therapy, 13 patients in progression before second line treatment, 3 patients in complete remission, 10 refractory patients, 9 patients treated with ibrutinib, 16 patients treated with idelalisib. To monitor changes in ROR1 expression we tested serial samples from 5 CLL patients (median follow up 76 months (66-131), median number of sampling points 12 (5-18)). For surface ROR1 protein analysis we used 8-colour flow cytometry (modified MRD protocol: CD45/CD3/CD19/CD5/CD81/CD79b/CD22/ROR1) in all samples. To quantify *ROR1* mRNA expression changes within individual patients we performed qRT-PCR in separated CLL cells (>95% CD19+CD5+). CLL cells from samples in remission were separated immunomagneticaly (Whole Blood Anti-ROR1 MicroBead Kit, Miltenyi Biotec).
Results: Using multicolour flow cytometry we confirmed ROR1 antigen/protein presence on CLL cells in each analysed sample. The ROR1 antigen was detectable on residual CLL cells during disease remission with the ability to distinguish malignant population from healthy B-cells. Using qRT-PCR we detected significantly higher levels of *ROR1* mRNA in samples of treated patients ($p<0.01$). This observation was supported by analysis of *ROR1* mRNA expression in five patients tested consecutively in several time-points. We detected *ROR1* mRNA expression increase in disease progression before therapy and further increase during therapy administration. In case of remission induction we observed decrease of *ROR1* mRNA level. In patients treated with ibrutinib or idelalisib we observed steep increase of ROR1 expression compared to patients treated with FCR regimen.
Summary/Conclusions: ROR1 protein remains detectable on CLL cells during disease course even in complete remission. *ROR1* mRNA levels are highly influenced by therapy administration especially in the case of treatment with BcR inhibitors.

Supported by grants AZV-MZ-ČR 15-29793A, 15-31834A, and 15-30015A, MUNI/A/1106/2016 and CEITEC 2020 (LQ1601) MŠMT ČR.

E1010

NORMAL SERUM PROTEIN ELECTROPHORESIS IDENTIFIES AN EXCELLENT PROGNOSIS GROUP AMONG IGHV MUTATED PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA, WITH A MEDIAN TFS OVER 18 YEARS

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Background: Approximately 36% of patients with chronic lymphocytic leukemia (CLL) have abnormal serum protein electrophoresis (SPE), either with hypogammaglobulinemia or with monoclonal immunoglobulin (Ig) peak. In this study, we compared locally recruited patients with normal and abnormal SPE.
Aims: The aim was to identify prognosis parameters.
Methods: A total of 189 patients (132 abnormal SPE and 57 normal SPE) were studied. Diagnoses were performed between 1980 and 2015. Prognosis parameters investigated were IGHV mutation status, presence of SF3B1, NOTCH1 or BIRC3 mutations (determined by high throughput and Sanger sequencing), and cytogenetic abnormalities such as del17p, del11q, del13q and trisomy 12 (assessed by standard caryotype, FISH analysis and QMPSF).
Results: In this series, 73%, 19% and 8% of patients were at Binet stage A, B and C respectively, and 30% had a normal SPE at diagnosis. Ninety six percent of patients with normal SPE were at Binet stage A, *versus* 63% of patients with abnormal SPE (Chi2 test : $p<10^{-4}$). Median lymphocytosis at diagnosis was lower in patients with normal SPE (12,82 G/L *versus* 19,54 G/L in abnormal SPE, Fisher test : p=0,00169). Regarding genetic prognosis factors, we found that 58% of cases with normal SPE had a good prognosis profile (mutated IGHV and/or isolated del13q, with no other genetic abnormality detected), meanwhile 65,2% of patients with abnormal SPE exhibited at least one poor prognosis marker (unmutated IGHV, mutation of SF3B1, NOTCH1, del17p, del11q or trisomy 12, Chi2 test : $p<10^{-4}$). In patients with normal SPE, only 3,5% cases were SF3B1 mutated against 15,2% in case of abnormal SPE (Chi2 test : p=0,002). Among other strong differences, 10,5% patients with normal SPE had a trisomy 12 against 18,2% for abnormal SPE. Isolated del13q was found in 38,6% and 21,2% of cases with normal and abnormal SPE respectively. Mutated IGHV status was found in 65% in normal SPE and 56% with abnormal SPE. Compared to the whole series, IGHV repertoire analysis shows bias in IGHV1-2, and IGHV4-34 rearrangements, with decreases usage of IGHV3-21 and IGHV3-48. Treatment free survival was markedly increased in patients with normal SPE (median of 10,0 years *versus* 3,0 years for normal and abnormal SPE respectively, log rank test : $p<0,0001$). Moreover, while patients with mutated IGHV had a median TFS of 8 years (against 3 years for unmutated patients), those with normal SPE and mutated IGHV had a median TFS over 18 years. Patients with mutated IGHV and abnormal SPE had a

median TFS of 4 years (log rank test : p=0,0003). Thus, patients with normal SPE and IGHV mutated status constitute a group with excellent prognosis.
Summary/Conclusions: In conclusion, normal SPE was associated with good outcome with decreased accumulation of side genetic events (in particular, SF3B1 mutations). This analysis shows a bias in IGHV repertoire according to SPE status. These results also clearly suggest that patients with a normal SPE and mutated IGHV have an extremely quiet CLL natural history. This could be either due to the weaker activity of the disease and/or to the absence of adverse consequences of a concomitant paraprotein.

E1011

HSP70 EXPRESSION IS MODULATED BY ITS MASTER REGULATOR HSF1 VIA MAPKS AND PI3K/AKT/MTOR PATHWAYS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: The search for molecules involved in apoptosis resistance/increased survival of B cells from Chronic Lymphocytic Leukemia (CLL) is still ongoing since this disease remains not definitively understood. We recently found that the Heat Shock Protein of 70kDa (HSP70), expressed in response to a wide variety of stress signals and allowing cells to survive to lethal conditions, was particularly overexpressed in neoplastic B cells from CLL. Moreover, the Heat Shock Factor 1 (HSF1), the major responsible for the transcription of HSP70, is itself overexpressed in CLL and strictly correlated to HSP70. In response to stress, HSF1 becomes phosphorylated, forms homotrimers, binds DNA and activates heat shock gene transcription. HSF1 is regulated by a fine balance of activatory/inhibitory phosphorylations mediated by kinases belonging to pathways triggered by RAS (*i.e.* PI3K/AKT/mTOR and RAF/MEK/ERK).
Aims: Since HSP70 is overexpressed in CLL neoplastic B cells and most of "HSF1-phosphorylating actors" belong to signalling pathways taking part from RAS, being the PI3K/AKT/mTOR and the RAF/MEK/ERK pathways, we are herein aimed at gaining information and dissecting this network in CLL B cells.
Methods: In a Reverse Phase Protein Array (RPPA) study, previously performedin B cells from 57 CLL patients and 11 healthy subjects, we assessed the activation/expression of key signalling proteins. Herein, we focused on HSP70, AKT-Ser473, mTOR-Ser244, GSK3a/b-Ser21/9, CDK2, CREB-Ser133, MEK1/2-Tyr217-221, ERK1/2-Thr202/Tyr204, NFkB-Ser536, p38MAPK-Thr180/Tyr182, SAPK-JNK-Thr183/Tyr185 and PDK1-Ser241. Cluster and separated analyses have been performed.
Results: We divided our patients in HSP70-high and HSP70-low considering as cut-off the value of the median of HSP70 expression levels calculated by RPPA and demonstrated that the examined proteins behave in a different way between patients expressing high or low levels of HSP70. HSP70-high patients present high Akt-Ser473, an inhibitor of GSK3a/b that, in the inhibited form, prevents HSF1 inhibition. By contrast, HSP70-low patients have high MEK1/2-Ser217/221 and ERK-Thr202/Tyr204, known to negatively regulate HSF1. Intriguingly, p38MAPK-Thr180/Tyr182 which has been described to both activate and inhibit HSF1 at different sites, is overexpressed in those patients presenting low levels of HSP70.
Summary/Conclusions: These data would suggest that, in CLL, HSP70 expression is regulated by the modulation of HSF1 activity through the activation of one or the other way triggered by RAS. In particular, an activation of the PI3K/AKT/mTOR pathway leads, as result, to a higher expression of HSP70 while an activation of the RAF/MEK/ERK signalling rather results in HSP70 down regulation. The dissection of signalling pathways connected to HSP70-HSF1 axis in CLL will contribute to define the biology and understand the pathogenesis of this disease.

E1012

THE INTERPLAY BETWEEN TH17 AND TREGS: A NEW IMMUNOSUPPRESSIVE INSIGHT IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chronic lymphocytic leukemia (CLL) is the most frequent leukemia in the Western world and it is characterized by the clonal expansion of CD5 positive B cells. In CLL, different T cells dysfunctions have been described, probably related to the interaction with malignant B cells. TH17 and regulatory T cells (Tregs) are subpopulations of T lymphocytes which play a fundamental part in inflammatory response and immune tolerance. However, their role in the immunopathogenesis of CLL has not yet been fully clarified.
Aims: The aim of this study is to clarify the interplay between TH17 and Tregs in the pathogenesis of CLL.
Methods: After obtaining the patient's informed consent, peripheral blood was collected from 30 untreated CLL patients and 30 age-matched healthy volunteers (HV). Cytokine production was evaluated before and after a 48 h culture of CD4+ T cells in complete medium with IL-6 (o/n), followed by a 5 h stimulation with PMA, Ionomycin and Monensin (PIM), or with *Candida Albicans*. For cytokine secretion analysis, stimulated CD4+ T cells were analyzed by human IL-17 and IL-10 secretion assay. IL-23 plasma levels were evaluated by ELISA. For the analysis of Tregs and their subsets, stimulated PBMCs were stained with anti-CD4 FITC, anti-CD25 APC-Cy7, anti-FoxP3 APC and anti-Tbet PE or anti-RORγt PE or anti-GATA-3 PE. Statistical analysis were carried out using the paired and unpaired two-tailed Student's t tests and confirmed with the non parametric Wilcoxon signed-rank test.
Results: In CLL patients we observed a reduced production of IFN-γ and IL-4, respectively from TH1 and TH2 and an increase of IL-17A from TH17, compared to HV. All the observed differences were statistically significant. We also evaluated the ability of CD4+ T cells to secrete IL-17A, IL-10 or both. We reported a statistically significant increase in the frequency of CD4+ IL-17A-producing cells in CLL patients compared to HV, whereas the percentage of IL-17A+/IL-10+ cells remained unchanged. In order to evaluate the functional effects of the observed alterations, we analyzed IFN-γ+ CD4+ T cells-mediated response after stimulation with *C. Albicans* for 48 h, with or without depletion of IL-17A-secreting cells. The frequency of IFN-γ-producing T cells resulted statistically significant increased in patients than HV before IL-17A-secreting T cells depletion. Conversely, after IL-17A+ CD4+ T cells depletion, we didn't observe significant differences in term of IFN-γ production. We also observed increased IL-23 plasma levels in patients compared to HV. In addition our data highlighted a significantly higher frequency of CD4+ CD25^{high}FoxP3+ cells (Tregs) in CLL samples, with a statistically significant increase in Tbet+ Tregs, RORγt+ Tregs and GATA-3+ Tregs subpopulations (Figure 1).

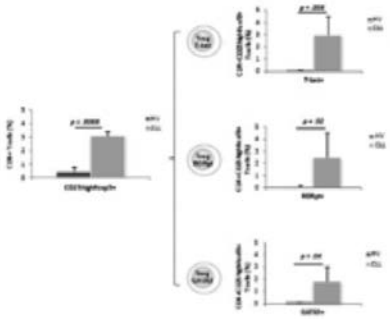

Figure 1.

Summary/Conclusions: Our results reported a down-regulation of IFN-γ and IL-4 producing T cells, associated to an increased frequency of Tregs and their subsets in CLL patients, probably trying to overcome the deficit of effector T cells. On the other hand, we observed a rise in IL-17A secreting T cells related to the increased IL-23 production by dendritic cells in order to restore TH17 pool, without changing the percentage of IL-17A+/IL-10+ cells. These data support the idea of the protective function of TH17 that show an effector and not a regulatory T phenotype. Starting from these observations, this study could pave the way to further researches and applications in the comprehension of the biological and regulatory mechanisms of TH17 and Tregs, supporting the study of a pioneering anticancer therapy in CLL.

E1013

LOW EXPRESSION OF CD25 IN CHRONIC LYMPHOCYTIC LEUKEMIA NOTCH1-MUTATED CASES INDEPENDENT OF CDK4/6 MISREGULATION

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Background: Recently, it has been shown that CDK6-mediated repression of CD25 is required for induction and maintenance of NOTCH1-induced T-cell acute lymphoblastic leukemia.
Aims: The aim of this study was to identify the NOTCH1 mutational status detected by deep sequencing in a cohort of 138 CLL patients and to correlate it with the immunophenotypic profile and CDK4 and CDK6 expression.
Methods: We performed targeted NGS sequencing of blood samples, collected at diagnosis, from 138 CLL patients. We designed a TruSeq Custom Amplicon

containing 13 genes and covering 28.099 bases. Paired-end sequencing was performed with MiSeq v2.2 chemistry, and a mean depth of 998 reads/base was obtained. Every patient underwent, at baseline, a flow cytometry characterization with a panel including (sIg)λ, (sIg)κ, CD19, CD5, CD11b, CD81, CD10, CD79b, CD29, CD38, FMC7, CD22, CD45, CD103, CD11c, CD25, ZAP70, CD11a, and CD24. CDK4 and CDK6 expression levels were quantified by RT-qPCR.
Results: With a median age of 66 y.o. (range, 31-89) and a slight male predominance, the median follow up time of our cohort was 43 months (24-104). We found that 38/138 (28%) patients harbored at least one mutation, with NOTCH1 (n=16, 12%), ATM (n=12, 9%), TP53 (n=9, 7%), and SF3B1 (n=8, 6%), as the most frequently mutated genes. Those patients with a NOTCH1 mutation showed a lower CD25 expression (24 mean fluorescence intensity units (MFIu)) than those without a mutation (43 MFIu), p=0.03. We could not validate the recently reported association between the presence of NOTCH1 mutations and a low expression of CD20. In our cohort, the MFI expression in NOTCH1 mutated and non-mutated patients was 163 and 146 units, respectively (p>0.05).We measured CDK4 and CDK6 expression in the CD19+ sorted fraction RNA of 7 NOTCH1 mutated cases and 11 non mutated cases, without finding significant differences (0.26 *vs* 0.27 for CDK6, 0.025 *vs* 0.022 for CDK4; p>0.5 in both cases).
Summary/Conclusions: We found a significant inferior expression of CD25 when activating NOTCH1 mutations are present in CLL patients. The relationship found between these two variables, with an inversed direction to that found in physiological conditions, has also been shown in the setting of NOTCH1-mutated T acute lymphoblastic leukemia. In CLL cases, it seems to be independent of CDK4/6 expression, prompting further studies assessing CDK4 and CDK6 regulators.

E1014

GENE MUTATIONS ANALYZED BY NEXT-GENERATION SEQUENCING ALLOW US TO DEFINE THE PROGNOSIS OF CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS WITH EARLY-STAGE DISEASE AND 13Q DELETION

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Background: Next-Generation sequencing (NGS) studies have revealed a number of recurrently mutated genes in chronic lymphocytic leukemia (CLL). It is reasonable to argue that evaluation of the newly gene mutations as prognostic markers would help to improve prognostication of CLL patients. Interestingly, gene mutations could help us to refine the prognosis in the group of CLL patients with other prognostic markers associated with good prognosis.
Aims: To analyze the presence of mutations of a panel of genes by NGS and its prognostic impact in patients with CLL, focusing in the groups of patients with good prognosis characteristics.
Methods: Amplicon-based-NGS was performed using 454 platform in 147 CLL patients to evaluate the mutational status of 6genes (*TP53*, *NOTCH1*, *SF3B1*, *XPO1*, *FBXW7* and *MYD88*). Samples were obtained at diagnosis or before treatment in all cases. 70.1% were Binet A and 53% had 13q deletion (13q-). A cut-off 2% was applied to define variants. All the mutations were validated.
Results: 1. NGS analysis showed that 37.4% of CLL patients (55/147) showed mutations in any of the analyzed genes. The frequency of mutations was 16.3% for *NOTCH1*, 10.2% for *SF3B1,* 6.8% for *TP53*, 4.8% for *XPO1*, 3.4% for *FBXW7*, and 1.4% for*MYD88*.The presence of mutations in any of these genes except to *MYD88* (mutated CLL) was significantly associated with clinical progression (60.0% for mutated CLL *vs.* 38.2% for unmutated CLL; *P*=0.05). Interestingly, mutated CLL patients showed a shorter time to first treatment (TFT) than unmutated CLL patients (30 months *vs.* 88 months; *P*=0.006). By contrast, *MYD88* mutations were detected in CLL with mutated *IGHV* and 13q-. 2. Of note, 23.6% of the mutations had a mutational load of ≤15% and thus would not have been detected by capillary Sanger sequencing. CLLs with mutations in ≤15% of cells had also a shorter TFT than patients without mutations (18 *vs.* 88 months; *P*=0.018), and similar to CLL patients with mutations in >15% of cells (*P*=0.370). In addition, 14.5% of mutated CLL patients showed 2mutations.Patients with more than one mutation had a shorter TFT than CLL patients with one mutation (7 months *vs* 31 months). 3. In the group of CLL patients with Binet stage A, 32.8% of them showed mutations in any of the analyzed genes. Interestingly, CLL patients Binet A with mutations (except to *MYD88*) showed a shorter TFT than CLL patients without mutations (31 *vs* 131 months, P<0.001). Besides this, CLL with 13q- as the sole cytogenetic alteration and gene mutations had also a shorter TFT that unmutated 13q- CLL patients (*P*=0.002).
Summary/Conclusions: 1) CLL patients with mutations in *TP53*, *NOTCH1*, *SF3B1*, *XPO1* and *FBXW7* show a worse prognosis than CLL patients without mutations. 2) Gene mutations in *TP53*, *NOTCH1*, *SF3B1*, *XPO1* and *FBXW7* in a low percentage of the cells are associated with a shorter TFT. 3) Among CLL patients with good prognostic characteristics (Binet A and 13q-), gene mutations help us to define the prognosis of the patients.

E1015

ALTERED COMPLEX C5 IS ASSOCIATED WITH COMPROMISED COMPLEMENT ACTIVITY IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: The therapeutic monoclonal antibodies used for the treatment of Chronic lymphocytic leukemia (CLL) mediate anti-tumor effects through several mechanisms: complement-mediated cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), and phagocytosis. CDC efficacy thus depends on the expression level of the target B-cell antigen, the integrity of apoptotic cascades within tumor cells, the functional capacity of effector cells, and the availability and activity of the complement (C) system. Published data indicate deficiency of one C protein or more in most CLL patients, as well as additional factors that may affect C activity. The role of structural abnormalities of C complexes in affecting C function has not been investigated.
Aims: To study the structural integrity of circulating C complexes, focusing mainly on C5, and to establish its importance for C activity in CLL.
Methods: Blood samples were collected from 36 naïve CLL patients and 10 healthy controls (HC). Biochemical and haematological parameters, and CLL staging were recorded. The isoforms of two key C components, C3 and C5 were studied by Western blot analysis. The activity of the C system before and after in-vitro activation via the classical or alternative pathways was followed by the levels of C5b-9, the terminal product of C activation. C activation was also studied in combination with C5-deficient serum or after supplementation with normal C5. In-vitro activation data were associated with the presence of C5 isoforms.
Results: Differences in C5 pattern were found in some of the CLL patients. Specifically, the C5 complex that exists as a single protein band in all HC and in 56% of CLL patients appeared in 44% of the patients as a clear double-band. No clear differences were observed in C3 pattern in the patients. Higher basal levels of C5b-9 were found in CLL patients with abnormal pattern of C5 (3946±758 SEM ng/ml) compared with both HC (682±158 SEM ng/ml) and CLL patients presenting normal C5 pattern (2363±655 SEM ng/ml). In-vitro C activation via the classical pathway was inversely correlated with basal activity, and was significantly lower (p<0.03) in the CLL patients with altered C5 compared to HC and CLL patients with normal C5. In-vitro activation via the alternative pathway was similar in all subjects' groups. C activity in C5-deficient serum supplemented with 33% sera from patients with abnormal C5 was significantly lower compared to the activity observed after supplementation with serum from HC or from patients with normal C5. Activity after supplementation with normal C5 (commercial) was significantly lower in sera from CLL patients with abnormal C5 pattern compared to sera from the other subjects' groups.
Summary/Conclusions: The data indicate a possible link between the activation potential of the C system in CLL patients and alterations in the complex structure of C5. The differences in C activation via the classical and alternative pathways may indicate disturbance in the classical pathway in patients with abnormal C5. The exact mechanisms by which abnormal C5 distracts the C activity need further clarification. Yet, the appearance of abnormal C5 in CLL patients with disturbed C activity bears the potential to develop a marker which will assist in identifying patients who are likely to be less responsive to future immunotherapy treatment due to compromised CDC. Development of such a marker may assist clinicians in refining and personalizing the immunotherapeutic approach, improving CDC and consequently the therapy results.

Chronic lymphocytic leukemia and related disorders - Clinical

E1016

ASSOCIATIONOF CPG-STIMULATED KARYOTYPE WITHTIME-TO-FIRST TREATMENT FOR CLL

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Background: Prognostic factors correlate with clinical outcomes, independent of treatment. B cell receptor (BCR) signaling pathway inhibitors can nullify the prognostic impact of some markers, such as *IGHV* mutation status.CpG-stimulated metaphase karyotype can identify clonal cytogenetic abnormalities in CLL that may not be seen with standard non-stimulated karyotype or by FISH. Complex cytogenetics, defined as 3 or more chromosome abnormalities in 2 or more metaphases was the highest-risk feature for shorter progression-free and overall survival in patients receiving ibrutinib for relapsed/refractory CLL. Complex karyotype is not uncommon among relapsed/refractory CLL cases, particularly those who previously received genotoxic chemotherapy.

Table 1. Continuous and Categorigal Patients Characteristics.

Continuous Characteristic	*n*	*Number (range)*
Age, median (yrs) **Sex,** Male	**500**	62 (34-91) 334 (67)
WBC, ALC(K/µl), **HGB** (gm/dl) median	**498**	20.5 (2.3-399) 14.8 (0-24) 13.7 (1-17.8)
PLT, median (K/µl)	**497**	182 (18-497)
LDH, (IU/dl) **B2M,** (mg/l) median	**493 / 486**	502 (48-3294)
Categorical Caracteristic	***n***	***Number (%)***
Rai Stage	**468**	
0 I II		390 (78)
III IV		78 (16)
IGHV-MS	**403**	
UnMutated		180 (45)
Mutated		223 (55)
FISH	**495**	
Del17p		33 (7)
Del11p		75 (15)
+12		78 (16)
None		101 (20)
Del13q		208 (42)
CpG-stim. Karyotype	**501**	
Complex3		35 (7)
Complex2		36 (7)
Single		83 (17)
Diploid		347 (69)
BM Zap70-IHC / CD38 Flow	**277 / 388**	
Positive		136 (49) /127 (33)
Negative		141 (51) / 261(67)
Treated	**501**	164 (33)

WBC=white blood cell; **ALC**=absolute lymphocyte count; **HGB**=hemoglobin; **PLT**=platelet; **LDH**=lactate dehydrogenase; **B2M**=beta-2 microglobulin

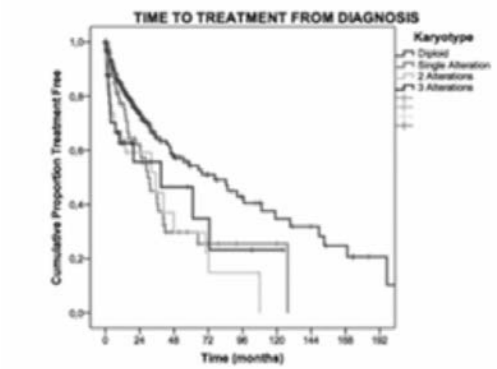

Figure 1.

Aims: The aim of this study is to report the incidence and the impact of CpG-stimulated karyotype in the treatment of naive CLL.

Methods: We evaluated 501 treatment-naïve patients with CLL at MDACC between July 2013 and June 2016. CpG-stimulated metaphase karyotype of CLL cells from blood or bone marrow was performed by culture of mononuclear cells for 72 hrs in media containing CpG-685 (20ug/ml), phorbol 12-myristate 13-acetate (PMA; 0.04ug/ml) and Pokeweed mitogen (PWM; 0.1ug/ml). Banding and analyses were by standard laboratory procedures. Twenty metaphases were analyzed per culture and patients were categorized as having diploid karyotype, a single, 2 or 3 or more(complex) clonal chromosome abnormalities present in more than 1 metaphase by CpG-stimulated karyotype.The frequency and distribution of chromosome abnormalities with other prognostic factors and time-to-first treatment from diagnosis (TTFT) were analyzed (Table + Firgure).

Results: The majority (69%) of patients had diploid cytogenetics. Higher-risk prognostic features such as del17p, del11q, unmutated*IGHV* and ZAP70 expression were associated with presence of complex karyotype abnormalities. Shorter TTFT from diagnosis was associated with 1, 2, and complex clonal chromosome abnormalities compared to diploid karyotype (p=.0005). A model was developed, which identified patient characteristics independently associated with shorter TTFT including: 1 or more clonal chromosome abnormality by CpG stimulated karyotype; unmutated*IGHV*; 3 involved lymph node sites; and CD38 expression (>30%).

Summary/Conclusions: In conclusion, CpG-stimulated karyotype identified 1 or more clonal chromosome abnormality in nearly a third of untreated patients and was a significant independent prognostic factor for TTFT. Models for TTFT may be useful in identifying patients at high-risk for needing treatment sooner and thereby useful for early intervention clinical trials.

E1017

COMPARISON OF THE CHRONIC LYMPHOCYTIC LEUKEMIA INTERNATIONAL PROGNOSTIC INDEX (CLL-IPI) WITH THE BARCELONA-BRNO PROGNOSTIC MODEL: ANALYSIS OF 1299 NEWLY DIAGNOSED CASES

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Background: In the last two decades, a plethora of clinical, serological and biological markers have been identified that are significantly associated with the prognosis of chronic lymphocytic leukemia (CLL) patients. Recent research has focused on the development of scoring systems capable of integrating the major prognostic parameters. A recent prognostic index called CLL International Prognostic Index (CLL-IPI), built on clinical, serological, and biological parameters (TP53 deletion and/or mutation, IGHV mutational status, β2M, clinical stage, and age) has been proposed and validated. Recently, a Barcelona-Brno CLL group, with the aim of simplifying the CLL-IPI, proposed a prognostic model comprising only two biomarkers (IGHV mutational status and FISH cytogenetics).

Aims: We performed a comparison of the CLL-IPI with the Barcelona-Brno prognostic model in an independent series of Italian and United States (U.S.) patients.

Methods: Databases from 4 Italian and 1 U.S. centers including roughly 3700 newly diagnosed CLL patients were used to compare the CLL-IPI with the Barcelona-Brno prognostic model. Baseline data regarding age, Rai stage, IGHV mutational status, β2M and fluorescence *in situ* hybridization (FISH)-detected cytogenetic abnormalities were available for 1299 cases. Del17p was used as the sole marker of *TP53* status. The CLL-IPI and the Barcelona-Brno prognostic model were calculated using the methods proposed. The accuracy of the prognostic models was assessed by the Harrell C index (an index of discrimination), the explained variation in mortality (an index combining calibration and discrimination), and the Akaike information criterion (AIC, an index comparing two non-nested prognostic models). The lower the AIC, the higher the prognostic accuracy of a predictive model.

Results: The median age of the 1299 patients was 63 years (range 27-92) with 61.3% males. The majority of patients had Rai stage 0 (57.9%). According to the CLL-IPI, 51.3% of patients were classified as low-, 28.7% as intermediate-, 16.2% as high-, and 3.8% as very high-risk. The 5-year OS probabilities were: 95% for low-risk, 89.9% for intermediate-risk, 70.1% for high-risk, and 32.8% for very high-risk cases (P<0.0001; Harrell C index=73%;P<0.001) (Figure 1A). According to the Barcelona-Brno prognostic model, 58.1% of patients were classified as low-, 31.8% as intermediate-, and 10.1% as high-risk. The 5-year OS probabilities were: 92.2% for low-risk, 83.6% for intermediate-risk, and 68.2% for high-risk cases (P<0.0001; Harrell C index=65%; P<0.001) (Figure 1B). The AIC showed the superiority of the CLL-IPI compared to the Barcelona-Brno prognostic model in predicting OS (CLL-IPI, AIC=3432.167 *versus* Barcelona-Brno prognostic model, AIC=3549.492). Accordingly, the explained variation in mortality provided by the CLL-IPI was 42% (P<0.001), a figure higher than that due to the Barcelona-Brno prognostic model

(21%,P<0.001), indicating that the CLL-IPI had a higher prognostic accuracy for mortality compared to that of the biomarkers-only prognostic model. Then, we also compared the ability of the two scores to predict TTFT in newly diagnosed patients. The Harrell C-index of the Barcelona-Brno prognostic model was 0.70 (P<0.001), lower than that of the CLL-IPI score (0.73,P<0.001). The AIC showed the superiority of the CLL-IPI compared to the Barcelona-Brno prognostic model in predicting TTFT (CLL-IPI, AIC=5960.503 *versus* biomarkers-only prognostic model, AIC=6010.929). Accordingly, the explained variation provided by the CLL-IPI was 33% (P<0.001), a figure higher than that achieved by the Barcelona-Brno prognostic model (28%,P<0.001), indicating that the CLL-IPI had a higher prognostic accuracy for predicting TTFT as compared to that of the Barcelona-Brno prognostic model.

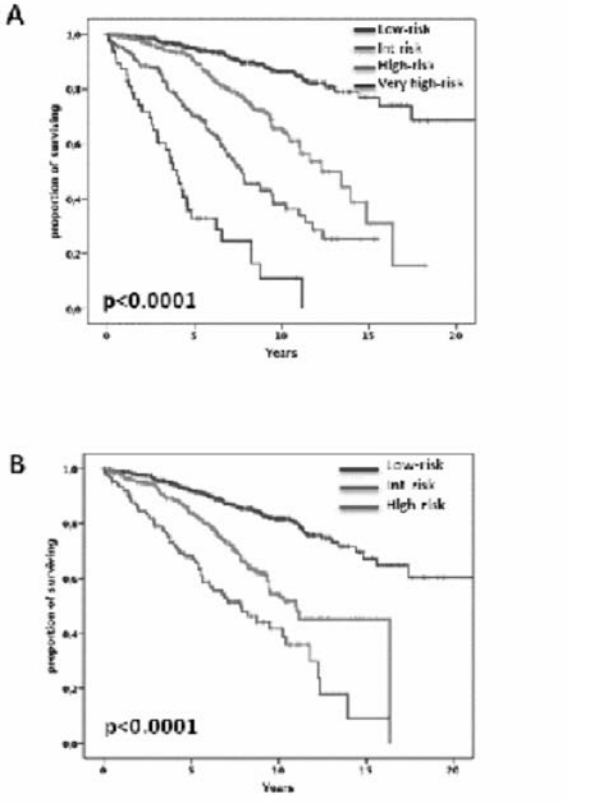

Figure 1.

Summary/Conclusions: Our results confirm the validity of both scores (CLL-IPI and biomarkers-only prognostic model) to predict survival and TTFT among patients with previously untreated CLL. Moreover, we have demonstrated that the CLL-IPI which combines clinical and serological data with biological parameters has a higher accuracy for predicting prognosis and TTFT of CLL patients than the Barcelona-Brno biomarkers-only prognostic model.

E1018

PRELIMINARY RESULTS OF S55746/BCL201 (A NEW BCL2 INHIBITOR) IN RELAPSED OR REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS AND EFFECT OF CALIBRATED MODERATE MEAL ON THE PHARMACOKINETICS

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Background: BCL-2 is an anti-apoptotic protein overexpressed in chronic lymphocytic leukemia (CLL). BCL-2 is responsible for apoptosis machinery dysregulation and contributes to chemotherapy resistance. S55746/BCL201 is a novel, oral, selective BCL-2 inhibitor.
Aims: The current first-in-human study evaluates the safety and aims to establish the recommended phase 2 dose; main secondary objectives include evaluation of pharmacokinetics (PK), food effect, pharmacodynamics and preliminary activity in patients (pts) with relapsed or refractory CLL.
Methods: S55746/BCL201 as single agent is being investigated in a phase I (EUDRACT, NCT02920697), open label, multicenters, international dose escalation trial. S55746/BCL201 was initially administered in fasting condition, once daily (in 21-day cycle) until progressive disease (PD) or unacceptable toxicity. A tumor lysis syndrome (TLS) prevention protocol was implemented. After giving informed consent pts could receive 50 to 2000mg according to a modified version of the Continual Reassessment Method for dose allocation process. In the food effect part of the study, 7 non-Hodgkin's lymphoma patients received a film coated 200mg tablet under fasting condition (*i.e.* after 10-hour fasting period) and after a calibrated moderate meal the day after.
Results: As of February 2017, 12 CLL pts have been treated (median age 67 years [range 52-82]). On these 12 pts, 5 presented a bulky disease, 1 a 17p13 deletion, 4 a 11q deletion, and 1 a p53 mutation. CLL pts were dosed up to 700mg, with a median duration on treatment of 79 days. Median number of prior regimens in CLL pts was 4 (range 2-5). Preliminary PK results in fasting pts showed that exposure increased linearly but with some inter-individual variability. The most frequent (≥2 pts) grade 3/4 adverse events (AEs) were neutropenia (n=2) and thrombocytopenia (n=2). AEs possibly related to the study drug were reported in 4 pts: neutropenia (n=2), neutrophil count decrease (n=1), fatigue (n=1), dyspnea (n=1), gingival bleeding (n=1) and left ventricular ejection fraction (LVEF) decrease (n=1). No clinical or laboratory TLS were reported. One patient in the 700mg cohort experienced a DLT (asymptomatic LVEF decrease grade 2 recovered within 2 weeks). At 700mg, a decrease in lymphocytes count from baseline (>50%) was observed in 3 out of 4 patients associated with a decrease in the sum of the product of the diameters of lymph nodes (from 23% to 40%). This decrease in lymphocytes count, started from cycle 1, and may be correlated with an induction of apoptosis in CLL cells (4 hours post first dose), detected by flow cytometry in CD19+/AnnexinV+ cells. Two CLL pts are ongoing after having completed their 3rd cycle; 10 pts have withdrawn from the study: 7 due to PD, 1 for lack of efficacy and 2 due to AE. The non-compartmental pharmacokinetic analysis of the food effect cohort (5 assessable pts) demonstrated that S55746/BCL201 PK is modified by the ingestion of a moderate meal (400-500 kcal with fat contributing to 150 kcal). The median Tmax was delayed from 1.5h to 4h when administered with food. Compared with fasting condition, Cmax and AUC increased by approximately 6-fold following a moderate meal. Based on these results, a protocol amendment to the clinical trial has been implemented in order to further investigate the administration with food in a new dose escalation.
Summary/Conclusions: S55746/BCL201 monotherapy showed first signs of activity across the tested dose levels with an acceptable safety profile so far. Based on PK food interaction results, dose escalation in the fed state has started.

E1019

INCREASED VIRUS-SPECIFIC IMMUNE RESPONSES PARALLELED BY A PNEUMOCOCCUS-SPECIFIC-IMMUNODEFICIENCY STATE AND HYPOGAMMAGLOBULINEMIA: ALREADY EMERGE IN HIGH-COUNT MONOCLONAL B LYMPHOCYTOSIS PRIOR TO CLL

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Background: Patients diagnosed with chronic lymphocytic leukemia (CLL) display a high incidence of infections, due to an associated immunodeficiency state that includes hypogammaglobulinemia. Even more, it has been recently shown that the earlier stages of disease, *i.e.* high-count monoclonal B lymphocytosis (MBL^{hi}), subjects also have increased risk for infection.
Aims: To evaluate the status of the humoral immune response in CLL at different disease stages, as well as in pre-leukemic MBL^{hi} and MBL low count (MBL^{lo}) cases, *vs* healthy controls, through quantitation of soluble plasma levels of specific antibodies against ubiquitous and pulmonary infection-associated pathogens.
Methods: A total of 249 subjects (119 males/130 females; aged 68±11y) including 91 healthy donors, 71 CLL-like MBL^{lo}, 29 CLL-like MBL^{hi} and 58 CLL cases (32 Binet A, and 26 Binet B/C patients) were studied. Detection of clonal CLL(-like) B cells was performed by high-sensitive 8-color flow cytometry. Quantification of plasma antibody isotypes and specific immunoglobulins against CMV (cytomegalovirus), EBV (Epstein Barr Virus), influenza virus and *S.pneumoniae* were performed by nephelometry and commercial ELISA kits, respectively. Individuals who had received vaccination against Influenza and/or Pneumococo were excluded from the analysis of the immunoglobulin-specific titers against the corresponding pathogen, respectively. Plasma CMV and EBV DNA load was measured using commercial kits.
Results: Total immunoglobulin (Ig) titers tended to decrease with disease progression, independently of the isotype. In contrast, specific IgM and IgG titers against CMV, EBV and Influenza virus did not vary among groups, with the

exception of VCA-EBV IgG titers, that were higher in CLL *vs* the other groups. Strikingly, the IgG levels for the three viruses tended to gradually increase, from healthy individuals to stage B/C CLL. These findings were more pronounced ($p<0.05$) for IgG and to a lesser extend also for IgM, when the ratios between the virus-specific Ig/total Ig titers of the same isotype were calculated, except for Influenza-specific IgG, that showed the same trend but without statistical significance. Regarding CMV DNA load, only 3/177 individuals -1 MBL^{hi} and 2 CLL- were found to be positive (below the limit of quantitation), while EBV DNA load was detected in plasma from 7/191 (all being Binet A CLL) at median levels of 3.6 copies/ul. In contrast to the virus-specific Igs, IgG plasma levels against *S.pneumoniae* progressively diminished through progression of the disease, in parallel to the overall lower gammaglobulin levels.

Summary/Conclusions: Both MBL^{hi} and CLL patients present relatively high levels of specific Ig against human host viruses in parallel to progressively lower levels of anti-*S.pneumoniae* antibodies, which might reflect (asymptomatic) chronic reactivation of humoral immune responses against host viruses and progressively decreased protection against other microorganisms, denoting a severe pathogen-specific humoral immunodeficiency state not reflected by the overall plasma immunoglobulin levels. Alternatively, these results might point out a potential role of ubiquitous viruses in the pathogenesis of the disease. Further analyses are necessary to establish the potential relevance of such asymptomatic humoral immune responses against host viruses in the expansion of the tumor B-cell clone and progression from MBL to CLL.

E1020

AN EXTENSIVE MOLECULAR CYTOGENETIC CHARACTERIZATION IN HIGH-RISK CHRONIC LYMPHOCYTIC LEUKEMIA IDENTIFIES KARYOTYPE ABERRATIONS AND TP53 DISRUPTION AS PREDICTORS OF OUTCOME AND CHEMOREFRACTORINESS

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Background: Chronic lymphocytic leukemia (CLL) is a heterogeneous disease, running an indolent course in some patients and a clinically aggressive course in others. Risk assessment is important in clinical practice and prediction of outcome and response to treatment is very useful in an era in which several chemoimmunotherapy combinations and effective mechanism-driven treatments are available.

Aims: We investigated whether an extended genetic characterization including mutational screening by next generation sequencing (NGS) and karyotype analysis could allow for a refinement of our capability to predict outcome in newly diagnosed CLL patients with high-risk features, as defined by the presence of unmutated IGHV gene and/or 11q22/17p13 deletion by FISH and/or TP53 mutations.

Methods: 101 patients were included in this study. TP53 disruption was defined by the presence of 17p13 deletion by FISH and/or TP53 mutation by NGS. Cytogenetic analysis was performed using CpG-oligonucleotide DSP30. Each patient was categorized according to the following classification: favorable group (isolated 13q14 deletion or normal karyotype), unfavorable group (deletions of 11q22 or 17p13, or complex karyotype, ie, at least 3 chromosome aberrations); intermediate group (all other karyotypic abnormalities). A cut-off of 98% homology to the germline sequence to discriminate between IGHV mutated and unmutated cases. Mutational screening was performed with Ion Torrent PGM NGS platform on 20 CLL-related genes by using a 5% cut off.

Results: Cytogenetic analysis showed favorable findings in 30 patients, unfavorable in 34 cases and intermediate in 36 cases. A complex karyotype was present in 21 patients. By NGS, 95 somatic mutations were observed in 56/101 (55.4%) cases; 80 missense mutations, 5 nonsense mutations and 10 frameshit deletions. 16 cases (15.8%) showed mutations in the TP53 gene, 11 (10.9%) in the NOTCH1 gene, 11 (10.9%) in the SF3B1 gene, 8 (7.9%) in the ATM gene, 5 (4.9%) in the BIRC3 gene, 5 (4.9%) in the PTEN gene, 4 (4.0%) in the MYD88 gene, 4 (4.0%) in the BRAF gene, 4 (4.0%) in the POT1 gene, and 18 (17.8%) cases in the remaining 11 genes. 26/56 (46.4%) mutated patients presented two or more mutations. The presence of mutations was associated with unmutated IGHV status ($p=0.040$) and the complex karyotype ($p=0.047$). TP53 disruption correlated with the presence of ≥2 mutations by NGS ($p=0.001$) and a complex karyotype ($p=0.012$). By multivariate analysis an advanced Binet stage ($p<0.001$) and an unfavorable karyotype ($p=0.001$) predicted for a shorter time to first treatment (TTFT), while TP53 disruption ($p=0.019$) and the unfavorable karyotype ($p=0.028$) predicted for a worse overall survival (OS). A shorter time to chemorefractoriness (TTCR) was associated with TP53 disruption ($p=0.001$) and unfavorable karyotype ($p=0.025$). Patients with both unfavorable karyotype and TP53 disruption presented a dismal outcome (median OS and TTCR of 28.7 and 15.0 months respectively).

Summary/Conclusions: A comprehensive analysis of chromosomal aberrations and gene somatic mutations in high-risk CLL showed that the cytogenetic profile was independently associated with a shorter TTFT, OS and TTCR. Since karyotyping using novel mitogens may contribute to the refinement of prognosis in high-risk CLL patients, the introduction of this technique in future CLL trials seems warranted to identify those patients that could be ideal candidates for consolidation treatment or novel treatment combinations.

E1021

SHOULD CLL-IPI BE USED TO ASSESS OVERALL SURVIVAL OF EVERY CLL PATIENT? A SYSTEMATIC REVIEW AND META-ANALYSIS

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Background: A weighted grading approach based on five independent prognostic factors (i.e, *TP53* status, IGHV mutational status, ß2-microglobulin, clinical stage and age) has been used by an international Working Group to generate the chronic lymphocytic leukemia international prognostic index (CLL-IPI). Although the robustness of CLL-IPI has been confirmed in independent validation studies it remains unclear whether CLL-IPI has the greatest validity and should be preferred to guide clinical decision in CLL.

Aims: To shed light on this important research question, we conducted a systematic review which includes all published studies which used CLL-IPI to prognosticate overall survival (OS) in CLL.

Methods: A comprehensive MEDLINE search using "CLL-IPI" as Medical Subject Headings (MESH) allowed to identify at the cut-off time of February the 28, 2017 "seven hits" with only "four" citations considered pertinent. The search extended to the conference proceedings of annual meetings of ASH, EHA and ASCO of last two years recognized "three" additional citations.

Results: Overall 6720 patients from seven evaluable studies were suitable for the present analysis aimed at assessing the impact of CLL-IPI on OS. The majority of patients (4953 or 73.7%) came from studies of external validation of CLL-IPI while 17% (1192) and 8.5% (576) had been used to generate (training set analysis) and to internally validate the model. Patient distribution into the four risk categories of CLL-IPI was heterogeneous thus reflecting the CLL phase (*i.e.*, at diagnosis, at time of first treatment and at relapse) of patients within different studies. Accordingly, patients diagnosed as having low-, intermediate-, high- and very high-risk CLL-IPI ranged respectively between 9% and 58%, 25% and 39%, 14% and 52% and 2% to 9%. Next we evaluated the 5-year OS of patients stratified into each of the four CLL-IPI risk groups using either "Q" or "I^2" test to assess the heterogeneity across different studies. The 5-year survival probability was 91% for low-risk group (95% CI, 90-91%; Q=55.2; P< 0.00; I^2, 87%), 80% for intermediate-risk group (95% CI, 79-82%; Q=49.36; P<0.00; I^2, 86%), 60% for high-risk group (95% CI, 57-62%; Q=42.78; P<0.00; I^2, 84%) and 32% for very high-risk group (95% CI, 27-38%; Q=18.1; P=0.01; I^2, 67%).

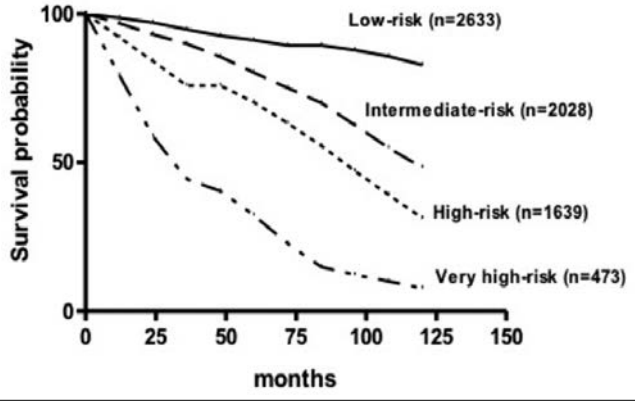

Figure 1.

Summary/Conclusions: In this comprehensive review and meta-analysis of studies thus far published on CLL-IPI we confirmed the value of this novel model to predict OS whatever the CLL phase (fig 1). The prognostic impact of CLL-IPI needs an extensive validation in patient cohorts receiving therapy with B-cell receptor or bcl-2 inhibitors. Nonetheless, in a study of relapsed/refractory CLL included in this analysis the PI3K-inhibitor idelalisib was not able to overcome the impact of CLL-IPI risk categories on OS.

E1022

IBRUTINIB FOR CHRONIC LYMPHOCYTIC LEUKEMIA: IMPACT OF THE CANADIAN YOU&I™ PATIENT SUPPORT PROGRAM ON TREATMENT ADHERENCE

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Background: Oral anticancer medications (OAMs) present several advantages compared with intravenous cytotoxic chemotherapy, including greater convenience for the patient. However, OAMs require that a patient be actively involved in regular drug administration over an extended period of time (Schneider SM, *et al.* Semin Oncol Nurs. 2011;27(2):133-141). Adherence to OAMs significantly impacts patient outcomes; poor adherence may result in inferior survival and outcomes, higher hospitalization rates, treatment resistance, and increased healthcare costs (McCue DA, *et al.* Pharmacotherapy. 2014;34(5):481-494). The Canadian YOU&i™ patient support program (PSP) was developed to improve adherence to long-term ibrutinib therapy using research-proven techniques for promoting positive behavioral changes, *i.e.* cognitive behavioral therapy, psycho-social support, and a nurse coaching component. Results from the program are presented below.
Aims: To evaluate patient adherence to ibrutinib, and patient and physician satisfaction with the YOU&i™ PSP
Methods: Using evidence-based literature reviews and global/local market research, various patient-centered barriers to treatment adherence were identified. Risk for non-adherence was calculated using the Morisky Medication Adherence Scale© score, which informed nurse coaching frequency. Adherence was delineated by prescription refill compliance. Patient and physician questionnaires were used to gauge satisfaction with the YOU&i™ PSP.
Results: As of 20 January 2016, a total of 903 patients with CLL were enrolled in the YOU&i™ PSP. A total of 552 patients were included in the adherence analysis. Of these, 86% opted in to receive the nurse coaching component. At 2 months from treatment initiation, patients who received nurse coaching demonstrated an adherence rate of 92.3%, as compared with 63.5% for patients who did not receive nurse coaching (95% CI, 17.5-41.0; p <0.0001). At 3 months the adherence rates were 89.9% *vs* 60.8% (95% CI, 17.5-41.4; p <0.0001). By 9 months, adherence rates were 81.7% *vs* 71.1% (95% CI, -4.4 to 28.4; p =0.141). At study conclusion, 12 month adherence rates were 76.6% *vs* 72.2% (95% CI, -18.9 to 32.4; p =0.715). Discontinuation rates were similar in all patients, regardless of nurse coaching status at 9 and 12 months. Patients reported satisfaction rates of >90% in surveys conducted at both 3 months and 12 months of program enrollment. Of physicians surveyed at 3 months, 96% reported that the YOU&i™ PSP was helpful in supporting patient needs.

Figure 1.

Summary/Conclusions: The current analysis provides insight into adherence patterns of patients on long-term ibrutinib treatment. These results are consistent with the literature showing that PSPs like the YOU&i™ PSP can help to improve adherence rates (Schneider SM, *et al.* J Adv Pract Oncol. 2014;5(3):163-172). The information obtained from long-term adherence data can help to inform future trials examining patterns of adherence with OAMs. Nurse coaching may be helpful in supporting early adherence by addressing side effects that occur more frequently at treatment initiation. Moreover, changes in disease or health status that arise over the first 12 months of therapy may provide information that allows a PSP to adapt to patients' evolving needs over the treatment journey. A better understanding of long-term adherence patters may allow programs such as the Canadian YOU&i PSP™ to target adherence support more precisely, thereby optimizing patient outcomes.

E1023

TREATMENT AND 17P DELETION TESTING PATTERNS IN COMMUNITY PRACTICE FOR PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) IN THE UNITED STATES

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Background: CLL is the most common type of leukemia in adults in the US. Traditionally, chemotherapy - and chemo-immunotherapy (CIT) have been the treatment mainstay. Historically, CLL patients (pts) with high-risk genetic features (e.g., 17p deletion (del(17p)) have poor prognosis and few treatment options. Over the past few years, oral-targeted therapies have been approved in the US for CLL pts, including those positive for del(17p).
Aims: This study used an electronic health record database to characterize treatment and del(17p) testing patterns in the first 2 lines of therapy (LoT) for CLL pts initiating treatment between 2011–2016. The association between del(17p) testing and utilization of targeted therapies was also explored.
Methods: This was a retrospective observational study in CLL utilizing a large, longitudinal, demographically and geographically diverse database of US cancer pts (Flatiron Health 12/2016). An analytical cohort of pts treated at community practice sites who initiated 1st LoT after Jan 2011 was developed. Two sub-cohorts of pts who initiated 1st LoT before and after 2014 were also identified reflecting the approval timeline of oral-targeted therapies in the US.
Results: As of Dec 2016, 3,140 pts with CLL were included in the broad Flatiron Health CLL cohort. The results of this analysis are based on the analytical cohort that included 1,700 pts who initiated 1st LoT during 2011–2016, of which 1,134 (66.7%) pts initiated therapy after 2014. Second LoT was initiated in 622 pts during 2011-2016, with 472 (75.9%) initiations occurring after 2014. Average age at CLL diagnosis was 66.9 years; 63.2% of pts were male; 70.2% were Caucasian. Over 2011–2016, the rate of genetic testing by cytogenetics or fluorescence *in situ* hybridization (FISH) before initiation of 1st LoT was 67.4%. The rate of del(17p) specific testing before 1st LoT was 59.1% (an increase from 38.9% in 2011 to 64.4% in 2016). Among those who were tested before 1st LoT, 12.5% tested positive for del(17p). In the sub-cohort of pts who initiated 1st LoT after 2014, ibrutinib monotherapy replaced fludarabine, cyclophosphamide and rituximab (R) combination as the 3rd most frequent (13.0%) 1st LoT in pts with CLL, after bendamustine/rituximab (BR) combination (29.8%), and rituximab (R) monotherapy (17.7%). In contrast, ibrutinib became the most common (43.9%) 1st LoT in newly diagnosed CLL pts with del(17p), followed by BR (24.5%) and R (7.1%). 36.6% of pts initiated 2nd LoT by December 2016. The three most common treatment sequences from 1st LoT to 2nd LoT were CIT to B-cell receptor pathway inhibitor containing therapy (16.4%), CIT to CIT (14.0%), and immunotherapy (IT) to IT (12.4%). Overall, the utilization of oral-targeted therapies has steadily increased since 2014, and multivariate analyses indicate that the presence of del(17p) is strongly associated (OR=8.7 and 2.8 for 1st and 2nd LoT, respectively) with this choice once these agents became available in 2014.
Summary/Conclusions: Considerable treatment pattern changes were observed for CLL pts in the US community practice due to the adoption of newly approved targeted therapies. Presence of del(17p) is strongly associated with choosing a targeted therapy regardless of LoT. Future research is needed to determine how differences in pt and disease characteristics and cytogenetic testing patterns influence treatment decisions and associated outcomes.

E1024

SINGLE-AGENT IBRUTINIB *VS* REAL WORLD TREATMENT FOR PATIENTS WITH TREATMENT-NAÏVE (TN) CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): AN ADJUSTED COMPARISON OF RESONATE-2™ WITH THE CLLEAR AND LYON-SUD DATABASES

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Background: The phase 3 RESONATE-2™ study demonstrated significant improvement of progression-free survival (PFS) and overall survival (OS) with ibrutinib (ibru) *vs* chlorambucil (chl) in TN (aged ≥65 years) CLL patients. In the absence of direct comparison of single-agent ibru with other frequently used treatments in this patient population, additional comparative evidence against standard of care as observed in clinical practice can provide useful insights on the relative efficacy of ibru.
Aims: To investigate the relative treatment effect on PFS and OS for ibru *vs* real world (RW) treatment in daily clinical practice in TN CLL patients by adjusted comparison of patient-level data from RESONATE-2™ *vs* RW data from the CLLEAR (Chronic Lymphocytic Leukemia Registry) and Lyon-Sud databases.
Methods: CLLEAR holds medical records for CLL patients from seven academic centers across the Czech Republic. Lyon-Sud database holds medical records for CLL patients from the academic French hospital Lyon-Sud. Patients initiated on CLL frontline therapy were selected from CLLEAR and Lyon-Sud using the same inclusion-exclusion criteria as for RESONATE-2 (excluding patients with age<65 and with del17p positive status). PFS and OS were com-

pared between ibru and RW treatment using patient-level data from RESONATE-2™ (n=136) and pooled patient-level data from the two cohorts. To adjust for differences in patient characteristics between the trial population and both cohorts, a multivariate Cox proportional hazards model was fitted on patient-level data to estimate the hazard ratio (HR) for ibru *vs* RW treatment, with age, sex, disease stage (based on RAI/BINET), and deletion 11q presence/absence included as covariates.

Results: Median age at treatment initiation for CLLEAR (n=418) and Lyon-Sud (n=110) was 73 and 71 years, respectively, *vs* 73 for Ibru patients from RESONATE-2™. The proportion of male patients was 63% in CLLEAR and 57% in Lyon-Sud *vs* 65% in RESONATE-2™. The median follow-up was 35.7 months (mo) for Lyon-Sud and 16.8 mo in CLLEAR *vs* 28.1 mo for RESONATE-2™. Adjusted HR for ibru *vs* physician choice in CLLEAR and Lyon-Sud were 0.23 [95% CI: 0.14, 0.39] and 0.25 [0.14, 0.43] for PFS, and 0.29 [0.11, 0.79] and 0.39 [0.18, 0.83] for OS, respectively. Fludarabine+cyclophosphamide+rituximab (FCR; n=117), bendamustine+R (BR; n=91), Chl alone (n=43), Chl+R (n=45), and other R-containing regimens (n=154) were the most commonly used treatment regimens across both RW cohorts. Older age, male gender, advanced disease stage and del11q positive status were independent risk factors for PFS and OS. The adjusted HRs (pooled estimates) for ibru *vs* the two most commonly used regimens were 0.30 [0.17-0.53] (FCR) and 0.33 [0.16-0.68] (BR) for PFS, and 0.44 [0.20-0.95] (FCR) and 0.33 [0.13-0.83] (BR) for OS (**Figure 1**). Estimates of HR *vs* regimens in the cohorts were consistent across both databases.

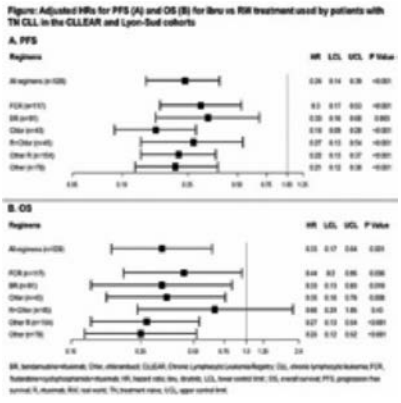

Figure 1.

Summary/Conclusions: This adjusted comparison of patient-level data from RESONATE-2™ with RW data from CLLEAR and Lyon-Sud demonstrates ibru to be more effective compared with RW treatment, with a 4.1-fold improvement in PFS and a 3-fold improvement in OS. When comparing ibru with the most commonly used RW treatments, statistically significant benefits for ibru were consistently observed *vs* all treatment regimens on PFS and for most comparisons on OS. These results further support the existing evidence that ibru significantly improves PFS and OS *vs* common regimens used in TN CLL settings, and has important implications for clinical practice.

E1025

CHARACTERISTICS, TREATMENT, AND OUTCOMES OF ≥80 YEAR OLD PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) ENROLLED TO PROSPECTIVE TRIALS OF THE GERMAN CLL STUDY GROUP

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Background: People over 80 years are the fastest growing age group in western populations. Clinical management of ≥80 year old patients (pts) with CLL remains a challenge due to the very limited amount of data currently available for this age segment. Two retrospective studies reported observational data on characteristics, treatment, and outcomes of ≥80 year old pts not enrolled in a clinical trial (Bairey et al., Meunier et al.). Comparably little is known about ≥80 year old pts who were treated for CLL within clinical trials, however.
Aims: To study the characteristics, treatment, and outcomes of pts aged ≥80 years who received their first therapy within prospective trials of the German CLL Study Group (GCLLSG).
Methods: Trial populations of seven clinical trials of the GCLLSG (CLL1, CLL5, CLL7, CLL8, CLL9, CLL10, CLL11; total N=3552) were reviewed and screened for pts ≥80 years at frontline treatment. Clinical, laboratory, and genetic data of identified pts were pooled. Time-to-event data were analysed by Kaplan-Meier methodology. Independent prognostic factors for survival were identified by multivariate analysis using Cox regression modelling with stepwise selection procedures.
Results: Among 3552 reviewed GCLLSG trial participants, 152 were aged ≥ 80 years at initiation of firstline treatment. A majority of these pts were identified from CLL11 (n=132) while the remaining were from CLL1 (n=3), CLL5 (n=1), CLL7 (n=3), CLL8 (n=2), CLL9 (n=9), and CLL10 (n=2). Median age was 82 years (range 80-90). Concomitant diseases were present in 99% of the pts and median cumulative illness rating scale (CIRS) score was 8 (0-18). Median creatinine clearance was 46 mL/min (range 17-99 mL/min). Identified genomic aberrations were 13q deletion as a sole abnormality in 27%, trisomy 12 in 18%, 11q deletion in 9%, and 17p deletion in 16% of pts. IGHV was unmutated in 69% of the pts. Distribution of CLL-IPI risk groups was as follows: 6% low, 19% intermediate, 61% high, and 14% very high. Most pts had Binet stage B (36%) or C (43%). Chemoimmunotherapy with chlorambucil plus obinutuzumab (CLB-OB) or chlorambucil plus rituximab (CLB-R) was administered to 61 (40%) and 56 (37%) pts, respectively. Remaining pts received chlorambucil alone (CLB, n=19), fludarabine (F, n=10), fludarabine/cyclophosphamide (FC, n=1), fludarabine/cyclophosphamide/rituximab (FCR, n=2), or bendamustine/rituximab (BR, n=3). Rates of grade 3 or 4 neutropenia and infections were 35% and 13%, respectively. Premature treatment discontinuations occurred in 15% of cases and were mostly due to adverse events. The total overall response rate was 92% with 13% complete remissions. Median observation time for all pts was 40.7 months. Median progression-free survival (PFS) and treatment-free survival (TFS) were 17.2 and 32.3 months, respectively. A total of 47 pts (31%) received at least one further line of treatment. Median overall survival (OS) was 48.3 months, with adverse events (22%) and progressive CLL (16%) being the most frequent causes of death. Standardized mortality ratio was calculated and showed a 1.99 (CI 1.54-2.53) increased risk of death as compared to an age- and sex-matched general population. Independent prognostic factors for OS were 17p deletion and elevated serum thymidine kinase.

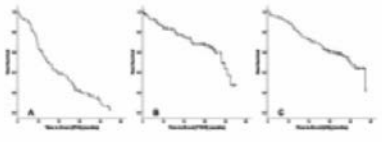

Figure 1.

Summary/Conclusions: Findings suggest that antileukemic therapy (including chemoimmunotherapy) is feasible and efficacious in ≥ 80 year old pts with CLL. However, such pts are stll highly underrepresented in clinical trials and even with modern treatment live shorter than age-matched controls of the general population. Broader recruitment of these pts to prospective trials and evaluation of targeted therapies therefore appears imperative to improve outcome of CLL in this age segment.

E1026

THE ROLE OF CD200 IN THE DIAGNOSIS OF CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Clinic, morphologic, immunophenotypic and genetic features are the basis for the diagnosis of B-cell malignancies. It is considered that the diagnosis of CLL requires the presence in peripheral blood of >5x10^9/L monoclonal B lymphocytes with a distinctive immunophenotype (*i.e.* SmIg^{weak}, CD5$^+$, CD19$^+$, CD23$^+$). Based on immunophenotypic characteristics, Matutes et al devised in 1994 a immunophenotypic score based on a few markers (CD5$^+$, CD23$^+$, FMC7$^-$,SmIg^{weak}and CD22^{weak}) each one of them receiving a score of 1 if present or 0 if absent. A total score of 4 or 5 is typical of CLL whereas those cases scoring 0 or 1 correspond to other B-cell malignancies, mostly lymphomas. Nevertheless, clinical and immunophenotypic features of CLL may overlap with other B-cell malignancies. CD200 has been described as a marker potentially useful to distinguish CLL from other CD5$^+$B-cell malignancies.
Aims: The aim of this study was to analyze whether the addition of CD200 to the Matutes score improves the diagnostic accuracy of CLL.
Methods: We prospectively assessed the immunophenotype of 99 peripheral blood samples of patients with suspected lymphoproliferative disorders between November of 2015 and January of 2017. Immunophenotyping was performed using a Canto Flow Cytometer (Becton Dickinson) and samples were stained with routine combinations plus CD200. The Matutes Score was calculated as follows: FMC7, CD22 and CD79b were considered score 1 in CLL when the positive cells were <30%, CD5 and CD23 were considered score 1 when the positive cells were ≥30%. The cut-off used for CD200 was calculated by Receiver Operating Characteristics (ROC). CD200 was scored as 1 when the positive cells were >96%. Mean Fluorescence Intensity Ratio

(MFIR) was calculated as a relative expression between MFI positive population and MFI negative population. McNemar´s test was used to assess statistical significant differences in accuracy among individual markers and scoring systems. The treating physician made the final diagnosis of the different B-cell malignancies according to IWCLL and WHO criteria. Logistic regression including sensitivity, specificity and accuracy values, were used to evaluate statistical differences in diagnosis precision between different combinations of markers as well as individual markers.

Results: Flow cytometry analysis was performed in 99 patients, including 62 cases with a diagnosis of CLL (62.6%) and 37 cases with a "non-CLL" diagnosis (37.4%). Matutes score was 4-5 in all CLL cases and ≤3 in "non-CLL" cases. CD200, CD23 and CD5 were the most consistent markers for CLL (90.3%, 96.8% and 100.0% of sensitivity respectively). Moreover, CD79b and FMC7 had also a good discriminant value (80-85% sensitivity). For "non-CLL" cases the most reliable markers were SmIg, FCM7 and also CD200. The analysis of the accuracy is shown in the table. Of note, CD200 as a single marker was found to be a reliable marker for distinguishing CLL and "non-CLL" cases (90.9%; p<0.001; 90.3% sensitivity, 91.9% specificity) showing a significantly higher accuracy than CD5, CD23 and SmIg as individual markers (p<0.001). The accuracy of CD200 did not vary when comparing% of positive cells and MFIR. In contrast, the accuracy for SmIg significantly increased from 67.7% to 78.8% when using MFIR values (according to the cut-off established by ROC curves), being lower in CLL than in "non-CLL" cases (71.0% *vs* 86.5%, p<0.001). Finally, the addition of CD200 to the Matutes score system and using a cut off ≥4, improved its accuracy from 88.9% (95% CI: 88.2-95.6) to 98.0% (95% CI: 94.7-100.0) and showed a better sensitivity.

Table 1.

Marker	Score punctuation				CLL vs "Non-CLL" % (95% CI)*	p-value*	CLL vs "Non-CLL" % (95% CI)Δ	p-valueΔ
CD200	Negative	0	Positive	1	90.9 (84.7-97.1)	<0.001	90.9 (84.7-97.1)	<0.001
FMC7	Negative	1	Positive	0	85.9 (78.5-93.2)	<0.001	90.9 (84.7-97.1)	<0.001
CD79b	Negative	1	Positive	0	84.8 (77.3-92.4)	<0.001	88.9 (82.2-95.6)	<0.001
CD5	Negative	0	Positive	1	82.8 (74.9-90.8)	>0.05	83.8 (76.1-91.6)	<0.001
CD23	Negative	0	Positive	1	82.8 (74.9-90.8)	<0.001	87.9 (80.9-94.8)	<0.001
SmIg	Weak	1	Moderate/Strong	0	67.7 (58.0-77.4)	<0.001	76.8 (67.9-85.6)	<0.001

* % of positive cells; Δ MFIR

Summary/Conclusions: These results confirm CD200 as a valuable marker in the diagnosis of CLL

E1027

COMPARISON OF CHROMOSOME BANDING ANALYSIS AND GENOMIC MICROARRAY TECHNIQUES FOR THE DETECTION OF COMPLEX KARYOTYPES IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Well-established poor prognostic factors in chronic lymphocytic leukemia (CLL) include deletions in 11q22 and 17p13 (del*ATM* and del*TP53*) by FISH. Genomic complexity detected by either chromosome banding analysis (CBA) or genomic microarray techniques, but underestimated by FISH, predicts an impaired outcome in CLL. Of note, none of these techniques is still routinely performed and standard criteria for risk stratification based on genomic complexity are lacking.

Aims: 1. To assess the complexity detected by genomic arrays in patients with complex karyotype by CBA; 2.To compare both methods regarding the number and type of aberrations detected in order to categorize patients based on genomic complexity.

Methods: A total of 24 CLL patients with complex karyotype (≥3 abnormalities, CK) by CBA were included [Median age: 73; 15 males (63%)]. Median time from diagnosis to CBA/microarray analysis was 3 months (range, 0-160), and 4 patients (16%) had received prior treatment. The cohort was enriched in del*ATM* and del*TP53* (37% and 42%, respectively). DNA from peripheral blood mononuclear cells or CD19+ lymphocytes was hybridized to Cytogenetics Whole-Genome 2.7M (n=2) or CytoScan HD (n=22) array, results were analyzed with Chromosomal Analysis Suite Software (Affymetrix). Number, size and type of aberrations detected were compared between techniques.

Results: A median of 3.5 aberrations (range: 3-9) were detected by CBA, being significantly lower than the copy number abnormalities (CNA) identified by microarrays (median 5, range: 1-28; P=0.018). The median size of the CNA was 5.4Mb (range: 0.1-174Mb). Current recommendations for microarray analyses suggest that only CLL known abnormalities and CNA >5Mb should be considered for clinical interpretation (Schoumans et al, 2016). When applying this cut-off, 42% of the initially detected CNA (74/177) were omitted and no significant differences in the number of abnormalities by each technique were found (P=0.334). CNA <1Mb did not involve any chromosomal altered region in the corresponding karyotype. Thus, their omission probably would not affect the stratification based on complexity. In contrast, most of the CNA between 1 and 5Mb involved small CNA associated to apparently balanced translocations by CBA, and in some cases revealed a higher genomic instability than the previously recognized by CBA (*i.e.* multiple deletions defined as a single one or a monosomy by CBA). Indeed, four cases showed chromothripsis not detected by CBA which has been associated with impaired outcome (Salaverria et al, 2015). Of note, genomic microarrays failed to detect some balanced translocations or subclonal aberrations by CBA, which probably were represented in a minor proportion of the sample but expanded during CBA culture. Thus, eight patients (21%) could only be considered complex by CBA, as by microarray analyses <3 CNA were detected. The present study is ongoing; additional cases have been collected in order to statistically assess the clinical impact on survival of the complexity detected by microarrays.

Summary/Conclusions: 1. The number of chromosomal abnormalities detected in CLL patients differs if assessed by CBA or genomic microarrays. 2. The current 5Mb cut-off to define clinically relevant CNA should be revised, as it could underscore genomic instability (contiguous small deletions, chromothripsis). 3. More studies should be performed to establish standard criteria for prognostic stratification of CLL patients based on genomic complexity consistent with the results from both techniques.

E1028

ABNORMAL SERUM FREE LIGHT CHAINS RATIO ASSESSMENT IN CHRONIC LYMPHOCYTIC LEUKEMIA: A SIMPLE YET POWERFUL TEST CORRELATING WITH CLINICAL OUTCOME AND MINIMAL RESIDUAL DISEASE

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Background: An abnormal serum Free Light Chain (sFLC) ratio has been shown to be significantly associated with poor outcome in chronic lymphocytic leukemia (CLL) Yegin ZA et al, Eur J Haematol 2010, suggesting that this parameter may discriminate different biological subgroups.

Aims: As the technic is easily implementable in routine lab and cost effective, we evaluated the sFLC levels (kappa + lambda) and kappa/lambda (K/L) ratio in CLL patients in this prospective study. The relationship between abnormal sFLC levels (K+L) and/or K/L ratio, minimal residual disease (MRD) assessed by flow cytometry (FCM) and disease evolution was evaluated.

Methods: Diagnosis was confirmed by 10-color FCM immunophenotyping of blood lymphocytes on a Navios (Beckman Coulter). Serum FLC kappa and lambda chains were measured by nephelemetry using the Freelite™ immunoassay. The normal free kappa chains level was defined as within the range of 3.3-19.4mg/L, and the normal lambda chains level within the range of 5.71-26.30mg/L. A normal sFLC kappa/lambda (K/L) ratio was therefore defined as between 0.26 and 1.65 (a ratio above 1.65 indicating an excess of kappa light chain, and a ratio below 0.26 indicating an excess of lambda light chain). The cumulative level of kappa plus lambda (K+L) was also evaluated. Most patients received combined chemo-immunotherapy or entered clinical trials whenever possible. The ROC methodology was used to establish the best cut-off value of sFLC ratio level to discriminate treated patients from those who remained treatment-free.

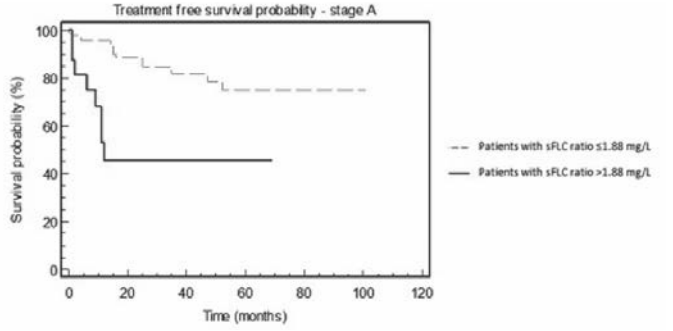

Figure 1.

Results: Main patients characteristics are detailed [N=147, M/F:75/72, 111 in early stage disease, Del 17p in 11 patients and Del11q in 15]. Median age was

69 years (range 34 to 86). Ninety patients were untreated during the follow-up period. Median follow-up duration was 30 months (range 0 to 101). Furthermore, sFLC measurement was assessed in 57 patients who progressed during the study and required treatment according to international guidelines. ROC curve analysis determines cut-off level of K/L ratio at 1.88. Abnormal sFLC was observed at diagnosis in 50.9% (N=29) of all treated patients. The mean ± SD ratio of sFLC in the untreated patients group and in the treated patients group was 1.51±2.08 and 2.80±3.75 respectively (p=0.0082). Considering the sFLC levels (kappa + lambda), the mean±SD in the untreated patients group and in the treated patients group was 29.1±17 and 53.0±41.9 respectively (p<0.0001). Treatment systematically induced a modification of the sFLC K/L ratio. Interestingly, after treatment completion, the persistence of an abnormal sFLC K/L ratio was associated with positive MRD determined by FCM with a 82% specificity and a 95% positive predictive value. Moreover, median time to treatment income for patients in early stage disease with ratio >1.88 was 12 months while it is not reached in those with ratio ≤1.88 (p<0.0001) (figure 1).

Summary/Conclusions: In this study we confirm the value of sFLC K/L ratio determination as a technically simple, standardized and cost-effective test to improve risk stratification of patients with low risk CLL at diagnosis, at the end of the treatment and during follow-up. Determination of the sFLC K/L ratio during the follow-up of treated patients provides additional information regarding the response to therapy in patients with an abnormal K/L ratio. In this study, persistence of an abnormal sFLC K/L ratio after treatment was strongly associated with positive MRD and could serve as a predictive as well as a prognostic biomarker for residual disease detection and clinical outcome.

E1029

PLATELET FUNCTION ASSAYS FOR STRATIFICATION OF BLEEDING RISKS IN CLL PATIENTS ON IBRUTINIB TREATMENT

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Background: Ibrutinib therapy in chronic lymphocytic leukemia (CLL) is associated with frequent bleeding complications, explained by inhibition of BTK, which mediates downstream signaling of GPVI and GPIb receptors in platelets. Detailed characterization of platelet functional impairment can help predict and possibly prevent severe bleeding on ibrutinib. Here we investigate platelet functional activity in CLL patients before initiation of ibrutinib and at different time points during treatment.

Aims: A longitudinal study on the impact of ibrutinib on platelet function, severity and frequency of bleeding.

Methods: Forty-three patients with relapsed and refractory CLL and 10 healthy donors were included in the study. Platelet functional activity was characterized by flow cytometry before and after activation with SFLLRN plus collagen-related peptide. Levels of CD42b, CD61, CD62P, PAC1, annexin V binding, and mepacrine release were determined. Aggregation with collagen, ADP and ristocetin were measured. All tests were performed before initiation of treatment, at weeks 2, 4, 8 and at 6 months. Bleeding complications were scored using ITP-specific Bleeding Assessment Tool.

Results: Among 43 CLL patients, 29 (67%) were men, the median age was 65 (range 31 to 83 years). Four patients with del (17p) received ibrutinib as a first line. In 39 previously treated patients the median number of prior treatments was 3 (range, 1-6). Del17p or TP53 mutation was found in 11 (25%) patients. Only 1 patient received anticoagulant and antiplatelet drugs. Median duration of ibrutinib treatment was 8.2 months (range 2.2-10.9). At least one bleeding episode occurred in 23 patients (53%). Among patients with bleeding, 14 (61%) had grade 1 events, 7 (30%) had grade 2 and two (9%) had grade 3 events. Bleeding frequency decreased with time on ibrutib; only 4 patients still had bleeding complications after 6 months. The patients with bleeding had significantly lower mean platelet count that those without (120 *versus* 170 thousands per microliter, P<0.0001) and higher lymphocytosis (74 *versus* 45, P<0.05). Their activation of integrins in response to stimulation was greatly impaired (9% *versus* 26%, P<0.0001; while the 95% confidence interval for healthy controls 63-137%), and there was significant difference in procoagulant activity as well (2% *versus* 5%, P<0.01; normal range is 7-35%). Importantly, the integrin activation allowed risk stratification: a person with more than 9% integrin activation had less than 10% risk to develop bleeding while the one with less than 9% integrin activation had a risk of more than 40%. There was no difference in dense- or alpha-granule release between the patient groups, and these indicators remained in their normal ranges. There were also significant differences in aggregation assay with ADP (25±16% *versus* 36±18% for bleeding and non-bleeding patients, p<0,001), collagen (38±19% *versus* 53±20%, P<0.001), and ristocetin (53±22% *versus* 62+20%, P=0.02). Interestingly, the patients with bleeding had negative correlation of the ADP-induced aggregation with leukocyte level.

Summary/Conclusions: Both classic aggregation assays and flow-cytometry-based techniques demonstrate impaired platelet function in the bleeding CLL patients compared with non-bleeding ones. The level of integrin activation appears to be the most sensitive and able to identify patients with different bleeding risks.

E1030

HYPOGAMMAGLOBULINEMIA IS A STRONG PREDICTOR OF TIME TO FIRST TREATMENT IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Management of chronic lymphocytic leukemia (CLL) dramatically improved since the introduction of novel therapies. Rarely patients requires treatment at diagnosis and approximately a third of patients will never require therapy. Predictive and prognostic factors are well known (IGHV, del11q, del17p, *TP53*); CLL-IPI score including age, clinical stage, beta2microglobulin, IGHV and deletion 17p and/or *TP53* mutation has been recently validated. It identifies 4 risk groups with significantly different time to first treatment (TTFT) and overall survival (OS). Hypogammaglobulinemia (HYPO) is a typical feature of CLL, with an incidence of 20-60% at diagnosis and a relationship with infections occurrence. Prognostic significance of HYPO at diagnosis has not been extensively evaluated in terms of OS and TTFT. Only IgG serum levels have been reported to be associated with TTFT but no data are available on other immunoglobulin classes (Ig).

Aims: To evaluate the impact of HYPO and single Ig classes on TTFT in a retrospective cohort of CLL patients and to assess the relationship between HYPO and CLL-IPI.

Methods: We retrospectively evaluated 698 consecutive CLL patients diagnosed at our Institution from 1983 till 2016. Data from laboratory, biological analysis and clinical stage were collected. We also evaluated immunoglobulin (Ig) status (*i.e.* IgG, IgM and IgA) at diagnosis and calculated CLL-IPI. HYPO was defined basing on our laboratory cut-offs (IgA 70mg/dl, IgG 700mg/dl, IgM 40mg/dl). However, as no recognized prognostic/predictive Ig cut off has been reported to date, we aimed to identify a prognostic threshold for each Ig class.

Results: From 698 patients assessed, 410 cases were evaluable for Ig values at diagnosis. IgA levels were lower than 70mg/dl in 17,4%, IgG lower than 700mg/dl in 22,2%, and IgM lower 40mg/dl in 33.7%. Forty-six percent of patients presented deficit of at least one Ig class, while 7.8% of patients had all Ig low. Each Ig deficit was related with a shorter TTFT with the following hazard ratios (HRs): 2.09 (1.45-3.03) for IgA (P<0.0001), 1.58 (1.10-2.27) for IgG (P=0.008) and 1.52 (1.09-2.13) for IgM (P=0.01) (Figure 1, A-B-C). However, only IgA deficit maintains statistical significance in multivariate analysis [HR 1.59 (1.08-2.35)]. A prognostic threshold for each Ig class was identified maximizing the differences in TTFT and the following values were obtained: 80mg/dl for IgA, 410mg/dl for IgG and 18mg/dl for IgM (Figure 1, D-E-F). Considering CLL-IPI, 186 patients presented IPI 0-1, 99 had IPI 2-3, 32 patients IPI 4-6, and 12 patients had IPI 7-10. Even in our series, CLL-IPI separated four risk groups with different TTFT and OS, suggesting that our cohort may be suitable to evaluate new prognostic factors. As regards the relationship between HYPO and CLL-IPI, we observed a correlation with IgA levels, using our laboratory cut-off. Moreover, we found a relationship among CLL-IPI and both IgA and IgM values, when using the newly validated Ig cut-off. Finally, CLL-IPI was a stronger prognostic factor for TTFT than HYPO in our analysis. However, the addition of IgA deficit to CLL-IPI appears to further improve CLL prognostication.

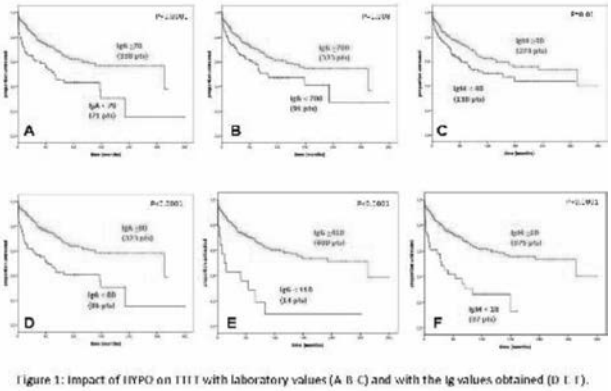

Figure 1: Impact of HYPO on TTFT with laboratory values (A-B-C) and with the Ig values obtained (D-E-F).

Figure 1.

Summary/Conclusions: In conclusion, HYPO significantly impacts on CLL prognosis. Moreover, even if CLL-IPI has a stronger prognostic value for TTFT compared to HYPO, the addition of IgA deficit appears to further improve CLL prognostication.

E1031

CLL: IS LYMPHOCYTE DOUBLING TIME (LDT) A RELEVANT PROGNOSTIC PARAMETER IN THE ERA OF PROGNOSTIC BIOMARKERS?

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Background: In CLL, tumor doubling time is reflected by the pace at which lymphocytes increase in blood (lymphocyte doubling time or LDT). However, since LDT is rarely available at the time of diagnosis, its role in assessing prognosis in patients in CLL is controversial.
Aims: To reassess the prognostic significance of LDT in a large series of patients.
Methods: Retrospective single-center study based on 629 patients diagnosed with CLL/SLL. LDT was measured at the time of diagnosis if prior WBC counts were available or calculated after diagnosis by linear regression analysis, usually over a treatment-free period of 2 months and including at least three WBC counts.
Results: 140 patients displayed short LDT (≤12 months) and 489 long LDT (>12 months). The median follow-up up was 13.4 years (6.1-22.5) and 11.2 years (2.2-30.9), respectively. Patients with short LDT were younger (p=0.005), had more advanced clinical stage (p<0.001), higher ALC (p<0.001), as well as increased serum LDH (p<0.001) and B2-microglobulin (B2M; p=0.035) levels and also a tendency towards lower levels of Hb and platelet counts. A short LDT was also associated with an increased expression of ZAP70 and CD38, unmutated *IGHV* (all p<0.001) and poor FISH cytogenetics (del17p, del11q) (p=0.002). Additionally, patients with a short LDT presented more frequently mutations in *NOTCH1* (p=0.008), *ATM* (p=0.029), *TP53* (p=0.035) and a tendency to more mutations in *SF3B1* (p=0.102). The proportion of patients treated in each group was markedly different [80% *vs* 46%] as it was the median time to treatment (TTT, 1.4 *vs* 9.4 years; p<0.001). Type of treatment (mainly, chemo[immuno]therapy) did not significantly differ between both groups and there was no significant differences in response rates (ORR 59% with 29% CR *vs* 69% with 29% CR; p=0.253). Overall survival (OS) was shorter in the group with short LDT (median: 7.2 *vs* 12.2 years; p<0.001). Univariate analysis demonstrated a significant correlation between OS and advanced clinical stage, age >70 years, increased B2M and LDH (p<0.001), short LDT, increased ZAP70 and CD38, unmutated *IGHV*, and high-risk FISH genetics (del17p, del11q) (all p<0.001). Likewise, mutations in *NOTCH1* (p<0.001), *SF3B1* (p=0.027), *ATM* (p=0.028) and *TP53* (p<0.001) were associated with OS. In a multivariate analysis including clinical stage, age, LDT, *IGHV*, ZAP70, FISH cytogenetics and *TP53* mutations (*NOTCH1* and *SF3B1* mutations were not included in the analysis because of the small number of patients in whom these markers were available), a short LDT maintained its prognostic value for OS (HR 2.7 (95% CI: 1.6-4.7), p<0.001) along with age >70 years (HR 5.2 (95% CI: 3.2-8.5), p<0.001), high-risk FISH (HR 2.2 (95% CI: 1.3-3.6), p=0.003), unmutated *IGHV* (HR 2.4 (95% CI: 1.5-4.0), p<0.001), and presence of *TP53* mutation (HR 2.0 (95% CI: 1.0-3.9), p=0.041).
Summary/Conclusions: This study shows that LDT continues being an independent prognostic parameter for OS in the era of biomarkers. In contrast, LDT did not correlate with response to therapy and, accordingly, cannot be regarded as a response predictor to chemo(immuno) therapy. Finally, LDT warrants investigation in the setting of novel therapies.

E1032

INDICATIONS FOR TREATMENT IN CHRONIC LYMPHOCYTIC LEUKEMIA: CLINICO-BIOLOGICAL CHARACTERISTICS AND PROGNOSTIC IMPACT

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Background: Chronic lymphocytic leukemia (CLL) is characterized by the progressive accumulation of monoclonal CD5$^+$ B cells in the bone marrow and lymphoid tissues. International guidelines recommend initiation of treatment only in case of infiltrative cytopenia, progressive splenomegaly or lymphadenopathy, short lymphocyte doubling time (LDT), B symptoms and/or refractory immune cytopenia (IC). These criteria are based on experts' consensus and considered equally relevant for treatment initiation, even though little evidence exists concerning the relative value of each individual criterion.
Aims: To describe the clinico-biological characteristics and prognosis of CLL patients according to the criteria that prompted the initiation of first-line treatment.
Methods: We identified 530 consecutive patients with CLL who required front-line therapy from 1978 to 2014 and had their indication(s) for treatment (IfT) recorded. Massive/progressive lymphadenopathy and massive/progressive splenomegaly were grouped together as lymphoid mass (LM). Infiltrative anemia and thrombocytopenia were categorized as marrow failure (MF). As 505 patients (95%) initiated therapy due to LM, MF or both, we decided to focus on these two groups. Patients whose IfT was both LM and MF were classified as MF following the logic behind Rai and Binet staging systems.
Results: Median age of the whole cohort was 62 years (range 22-93), and 63% of patients were male. Median follow-up from first-line therapy was 64 months (range, 1-433). Half of the patients had a single IfT, while the other half had two or more. IfT were LM in 72% patients, MF in 31%, short LDT in 29%, B symptoms in 19%, and IC in 3%. Compared to patients from the LM group, patients from the MF group were significantly older, had a significantly higher β2-microglobulin level (probably due to an age-related impaired renal function), and were more frequently treated with alkylating agents than purine analogues. Conversely, patients treated due to LM had more frequent adverse prognostic characteristics, such as higher ZAP70 expression, unmutated *IGHV* genes, and 11q deletion. The median OS of the entire population was 77 months (95% confidence interval [CI]=71-83) from first-line therapy and 108 months (95% CI=102-118) from diagnosis. Indication for treatment was significantly associated (p<0.001) with a shorter OS from first-line therapy: 63 months in the MF group (95% CI: 48-72), compared to 89 months in the LM group (95% CI: 80-106). This association remained significant after adjusting for age and β2-microglobulin concentration.

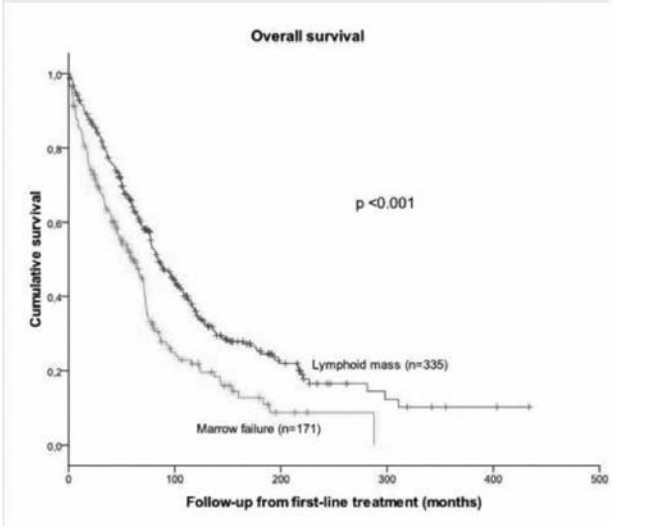

Figure 1.

Summary/Conclusions: All IfT are still regarded as equally important, although no solid evidence exists to support such statement. In our series, infiltrative cytopenia and/or progressive lymphadenopathy/splenomegaly constituted the IfT in most (95%) CLL patients. In spite of being enriched in favorable biological prognostic factors (mutated *IGHV* genes, low ZAP-70 expression and favorable-risk cytogenetics), MF patients had a shorter age-adjusted OS from first-line therapy compared to LM patients. Further studies should address whether this result also applies to patients treated with novel agents.

E1033

UNCOVERING PRIMARY TP53-DELETED CLONES WITH FISH THROUGH FACS-SUPPORTED PURIFICATION OF CHRONIC LYMPHOCYTIC LEUKEMIA LYMPHOCYTES

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Background: The presence of TP53-inactivation in chronic lymphocytic leukemia (CLL), namely through the deletion of all or part of the chromosomal region containing its locus, is a well-established marker of poor prognosis and chemoresistance to traditional chemotherapeutic agents. Fluorescence *in situ* hybridization (FISH) is a useful tool for the detection of the deletion. Nevertheless, its sensitivity is influenced by the number of blood-cell lineages that carry the aberration; the absolute count of deletion-positive cells; and the proportion of deletion-positive neoplastic cells relative to deletion-negative neoplastic cells and non-neoplastic cells, in the whole blood or bone marrow sample. The latter issue can be minimized by purifying the sample through the selection and separation of tumor cells, using techniques such as fluorescence-activated cell sorting (FACS).
Aims: In this study, we aim to evaluate the benefit of using purified samples of neoplastic CLL lymphocytes for the detection of TP53-deletion by FISH, when compared to full samples.

Methods: We reviewed all CLL samples that were submitted for the investigation of TP53-deletion through FISH, in our Lab, between January 1st 2011 and February 28th 2017. Results obtained on tests performed on whole mixed cellularity samples were compared with results obtained directly in FACS purified CLL clonal lymphocytes.
Results: We analyzed 410 samples tested for the deletion of TP53 in our Lab during the study period. The majority of patients (63.2%) were male. Although FACS separation of neoplastic cells was only introduced within the last two years of the study period, it accounted for 39.0% of all tested samples. This poor prognostic aberration was identified in 15.8% of patients in the overall cohort, with no differences in the incidence of a positive finding between mixed cellularity samples and FACS purified samples (15.6% *vs* 16.2%, respectively, p=NS). In contrast, the average proportion of positive cells within a positive sample was markedly different between mixed cellularity samples and FACS-processed samples, increasing nearly three-fold through the purification of the sample, from 24.0±15.9% to 62.9±33.3%, p<0.001. In fact, in 57.7% of all patients who were tested after FACS separation of CLL cells, the TP53-deleted clone was larger than 50% of neoplastic clonal lymphocytes, making it the primary clone.
Summary/Conclusions: We observed that the pre-processing of the sample through the FACS-supported purification of CLL neoplastic lymphocytes revealed that the TP53-deleted clone was nearly three-fold larger than suggested by the mixed cellularity sample, increasing from an average of a quarter of all cells, to nearly two-thirds. This finding uncovered that the TP53-clone was, in fact, the primary major clone within the neoplastic lymphocyte population in the majority of patients. Considering the poor prognosis conferred by the aberration, and its impact on current treatment decisions, it is quite significant to correctly identify a primary deletion-positive clone, instead of mislabeling it as a secondary minor clone.

E1034

PRIMARY PEGFILGRASTIM PROPHYLAXIS *VERSUS* FILGRASTIM GIVEN "ON DEMAND" FOR CLADRIBINE - INDUCED NEUTROPENIA IN HAIRY CELL LEUKEMIA

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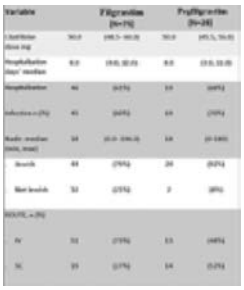
Background: Major advances in the treatment of patients with HCL were made in the 1980's after the introduction of two purine analogues: pentostatin and cladribine. Both these agents dramatically altered the clinical course and outcome of this disease and induced high response rates of 75-90%, with durable remissions and subsequent median relapse-free survival of up to 15 years. The major significant short-term toxicity of therapy with cladribine are neutropenia and neutropenic fever (NF). Based on the script data: 71% of patients experience grade 4 neutropenia (absolute neutrophil count [ANC] <500x109/l), and 42% develop NF. The latter complications may result in life - threatening infections, as well as hospitalization.
Aims: In this retrospective study, we compared the incidence and duration of neutropenia, NF and hospitalization in patients with HCL treated with cladribine followed by pegfilgrastim as primary prophylaxis *versus* daily filgrastim given "on demand "according to the absolute neutrophil count.
Methods: The study population included 202 patients with HCL, diagnosed and followed in 12 medical centers in Israel during 1985-2015. Patients were treated with cladribine, for 5-7 days given either sub-cutaneously or via intravenous infusion. Medical records were evaluated for details of disease at diagnosis, including date of diagnosis, age, sex, ethnicity,complete blood count results, and spleen size at diagnosis. The efficacy of pegfilgrastim and filgrastim was assessed by evaluating the incidence of neutropenia (defined as ANC <1000x109/l), number and length of hospitalizations due to NF, severity of infections and the number of days from the last day of therapy until recovery of ANC to >1000x109/l.
Results: Mean follow up was 7.5 years (0.1-40), with 5 and 10 years' survival of 96% and 90.62% respectively. The median age at diagnosis was 53 years, and 81.8% were males. First line therapy with cladribine was given to 159 patients (80.7%) and of these 50.3% required hospitalization for the administration of broad-spectrum antibiotics due to NF. The risk factor to develop NF was WBC< 0.6 109/l, and ANC<0.3109/l. Twenty eight patients were treated with pegfilgrastim as primaryprophylaxis 24 hours after the last day of therapy with cladribine, while 75 patients received filgastrim " on demand "due to neutropenia.Median hospitalization days, and Nadir duration was 8 and 18 days respectively in both groups (p=0.71, p=0.44).

Table 1.



Summary/Conclusions: Infectious complications post cladribine treatment, remains high, with an incidence of 50.3%. For all parameters analyzed, including the percentage of febrile patients, number of febrile days, and NADIR duration the results of primary pegfilgrastim prophylaxis and filgrastim given on demand were similar.Accordingly, we conclude that it remains the treating physician's choice to decide on which type of filgrastim to use and when to administer it.

E1035

REDUCED HEALTHCARE RESOURCE UTILIZATION IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA ACHIEVING COMPLETE REMISSION TO FIRST-LINE THERAPY

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Background: Most targeted therapies in the management of chronic lymphocytic leukemia (CLL) lead to high overall response rates but complete remissions are rare. Achieving complete remission (CR) is associated with improved clinical outcomes such as longer time to progression; however little is known about the economic benefits associated with achieving CR.
Aims: The objective of the study was to compare healthcare resource utilization among CLL patients initiated on first-line treatment who achieved CR *versus* those who did not.
Methods: This was a retrospective chart review study. From July to August 2016, 93 US oncologists/hematologists provided data abstracted from medical charts of their CLL patients who initiated a first-line CLL treatment between January 2010 and December 2014. The study collected patient demographics, clinical characteristics, response to first-line therapy, and the number of all-cause hospitalizations between first-line therapy initiation and end of the data follow-up (*i.e.*, patient's date of death, end of care, or data collection date, whichever occurred first). Patients were selected based on their best response to first-line therapy (*i.e.*, CR, partial remission [PR], stable disease [SD] and progressive disease [PD]) as defined by the physician according to iwCLL 2008 criteria. The targeted number of patients in each category was *a priori* determined based on rates of response observed in clinical trials. The incidence of all-cause hospitalization was compared between patients who achieved CR and those who did not (including patients with PR, SD or PD) using univariate and multivariate generalized linear models with a Poisson distribution. As patients had different follow-up, incidence rates were reported per-patient-per-month (PPPM). Multivariate regression models were adjusted for age, gender, selected comorbid conditions, time from CLL diagnosis to first-line initiation, and Eastern Cooperative Oncology Group (ECOG) status.

Table 1.

Table 1. Comparison of hospitalization between CR and non-CR cohorts

	CR IR PPPM	Non-CR IR PPPM	*Unadjusted IRR (95% CI)*	*Adjusted IRR (95% CI)*
All-cause hospitalization	0.006	0.021	3.30 (1.69-8.21)	2.44 (1.13-6.42)

** The number of hospitalizations was not available for a total of 29 patients (17 CR and 12 non-CR patients).*

Results: Patient-level data was collected for 179 patients who achieved CR and 151 patients who did not achieve CR (120 patients with PR, 25 with SD, and 6 with PD). Average time from CLL diagnosis to first-line initiation was 8.4 months for patients who achieved CR and 13.3 months for those who did not. The majority of patients were male (65%), the average age was 63 years, and 80% of patients had an ECOG of O or 1 at first-line therapy initiation. The medi-

an follow-up after first-line therapy initiation was 30 months. Over that period, patients who did not achieve CR had statistically significantly higher incidence of all-cause hospitalization compared to patients who achieved CR (0.021 *vs* 0.006 PPPM; unadjusted incidence rate ratio [IRR]=3.30, p<0.05). After adjusting for potential cofounders, the incidence of all-cause hospitalization was 2.4 times higher for patients who did not achieve CR compared to those who did (IRR=2.44, p<0.05).
Summary/Conclusions: Results from this study showed that achieving CR to first-line therapy (vs. not achieving CR) is associated with reduced frequency of all-cause hospitalizations. This suggests that, in addition to the clinical benefit associated with CR achievement, treatment strategies in CLL that improve CR may help reducing the economic burden of CLL management for both patients and payers.

E1036

RITUXIMAB (R) USED AS A SINGLE AGENT FOR AUTOIMMUNE HEMOLYTIC ANEMIA (AIHA) IN TREATMENT NAÏVE CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS INDUCES ALSO SIGNIFICANT DISEASE RESPONSE WITHOUT TOXICITY

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Background: There are very few effective treatment options for steroid refractory AIHA of CLL or for CLL patients(pts) that are unable to receive corticosteroids. R has been noted to be active in certain autoimmune hematologic disorders while experience with single-agent R in untreated CLL pts is very limited.
Aims: To report our experience concerning the use of R as a treatment of AIHA occurring during the clinical course of treatment naïve CLL pts by analyzing concomitantly its efficacy and safety as a single agent in CLL therapy
Methods: 15 pts diagnosed with CLL who received R due to AIHA were included in this study. Staging was performed at diagnosis (Binet system). Pts were placed on R at the standard dose I.V of 375mg/m^2 once weekly for 6 consecutive weeks because of contraindication of corticosteroids administration
Results: Pts' median age was 60 y(range, 42-83 y), (8 out of 15, males), 10 having disease stage A and 5 B. Two were presented with splenomegaly and 1 with B-symptoms. 12 pts (83%) had leukemic lymphocyte counts of more than 50x10^9/L. Median time from diagnosis, the AIHA diagnosis and to 1st R infusion was 59 mos. All 15 pts completed the 6-week course of R and were assessable for response. The median WBC and the median absolute lymphocyte count(ALC) before R administration and after the end of 6-week course are shown in the Table. Resolution of the AIHA effect was achieved in all pts whereas in 4 there was a persistence of positive DAT without evidence of active hemolysis. After the end of 6 weekly R infusions 13 out of 15 pts (86%) showed also disease response. 12 pts experienced PR (80%) and 1 CR (6%). All pts with advanced disease also responded entering PR. Resolution of splenomegaly was documented in both splenomegalic pts. After a median follow up of 84, 5 mos from CLL diagnosis, 14 pts are alive, 9 maintain their disease response while 5 were in need of therapy due to CLL progression, after a median time of 10 mos from the last R infusion. Among them 4 were placed on FCR (2CR, 2PR) and 1 on R-Bendamustine(PR). Median PFS has not reached. All pts received the entire first dose on day 1 of treatment. There was only a grade 3 infusion related reaction in a patient with WBC>400x10^9/L without need for hospitalization. None of the pts experienced severe tumor lysis syndrome, pulmonary insufficiency, myelosuppression or opportunistic infections.

Table 1.

Table: Median WBC and median ALC

	Before R	After 6 R
mWBC × 10^5/L (range)	63,7(6,9–521,4)	7,6(4,0-38,3)
mALC × 10^5/L (range)	58,0(2,4-480,0)	3,2(0,8-30,2)

Summary/Conclusions: A) R is an effective agent for AIHA treatment with concomitant significant activity against CLL and therefore could be the standard of care for CLL pts with AIHA, especially for the cohort of pts with comorbidities. B) We confirm previous data that: 1) single-agent R induces significant responses in treatment naïve CLL pts 2) R is well tolerated and its administration is not associated with myelosuppression or immunosuppression 3) R as a single agent could be an excellent first-line treatment option for pts who are very elderly or who have a poor performance status

E1037

ATTAINMENT OF COMPLETE REMISSION IS SIGNIFICANTLY ASSOCIATED WITH LONGER SURVIVAL OUTCOMES IN RELAPSED/REFRACTORY (R/R) CLL: A META-ANALYSIS

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Background: Chronic lymphocytic leukemia (CLL) is an incurable neoplasm of B lymphocytes, associated with a heterogeneous clinical course. Complete response (CR) with/without minimal residual disease in first-line chemoimmunotherapy has been associated with more favorable progression-free survival (PFS) and overall survival (OS). However, patients (pts) with R/R CLL and/or those with TP53 abnormalities (ie, 17p deletion and/or TP53 mutation) are less likely to achieve deep responses and experience poorer outcomes. Therefore, less is known about the relationship between CR and survival outcomes in R/R CLL pts.
Aims: To quantify this association, we generated meta-analytic estimates of PFS and OS reported in clinical trials using the proportion of study patients with CR as a predictor variable.
Methods: We performed a systematic literature review of PubMed/EMBASE up to Nov 2014 and congress abstracts 2012–2014. Randomized controlled trials and observational studies evaluating any treatment in R/R CLL pts were eligible for inclusion. Data were extracted from publications as median survival, the proportions of pts surviving at specific follow-up times, or individual event or censoring times from reported Kaplan-Meier (KM) curves, along with the proportion of pts with CR. Data were synthesized to estimate overall OS and PFS including population-level CR as a covariate using a Weibull proportional hazards model within a Bayesian meta-analysis framework.
Results: 74 published studies of treatment outcomes in R/R CLL pts were identified from the peer-reviewed literature and congress abstracts. 56 of these studies reported the proportion of CRs together with either OS or PFS outcomes and were included in the analysis. Individual pt data were extracted from KM curves of 29 studies generating 5176 individual pt OS and PFS data points in addition to 54 study-level data points including 3638 pts. There were no clinically meaningful differences in study or pt characteristics among the included studies that were not also associated with CR, our variable of interest. The hazard ratio (HR; and 95% credible interval, the Bayesian analog to confidence intervals) of survival for each 10% increase in CR among a study population was estimated to be 0.64 (0.60, 0.68). Estimated median OS for hypothetical populations with 0% CR, 25% CR, or 50% CR were 20.4 mo, 44.7 mo, and 61.9 mo. Corresponding median PFS estimates were 10.0 mo, 21.9 mo, and 30.3 mo. (Figure 1).

Figure. Weibull meta-analysis estimates of OS (A) and PFS (B) with median survival times for a population with 0% CR, 25% CR and 50% CR

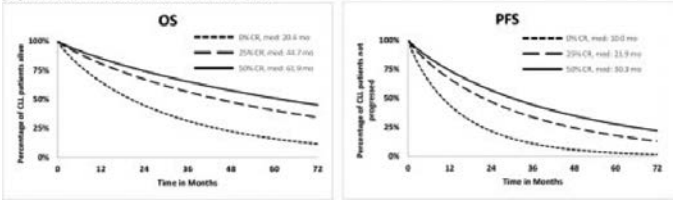

Figure 1.

Summary/Conclusions: The attainment of CR is significantly associated with longer OS and PFS outcomes in R/R CLL at the study level. Moreover this can be expressed linearly, with each 10% increase in CR rate corresponding to a 36% reduction in the risk of progression or death. To our knowledge, this is the first meta-analysis to quantify the relationship between CR and survival outcomes in R/R CLL pts. It must be noted that these results reflect the study (*population)* level CR *versus* survival association and therefore do not necessarily represent the expected survival gain associated with an *individual* achieving CR. Further, CR is less likely to be achieved in pts with TP53 abnormalities, a factor not explicitly considered in our analysis. These results synthesize data from 56 clinical trials and strongly support the importance of achieving CR to improve long-term outcomes in R/R CLL pts. In particular, given the negative association between CR and TP53 abnormalities, treatments focused on improving the likelihood of CR in these hard-to-treat pts are likely to confer the greatest impact on survival outcomes.

E1038

APLICATION OF THE CLL-IPI AND THE MDACC PRGNOSTIC INDEXES IN A LOCAL COHORT OF CLL PATIENTS

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Background: New prognostic scores have been developed in order to better discriminate the clinical course of CLL patients, along with Rai and Binet clinical staging systems. These scores, such as that proposed by the MDACC group, and recently the CLL-IPI combine clinical and biological variables with prognostic value.
Aims: In this study we investigated the validity and reproducibility of these scores in a local cohort of patients with CLL.
Methods: We made a retrospective analysis including 650 unselected CLL patients newly diagnosed and previously untreated from a single institution. The final analysis has been limited to the 486 cases with complete data to apply the MDACC score, and to the 258 cases with complete data to apply the CLL-IPI score.
Results: Median age was 67 years old (25-90). With a median follow-up time of 46 months, 394 patients were alive, and 187 had received any treatment for CLL at the moment of the analysis. Median overall survival (OS) of the whole series was 173 months (127-220), and median time to first treatment (TTFT) 106 months (82-130). The MDACC score was applied to 486 cases giving 0 to 9 points to each case according to: age, b2-microglobulin levels, absolute lymphocyte count, sex, Rai stage, and number of involved lymph node groups. As shown in the Table, stratification of patients using the MDACC score allowed the prediction of prognosis for both TTFT (P=0.000) and OS (P=0.000). 162 patients were classified as low risk, 302 as intermediate risk, and 21 as high risk. Due to missing data, the CLL-IPI score could only by applied to 258 patients giving 0 to 10 points to each case according to *17p* deletion, *IGHV* mutational status, β2-microglobulin, clinical stage, and age. As shown in the table, 126 patients were classified as low risk, 79 as intermediate risk, 46 as high risk, and 7 as very high risk. We also found significant differences in terms of OS (P=0.000) and TTFT (P=0.000) using this score.

Table 1.

	Number of patients	Overall survival (months)	P	Time to first treatment (months)	P
MDACC score			0.000		0.000
Low risk (1-3)	162	238 (IC 95%, 146-330)		133 (IC 95%, 110-155)	
Intermediate risk (4-6)	302	141 (IC 95%, 131-151)		81 (IC 95%, 46-115)	
High risk (≥7)	21	63 (IC 95%, 52-74)		2 (IC95%, 0-4)	
CLL-IPI score			0.000		0.000
Low risk (0-1)	126	238 (IC 95%, 147-330)		Not reached	
Intermediate risk (2-3)	79	144 (IC 95%, 128-162)		53 (IC 95%, 34-71)	
High risk (4-6)	46	74 (IC 95%, 57-91)		7 (IC 95%, 5-10)	
Very high risk (7-10)	7	32 (IC 95%, 21-42)		9 (IC 95%, 0-21)	

Summary/Conclusions: In this study we confirm that both scoring systems are able to discriminate patients in different prognosis subgroups. Both scores are also easily applicable in clinical practice. The new CLL-IPI score is able to distinguish subgroups of patients with worse prognosis including new factors (17p deletion and mutational status of IGHV).

E1039

CHRONIC LYMPHOCYTIC LEUKEMIA: PROGNOSTIC VALUE OF CLINICAL STAGES AND CLASSICAL PROGNOSTIC PARAMETERS DEPENDING ON TREATMENT MODALITY

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Background: Prognostication is a key component in the management of patients with chronic lymphocytic leukemia (CLL). Prognostic factors however may change as a result of the introduction of more effective therapies.
Aims: To investigate whether the prognostic value of classical parameters has changed over time.
Methods: Retrospective single-center study of prognostic factors and outcome in patients with CLL diagnosed before (n=454) and after (n=903) 1995 when purine analogs and subsequently chemoimmunotherapy (CIT) were introduced in CLL treatment at the Hospital Clínic, Barcelona.
Results: The median follow-up up was 8.3 years (0.1-33.0) for the overall series and 24.9 years (21.9-33.0) and 7.8 years (0.1-21.3) for patients diagnosed before and after 1995, respectively. Patients diagnosed before 1995 were older (p<0.001), had more advanced clinical stage (p<0.001), higher ALC (p<0.001), shorter LDT (p<0.001), and more often anemia (p=0.028), thrombocytopenia (p<0.001) and increased serum LDH levels (p=0.019) than those diagnosed thereafter. There were no differences in B2-microglobulin (B2M) levels and ZAP70 or CD38 expression. Mutated *IGHV* was more frequently detected in patients diagnosed before 1995 (75% *vs* 55%; p<0.001). The proportion of patients receiving treatment did not differ between groups [42% (38-47%) *vs* 46% (42-49%) at 6 years; p=0.08]. The type of therapy given to patients diagnosed before and after 1995 was: alkylating agents (91% *vs* 34%), purine analogs (4% *vs* 27%), CIT (0% *vs* 31%), other (5% *vs* 8%) (p<0.001). The response rate was lower in patients diagnosed before 1995 (57% with 9% CR *vs* 67% with 36% CR; p<0.001) and overall survival (OS) was shorter (median; 8.0 *vs* 10.1 years; p<0.001). The median OS in patients diagnosed before and after 1995 broken down by clinical stage was: stage A: 10.1 *vs* 10.9 years (p=0.1); stage B: 4.5 *vs* 9.2 years (p<0.001); stage C: 3.8 *vs* 3.8 years (p=0.2). In both groups of patients univariate analyses demonstrated a correlation between OS and clinical stage (both p<0.001), age >70 years (both p<0.001), B2M (both p<0.001), short lymphocyte doubling time (LDT) (both p<0.001), unmutated *IGHV* (both p<0.001), and ZAP70 (p=0.015 and p<0.001). High-risk FISH correlated with OS in patients diagnosed after 1995 (p<0.001). In patients diagnosed before 1995, the number of subjects with available FISH was too small for a meaningful analysis. In multivariate analysis (age >70 years, advanced clinical stage short LDT increased B2M, diagnosis before 1995) only age (HR 2.7 (95% CI: 2.1-3.4), p<0.001), LDT (HR 2.5 (1.9-3.2), p<0.001) and B2M (HR 2.8 (2.2-3.6), p<0.001) showed independent prognostic significance for OS. *IGHV* mutational status, ZAP70 and high-risk FISH cytogenetics correlated with OS but these variables were not included in multivariate analyses because of the many patients with missing information.
Summary/Conclusions: Survival of patients with CLL in intermediate-risk (stage B) disease has dramatically improved over the last years. In contrast, the outcome of patients with either low (stage A) or high (stage C) stage has not been significantly modified, indicating the need for more effective therapies in these patients. Importantly, the prognostic significance of classical prognostic variables has not changed after the introduction of more effective therapies. Finally, similar studies are warranted in patients treated with novel agents.

E1040

AN OBSERVATIONAL STUDY EVALUATING THE USE OF BENDAMUSTINE AS FIRST-LINE TREATMENT FOR CHRONIC LYMPHOCYTIC LEUKEMIA IN RUSSIA

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Background: Bendamustine has gained footing as a component of first-line therapy for chronic lymphocytic leukemia (CLL) due to its efficacy and favorable toxicity/tolerability profile for most patient types. There is a dearth of data on the effectiveness of bendamustine as first-line therapy in the Russian CLL patient population, which is needed to support drug cost reimbursement processes.
Aims: Evaluate effectiveness of first-line therapy with bendamustine for CLL in the Russian Federation.
Methods: This was a prospective, multicenter, observational study (NCT02110394) in adults (>18 yr) diagnosed with CLL who were receiving, or were scheduled to receive, first-line therapy with bendamustine plus rituximab. Patients who had prior CLL treatment (eg, chemotherapy, radiation) or had contraindications to bendamustine were excluded. Each subject was required to have 2 to 8 study visits, where Visits 2–6 reflected treatment cycles; total number of treatment cycles for each patient was determined by the study investigator. Interim and final evaluations were performed after 3 and 6 treatment cycles, respectively. Primary endpoints were overall response rate (ORR, patients achieving complete remission [CR] plus those achieving partial remission [PR]). Secondary endpoints were time to therapeutic failure, time to progression, progression-free survival (PFS), relapse or death after CR or PR, quality-of-life (EQ-5D questionnaire), and frequency of adverse drug reactions (ADRs).
Results: Of the 196 patients who enrolled between June 2012 and August 2015, 191 were included in the Safety Population (patients who received ≥1 dose of study drug) and 149 were included in the Full Analysis Set (FAS; patients in the Safety Population who had ≥1 response evaluation). Most patients in the FAS were male (59.7%); mean age was 61.5±8.9 yr. Overall, 35.6% of patients were ≥65 yr old and 80.5% had ≥1 comorbidity such as decreased renal function. The ORR was 83.2%; CR and PR rates were 59.7% and 23.5%, respectively. Generally, response rates were slightly higher than those reported in the Phase 3 pivotal trial (Knauf *et al. J Clin Oncol*. 2009). Eradication of minimal residual disease was achieved in 23 of the 84 evaluable

patients (27.4%). Overall, 80.3% of FAS patients did not experience therapeutic failure and 85.9% did not experience disease progression during the 2-year observation period. By the end of the study, median PFS had not been reached; 2-year PFS rate was estimated as 85.9%. Improvements from baseline were observed after 6 cycles of treatment across all EQ-5D domains. No relapses or deaths occurred in the FAS; however, 2 subjects in the Safety Population experienced fatal serious ADRs (myocardial infarction [n=1]; acute pneumonia, infections and toxic shock, and atrial fibrillation [n=1]). In concurrence with the Phase 3 trial results, hematologic disorders (19.9%; anemia, neutropenia, thrombocytopenia), most of which were Grade ≤2 in severity, were the most common ADRs (Safety Population; **Table 1**).

Table 1. Hematologic ADRs by CTCAE Grade.

Hematologic ADR	Grade ≤2 (%)	Grade ≥3 (%)
Anemia	49.2	0.9
Neutropenia	21.7	17.5
Thrombocytopenia	5.3	0.5

Summary/Conclusions: First-line therapy with bendamustine plus rituximab was well tolerated in this Russian CLL population, including elderly patients and patients with renal dysfunction or other comorbidities. Additionally, combination therapy resulted in high rates of treatment response in the CLL. These data confirm the value of bendamustine as a first-line agent for CLL in routine clinical practice in Russia.

Chronic myeloid leukemia - Biology

E1041

MUTAGENESIS OF BCR-ABL1 IS REQUIRED FOR RESISTANCE DEVELOPMENT IN DE NOVO CHRONIC MYELOID LEUKEMIA KCL-22 CELLS BUT NOT IN RELAPSED KCL-22 CELLS EXPRESSING BCR-ABL1 INDEPENDENT RESISTANCE

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Background: *BCR-ABL1* kinase domain (KD) mutations are an important mechanism of resistance of chronic myeloid leukemia (CML) patients developing during the tyrosine kinase inhibitors (TKI) treatment. However, mechanisms underlying KD mutation acquisition in TKI-resistant CML cells are not yet well understood.

Aims: We studied an acquisition of mutations in the KD after an exposure of *de novo* and relapsed (grown in optimal growing medium for 24 months) KCL-22 cells to imatinib (IM). In addition, we examined kinetics of mutated subclones in established IM-resistant KCL-22R culture after dose-reduction of IM. We also studied changes in the expression profile of KCL-22 cultures early after exposure to IM.

Methods: The occurrence and kinetics of expansion of *BCR-ABL1* mutant subclones were studied using next-generation deep sequencing in KCL-22 cells treated with 0.4 μM IM and in established IM-resistant KCL-22R cells at 4 μM IM. In other set of experiments, KCL-22R cells were sorted according to the CD38 expression to explore whether CD38 is associated with the acquisition of *BCR-ABL1* mutations as suggested by Wang *et al*. (2014). A protein array was used allowing analysis of 576 proteins per sample. DNA damage pathway-RT Profiler PCR arrays were applied for gene expression analysis.

Results: No *BCR-ABL1* KD mutations were detected in *de novo* untreated KCL-22 cells, however T315I and E255K appeared after the exposure of the cells to 0.4 μM IM. PCR array revealed increased expression of SUMO 1 ligase and ERCC2 involved in the nucleotide excision repair pathway. Notably, we also found a significant decrease of G2/M-checkpoint protein GADD45A whose deficiency is associated with mutagenesis (Hollander *et al*., 2001). During the first culture period, T315I slowly emerged whereas E255K was not detectable. Later, E255K-bearing cells also became detectable and increased over time. A similar time-dependent expansion of mutant-bearing sub-clones was seen in the KCL-22R cells growing at 4 μM IM. Interestingly, a mutant-clone switch from T315I to E255K in KCL-22R was accelerated after IM reduction from 4 μM to 1 or 2 μM. Moreover, the emerging of E255K sub-clone was accompanied by rapid decrease of CD38 expression in KCL-22R cells. Profiling of transitional KCL-22R culture, carrying both T315I and E255K sub-clones, revealed that T315I transcripts were expressed only in the CD38+ subpopulation, while E255K was detected only in CD38- cells. Unlike to *de novo* KCL-22 cells, *BCR-ABL1* mutations were repeatedly not detected in relapsed KCL-22 cells up to follow-up of 60 days after the cells exposure to 0.4 μM IM. Neither *BCR-ABL1* upregulation nor gene amplification was detected in these cells. We identified considerably upregulated (D7, DTX3, ETV6, GLUL, HCLS1, HIF1α, IGF1R, MAP2K7, MYH11, TP53) or downregulated (BAD, BID, MCL2 NOTCH3, PDKPK1) proteins early, 4 weeks after the exposure to IM. Increased expressions of HIF1α and IGF1R proteins are known to ensure proliferation, while decreased expressions of pro-apoptotic proteins BAD and BID enhance survival of CML cells in the presence of TKIs.

Summary/Conclusions: Our observation suggests the ability of KCL-22 cells to survive and proliferate early after the exposure to IM. *BCR-ABL1* mutations development seems to be related to a mutagenesis of imatinib on *de novo* KCL-22 cells, but not on relapsed KCL-22 cells that activated signaling pathways ensuring their survival and growing in the presence of tyrosine kinases inhibitor.

Supported by the project no. 00023736 and AZV 15-31540A of MZCR and ERDF OPPK CZ.2.16/3.1.00/28007.

E1042

FLOW-CYTOMETRY DETECTION OF CD26+ LEUKEMIA STEM CELLS IN PERIPHERAL BLOOD: A SIMPLE AND RAPID NEW DIAGNOSTIC TOOL FOR CHRONIC MYELOID LEUKEMIA

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Background: Diagnosis of Chronic Myeloid Leukemia (CML) implies documenting in bone marrow (BM) or in peripheral blood (PB) Philadelphia (Ph) chromosome by cytogenetics, molecular BCR-ABL1 fusion by FISH or BCR-ABL1 rearrangement by RT-PCR. In clinical practice, at the earliest, 24-72 hrs are needed to confirm CML by any of these assays. Lately, characterization of CML leukemia stem cells (LSCs) from BM samples of CML patients (pts) showed a specific co-expression of dipeptidylpeptidaseIV (CD26) within the $CD34^+/CD38^-/Lin^-$ stem cell fraction and CD26 appeared a robust biomarker for identifying CML LSCs within the normal BM compartment. We recently demonstrated that $CD34^+/CD38^-/CD26^+$ LSCs can be easily identified by flow-cytometry also in PB during treatment with tyrosine kinase inhibitors.
Aims: We investigated accuracy and specificity of flow cytometry PB $CD34^+/CD38^-/CD26^+$ LSCs identification as a new tool for the diagnosis of CML.
Methods: Pts with clinical suspicion of CML entered the study after written informed consent and all were evaluated for $CD26^+$LSCs, cytogenetics, FISH and/or BCR-ABL1 RT-PCR analysis. $CD34^+/CD38^-/CD26^+$ population was investigated in PB and when possible simultaneously in BM samples using a flow-cytometry 4-color staining procedure. $2.0x10^6$leucocytes were incubated with BD Pharmigen CD45V500 (c.2D1), CD34FITC (c.581), CD38APC (c.HIT2), CD26 (c.M-A261) and negative controls. Acquisition and analysis of at least $1.0x10^6$ $CD45^+$ cells were done by FACSCanto II with DIVA 8 software (BD, Biosciences). $CD26^+$ cells were identified by sequential gate. $CD45^+$ and $CD34^+$ gates were performed on viable cells identified by FSC/SSC light properties and $CD34^+/CD38^-$ population was gated applying a narrow gate excluding all $CD38^+$ cells (Fig.1).
Results: PB samples from 107 pts with myeloproliferative features were evaluated for $CD26^+$LSCs. Leucocytes median value was $52x10^9$/L (range 5-$408x10^9$/L). In 83/107 (77.5%) pts we showed $CD34^+/CD38^-/CD26^+$ LSCs in PB and in 83/83 (100%) the diagnosis of CML was confirmed by cytogenetics, FISH and RT-PCR analysis. Median value of circulating PB CD26/µL was 14 (range 0,27-698) and a positive correlation with leukocyte count ($p<0.01$) was found. In 53/107 (49,5%) pts analysis was performed contextually in BM samples. All $CD26^+$ PB-BM matched pairs (49/53) showed superimposable results in terms of absolute number of $CD26^+$LSCs/µL (19,18 and 18,73 respectively) while the percentage of $CD26^+$ cells within the $CD34^+/CD38^-$ fraction appeared lower in BM than in PB samples (median 28,18 and 37,33; range 0,87-77,14 and 5,59-99,57 respectively). In 24/107 (22.5%) PB samples and in 4/53 BM samples $CD26^+$ LSCs were not detected and none of these samples was found Ph or BCR-ABL1 positive. Pts with CD26 neg PB/BM samples were subsequently diagnosed as Idiopathic Myelofibrosis (12 pts), Myelodysplastic/Myeloproliferative disorders (7 pts) benign neutrophilia (5 pts). Of note, we additionally studied 4 PB+BM samples of 4 Ph+ acute lymphoblastic leukemia and all scored negative for $CD26^+$LSCs.

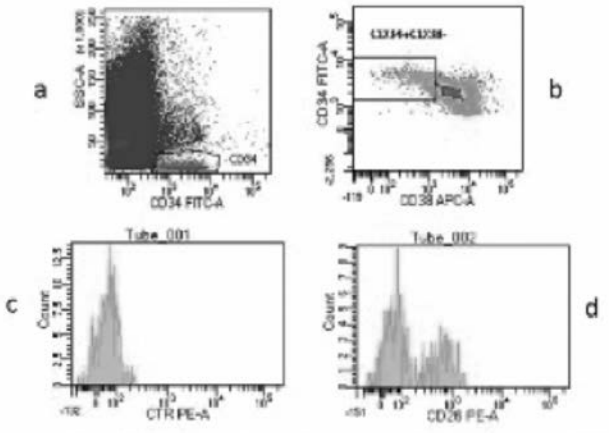

Fig 1 PB CD26⁺ LSCs evaluation in a representative CML patient. CD34 gating on viable cells identified by FSC/SSC light properties (a) $CD34^+CD38^-$ population gated applying a quite narrow gate that excluded also a proportion of CD38 dim cells (b). Negative control (c) and CD26 expression on $CD34^+CD38^-$ population (d). In the case here depicted the percentage of $CD26^+$ cells within the $CD34^+CD38^-$ fraction was 43,7% corresponding to 17,4/µL of circulating $CD26^+$LSCs

Figure 1.

Summary/Conclusions: Flow-cytometry evaluation of PB $CD34^+/CD38^-/CD26^+$LSCs is a feasible, very rapid (about 3 hrs from sample handling to results) and highly specific alternative/complementary diagnostic tool for CML. To validate these data in a larger cohort of patients we are developing a pre-titrated lyophilized antibody mixture (lyotube, BD Biosciences) to maximize sensitivity and to optimize standardization and working time, with the further aim to monitor stem cells minimal residual disease in CML patients.

E1043

LIPID PEROXIDATION AND INFLAMMATORY STATUS DURING TKI TREATMENT IN CHRONIC MYELOID LEUKEMIA PATIENTS: INTERIM ANALYSIS OF A PROSPECTIVE MULTICENTER STUDY

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Background: Evidences of increased cardiovascular (CV) events, mostly atherothrombotic, in Chronic Myeloid Leukemia (CML) patients (pts) treated with some Tyrosine Kinase inhibitors (TKIs) prompted physicians to carefully evaluate CV risk factors (CVRFs) in the choice of TKI. However, the pathogenesis behind CV events during TKIs is still largely unknown and even pts without overt CVRFs incur in CV events. We retrospectively showed that an induced "inflammatory status" during nilotinib treatment, together with genetic pro-atherothrombotic predisposition, may partly explain the increased incidence of CV (Bocchia, Oncotarget 2016). These data provided the rationale to start a multicentric "Prospective study of T**KI** induced pro-**A**therothR**O**mbotic status in CML. KIARO study" (Grant support: AIRC-ITT) including Chronic Phase CML pts treated with any first line approved TKI in which clinical, genetic and biochemical pro-atherothrombotic profiles were evaluated at diagnosis and during treatment.
Aims: This prospective study aims to confirm the possible role of genetic predisposition and behavior of specific pro/anti-inflammatory biochemical parameters in the atherosclerotic pathogenesis during TKIs treatment.
Methods: Enrolled pts were prospectively evaluated for: presence of traditional CVRFs, atherothrombotic episodes, presence of Single Nucleotide Polymorphisms (SNPs) associated to CV risk (Cardiokit) and plasma levels of several pro and anti-inflammatory cytokines. In this first interim analysis we focused on levels of LDL, oxidated-LDL (oxLDL), TNFα, IL-6 and IL-10 and the presence of SNPs of LDL-R (rs1122608), LOX-1 (rs3736235), and IL-10 (rs1800896) genes.
Results: 12 Italian Hematology Units participated to the study and up to date 95 CML patients were enrolled. We here report data from the first 43 patients on TKI treatment for at least 12 months (15 nilotinib, 14 imatinib and 14 dasatinib,. No CV events were recorded to date. At diagnosis, levels of LDL (143.5±13.2), ox-LDL (237.4±99.5), TNFα (3.91±2.51), IL-6 (1.96±0.99) and IL-10 (0.34±0.15) were evaluated for the whole cohort and according to the TKI treatment. No statistically significant differences were found in the expression of these variables between the 3 groups of treatment ($p>0.096$). Considering the genotype frequency, we confirmed in the whole cohort a correlation between basal levels of LDL, oxLDL and IL10 with the presence/absence of the detrimental G/G allele of LDL-R (H.R. 2.11, $p<0.01$), LOX-1 (H.R. 2.86, $p<0.01$) and IL10 (H.R. 1.85, $p<0.05$) polymorphisms. During TKIs treatment we observed increased levels of LDL ($p<0.05$) and oxLDL ($p<0.05$) only in the nilotinib cohort at 3 and 12 months of treatment, regardless of the concomitant use of CV medications. No differences in TNFα and IL6 levels during the first 12 month of treatment were detected in the 3cohort ($p<0.079$). Interestingly, IL-10 levels were significantly higher at 3 and 12 months of treatment in the imatinib and dasatinib cohort ($p<0.01$) respect to nilotinib ($p=0.094$).
Summary/Conclusions: This interim analysis, although still very preliminary, suggests that in nilotinb patients the high levels of LDL and oxLDL in combination with low levels of IL10, could induce a persistent pro-inflammatory/oxidative status potentially favoring atherothrombotic events. Additional biochemical and genetic data as well as prolonged clinical observation are needed to confirm this hypothesis. Patients enrolment and monitoring is ongoing.

E1044

TRANSCRIBED ULTRACONSERVED NONCODING RNAS (T-UCRS) IN CHRONIC MYELOID LEUKEMIA: EXPRESSION PROFILES ASSOCIATED WITH MOLECULAR RESPONSE TO THERAPY WITH TYROSINE KINASE INHIBITORS

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Background: Transcribed ultraconserved regions (T-UCR) are a novel class of long noncoding RNAs. Many classes of noncoding RNAs have been implicated in human tumorigenesis. In addition to the different expression profiles of T-UCRs that could be used to distinguish human leukemias and carcinomas, they have also been reported to have direct interactions with miRNA with an important regulatory effect in disease development such as chronic myeloid leukemia (CML).
Aims: In this study, we aimed at the correlation of T-UCR and miRNA:T-UCR pairs in CML, according to tyrosine kinase inhibitor (TKI) therapy, clinical risk scores and molecular response.
Methods: We analysed peripheral blood samples from 45 CML patients and 15 healthy controls. Two panels of 481 T-UCR and 752 miRNA probes were used for RT-qPCR analysis. Differential expression was evaluated using the Mann-Whitney test followed by Benjamini-Hochberg multiple testing correction.
Results: CML samples presented significantly different expression of uc.164 ($p<0.01$), uc.118 ($p<0.01$), uc.125 ($p<0.01$), uc.391 ($p<0.01$), uc.153 ($p<0.01$), uc.141 ($p<0.01$), uc.143 ($p<0.05$) and uc.145 ($p<0.05$), when compared to healthy controls. This latter T-UCR (uc.145) was associated with development and immune regulation pathways. We analysed Sokal, Hasford and EUTOS risk scores and found uc.236 ($p<0.0001$), uc.39 ($p<0.05$) and uc.7 ($p<0.05$) to be associated with EUTOS low risk. Concerning therapy, dasatinib was correlated with uc.187 ($p<0.005$). For imatinib doses, uc.4 ($p<0.05$) and uc.3 ($p<0.05$) inversely correlated with 400 and 800mg daily, respectively. Molecular response in CML samples presented a signature including uc.187 ($p<0.001$), uc.107 ($p<0.05$), uc.409 ($p<0.05$), uc.198 ($p<0.05$), uc.309 ($p<0.05$), uc.102 ($p<0.05$), uc.294 ($p<0.05$) and uc.361 ($p<0.05$). Major molecular response was identified by the altered expression of uc.198 ($p<0.05$), uc.215 ($p<0.05$) and uc.210 ($p<0.05$). The negative regulation of T-UCRs by miRNAs, involving T-UCR:miRNA interaction, was associated with upregulated (miR-720, miR-886-3p, miR-1274a, miR-101 and miR-129) and downregulated (miR-489 and miR-1973) microRNAs.
Summary/Conclusions: In the present study, we identified T-UCRs signatures and T-UCR:miRNA pairs associated with CML, risk scores, TKI therapy and molecular response. The expanded knowledge of RNA biology in general, together with the recent interest in the multitude of newly discovered elements such as T-UCRs, could help to improve CML therapy.

Financial Support: FEDER (Programa Operacional Factores de Competitividade – COMPETE) and FCT (Fundação para a Ciência e Tecnologia) through project PEst-C/SAU/LA0001/2013-2014.

E1045

MAINTENANCE OF LEUKAEMOGENIC POTENTIAL OF BCR/ABL+ CELLS REQUIRES PAK2 BUT NOT PAK1

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Background: p21-activated kinases (PAKs) are key nodes in oncogenic signalling pathways that control growth, survival, and motility of cancer cells. Their activity is increased in many human cancers and the increase is associated with a poor prognosis. To date, PAK deregulation has mainly been studied in solid tumours, where PAK1 and PAK4 are the main isoforms deregulated.
Aims: We aimed at identifying deregulations of PAKs in haematopoietic tumors and the potential contribution of individual PAKs to tumorigenesis.
Methods: We used a combination of *in silico* analysis of publicly available data of haematological patients, shRNA-mediated knockdown and *in vitro* transformation assays. In parallel, we analysed the tumorigenic potential of leukemic cells *in vivo* after knockdown of individual PAKs.
Results: We show that PAK1 and PAK2 are frequently deregulated in haematopoietic tumors, particularly in BCR/ABL$^+$ leukemia. In suspension, BCR/ABL$^+$ leukaemic (KU812) cells deficient for PAK1 and PAK2 undergo apoptosis, while the loss of either protein is well tolerated. When leukaemic cells are challenged to grow towards an extracellular matrix, the loss of PAK2 alone abrogates cell growth. PAK2-deficient cells fail to form colonies in growth-factor free methylcellulose and do not induce lymphomas *in vivo*.
Summary/Conclusions: PAK2 is the critical isoform in a BCR/ABL-driven disease. This finding suggests that the PAK2 isoform represents a promising target for the treatment of haematological diseases.

E1046

MIRNA PROFILING OF CIRCULATING EXTRACELLULAR VESICLES IN CML PATIENTS WITH MUSCULOSKELETAL PAIN ASSOCIATED WITH DISCONTINUATION OF TYROSINE KINASE INHIBITORS

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Background: Clinical trials of TKI discontinuation are still ongoing, approximately 60% of CML patients who achieved a deep molecular response for more than 2 years maintained a major molecular response after discontinuation of imatinib. However, the long-term prognosis and/or adverse events after TKI cessation remain unclear. Recent reports showed that transient musculoskeletal pain without consistent biochemical abnormalities occurs in approximately 30% of CML patients after stopping imatinib.
Aims: Recent evidences suggest that extracellular vesicles (EVs) that contain genetic element such as DNA, RNA, and miRNA, are important mediators of intercellular communication. We therefore studied molecular study to ascertain the possible correlation between musculoskeletal pain and EV-miRNA expression.
Methods: We investigated circulating EV-miRNAs in five CML patients who did not experience musculoskeletal events and five patients with musculoskeletal pain after stopping TKIs, as well as three healthy individuals. Peripheral blood was obtained approximately 3 months after successful TKI cessation in CML patients. Exosomes were extracted by using Total Exosome Isolation Reagent (Invitrogen, Carlsbad, CA, USA) and EV-miRNA profiling was performed with a TaqMan Low-Density Array (Thermo Fisher Scientific, Carlsbad, CA, USA), as reported previously. The relative expression level of each gene was calculated by using the comparative thresholds cycle (Ct) method. Synthetic spike control (ath-miR-159; Hokkaido System Science, Hokkaido, Japan) was used as an invariant control for EV-miRNA. This study was approved by the institutional review board of Tokyo Medical University (no. 930 approved 24 June 2008 and no. 3052 approved 9 June 2015).
Results: Three-way analysis of variance (ANOVA) performed for healthy controls and CML patients with and without musculoskeletal pain revealed EV-miR-140-3p to be the most significant value (P=0.00778). A t-test analysis using R software identified 10 differentially expressed EV-miRNAs for CML patients with and without musculoskeletal pain: seven miRNAs were upregulated (miR-107, miR-145, miR-140-3p, miR-539, miR-495, miR-299-5p, miR-425) and three were downregulated (miR-122, miR-218, miR-523) in CML patients with musculoskeletal pain. The up-regulated EV-miR-140-3p in all CML patients decreased after release of musculoskeletal pain.
Summary/Conclusions: CML patients with increased EV-miR-140-3p achieved levels similar to those of healthy controls after relief from musculoskeletal pain. It has been reported that there is an association between musculoskeletal pain and inflammatory indicators in some CML patients who stopped TKIs; however, we did not find any positive association. Although the number of CML patients in this study is too small to draw definite conclusions, further research should investigate whether upregulation of EV-miR-140-3p expression in peripheral blood is correlated with musculoskeletal events in CML patients after TKI cessation.

E1047

SOLUBLE AND MEMBRANE-BOUND RECEPTOR–LIGAND IMMUNE CHECKPOINTS AND CHRONIC MYELOID LEUKEMIA: CORRELATIONS WITH MOLECULAR RESPONSE AND TYROSINE KINASE INHIBITOR THERAPY

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Background: Blockade of immune checkpoints seems to unleash the potential of the antitumor immune response in a fashion that is transforming human cancer therapeutics. Soluble and membrane-bound receptor–ligand immune checkpoints are the most druggable forms using agonist antibodies (for co-stimulatory pathways) or antagonist antibodies (for inhibitory pathways). Although its implications in immune response during chronic myeloid leukemia are obvious, literature regarding chronic myeloid leukemia (CML) and immune checkpoints is scarce.
Aims: This study aimed at the analysis of lymphocyte subsets expression and plasmatic levels of immune checkpoint inhibitors during tyrosine kinase inhibitor (TKI) therapy in CML and its correlation with molecular response.
Methods: Peripheral blood samples from chronic phase CML patients (n=55), divided according to molecular response to imatinib, dasatinib, nilotinib, bosutinib, ponatinib and Interferon-alpha 2b (IFN-α 2b) therapy, were included in this study. Multi-parametric flow cytometry was used for the analysis of the

expression of several immune checkpoint inhibitors (BTLA, GITR, LAG-3, PD-1, PD-L1, PD-L2, TIM-3, CD137/4-1BB) by different T, B NK, monocyte and dendritic cell subsets. A 14-plex panel including BTLA, GITR, HVEM, IDO, LAG-3, PD-1, PD-L1, PD-L2, TIM-3, CD28, CD80, CD137 (4-1BB), CD27, and CD152 (CTLA-4) was analyzed by xMAP technology (Luminex®).
Results: Expression of CD137 by several lymphocyte subsets and PD-1 by regulatory T cells (Tregs) and natural killer (NK) cells were found significantly altered in CML patients under TKI therapy. These associations were observed for the cell population frequency expressing the receptor, and also for density of these molecules. Increased plasmatic levels of BTLA, HVEM, PD-1, PD-L1, and CD137 were associated with good molecular response to therapy. PD-1, PD-L1, TIM-3 and CD137 were found increased in patients that achieved MR4.5.
Summary/Conclusions: Some immune checkpoint inhibitors seem to be affected by TKI therapy in CML and their cell expression and plasmatic levels correlates to molecular response. Similar observations were described for other types of cancers, including solid tumors. Soluble and membrane-bound receptor–ligand immune checkpoints could represent interesting targets for future therapeutic monitoring and for pharmacologic interventions in CML.

Financial Support: FEDER (Programa Operacional Factores de Competitividade – COMPETE) and FCT (Fundação para a Ciência e a Tecnologia) through project PEst-C/SAU/LA0001/2013-2014.

E1048

TYROSINE KINASE INHIBITORS SIGNIFICANTLY CHANGE THE EXPRESSION OF POLYCOMB GENES IN CHRONIC MYELOID LEUKEMIA

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Background: It has been reported that, notwithstanding their clinical success, tyrosine kinase inhibitors (TKIs) are not able to eradicate the leukemic stem cell (LSC) in patients with chronic myeloid leukemia (CML). Different mechanism have been hypothesized, especially those linked to the niche (increased osteoblastic differentiation, angiogenesis, hypoxia...). The epigenetic control seems to be relevant, and our group previously identified a correlatio between the expression of some polycomb genes (PcGs) and response to TKIs, with BMI1 resulting a good predictive molecular marker (Crea, 2015).

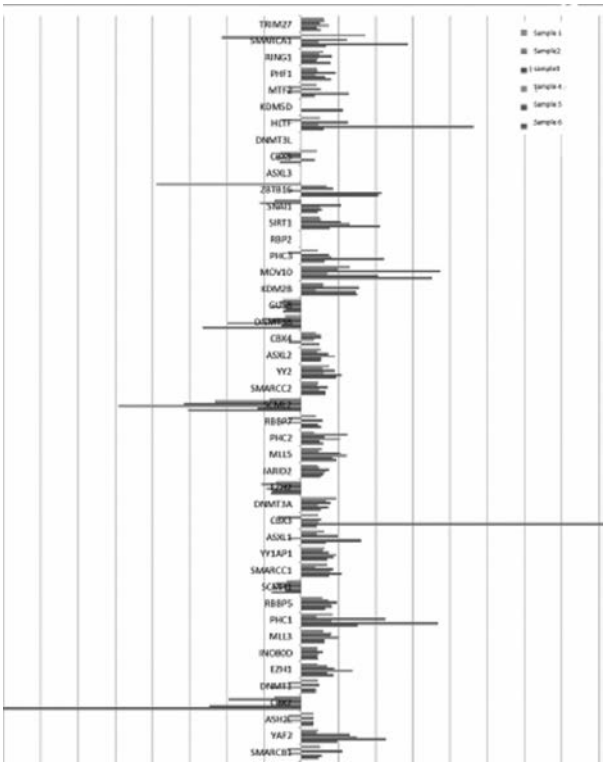

Figure 1.

Aims: In order to better understand the role of the PcGs genes in CML patients receiving TKIs, we analyzed the expression of 86 PcGs at baseline and after 6 months of therapy.
Methods: Buffy coats obtained from peripheral blood samples of 6 patients (5 receiving imatinib and 1 dasatinib) have been used for the RNA extraction; these RNAs were used for quantifying the BCR-ABL1/ABL1 ratio%IS, according to the European guidelines, and the expression of the chosen 86 PcGs by real-time PCR (PrimePCR pathway kit, Biorad, Milan, Italy) at diagnosis and after 6 months of treatment. Expression values were calculated using the 2DDCt method.
Results: At the sixth month of treatment, 5 patients were in optimal response and one was "warning", according to the 2013 ELN guidelines. After therapy, 55% of the tested PcGs resulted up-regulated and 23% of them in the majority of patients; whereas 3 genes (DNMT3B, SCML2, CBX2) were down-regulated in at least half of samples. The expression of 5% of PcGs was "mixed", up- or down-regulated in different samples. Among the up-regulated genes, some could be relevant from a biological point of view: 1) HLTF, a target for RUNX1, whose low expression in acute leukemia is correlated with poor outcome; 2) PHC2, able to silence the HOX genes, overcoming the multidrug resistance in myeloid models; 3) PCGF5, that is a marker of normal hematopoiesis; 4) MOV10, that has been reported to have an anti-viral activity, increasing levels of gamma interferon. This up-regulation is particularly interesting, because concerns all assessed samples and could explain our previous observation that Torque Teno virus replication does not occur in CML patients during TKIs therapy; 5) in the only "warning" patient, the up-regulation of SIRT1 was observed: this is in line with the observation that its up-regulation increases the oncogenic ability of K562 cells in a murine model. Among the down-regulated genes, could be relevant: 1) CBX2, that binding P16/p19 promotes the cell cycle progression; its down-expression could induce apoptosis; 2) DNMT3B, whose high levels have been reported in stem cells, and whose reduction could characterize the differentiation process; 3) ZBTB16, whose reduction could be a sign of the reduced osteoblastogenesis, one of the mechanisms responsible for the LSC preservation in the niche; 4) SMARCA1, it too correlated to the cell cycle progression. Finally, BMI1 levels resulted unmodified in 3 cases and increased in other 3.
Summary/Conclusions: We demonstrated that PcGs de-regulation occurs in CML patients during the treatment with TKIs, with possible pathogenetic implications. Huger series of patients will improve the biological suggestions coming from these preliminary data.

E1049

IDENTIFICATION OF PROGNOSTIC AND SUSCEPTIBILITY MARKERS IN CHRONIC MYELOID LEUKEMIA USING NEXT GENERATION SEQUENCING

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Background: Chronic Myeloid Leukemia (CML) is 20% of all leukemias diagnosed every year. Discovery of Imatinib Mesylate has brought a paradigm shift in treatment of Chronic Myeloid Leukemia, despite 15% - 20% patient showing resistance to this TKI. Therefore, it is important to identify susceptibility and prognostic markers, which can help us in predicting occurrence and prognosis of CML. We did Clinical Exome Sequencing, a panel of more than 4800 clinically important genes, in CML patients
Aims: To identify prognostic and susceptibility genetic markers in CML
Methods: Enrolled CML patients (n=18) were segregated as responders (n=10) and failures (n=8) as per ELN, 2013 guidelines. Healthy controls (n=5) were also enrolled. DNA from blood of subjects was subjected Next Generation Sequencing (NGS). Mutations present in one patient group and absent in opposite group were considered as prognostic markers, whereas rare mutations, present in more than 50% of enrolled patients and absent in healthy controls, were considered as susceptibility markers
Results: We discovered mutations in genes associated with cancer or cancer related functions in different patient groups as markers. Five of them: rs116201358, rs17882014, rs4014596, rs52897880 and rs2274329 in C8A, HLA-DRB1, UNC93B1, APOH and CA6 genes respectively, were present in responders; rs4945 in MFGE8 was present in failures. Mutations in HLA-DRB1 (rs17878951, rs11554462, c.239C>G), HLA-DRB5 (rs137863146), RPHN2 (rs193179333), CYP2F1 (rs116958555), KCNJ12 (rs76684759), FUT3 (rs151218854), BMO1 (rs28370522) and PRSS1 (rs144422014) were present in half or more patients
Summary/Conclusions: We discovered potential genetic markers, which can help in predicting response to IM as frontline therapy. Susceptibility markers can be used as panel for to configure individuals prone to CML

E1050

FEATURES OF THE A2455G POLYMORPHISM OF GENE CYP 1A1 IN PATIENTS WITH CML

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Background: Chronic myeloid leukemia (CML) is the most common myeloproliferative disorder characterized by the reciprocal translocation t (9; 22), (q34; q11), leading to the formation of chimeric oncogene BCR-ABL on the 22q-chromosome. It is known that the protein products of the genes of cytochromes ensure homeostasis at the cellular and tissue level, carrying out the metabolism of toxic compounds that can damage the genome of the cells. Prognostic value of certain genotypic variants of these genes in the formation of a number of neoplastic diseases, including leukemia. In individuals with weakened functional genotypes of A2455G polymorphism of CYP 1A1 gene expression of this enzyme and, consequently, inactivation of xenobiotic must occur very slowly, thus creating conditions for adverse action of harmful metabolites in the genome of the cells. Currently, the scientific literature discusses the role of the negative allele A2455G polymorphism of CYP 1A1 gene in the development of hematological malignancies. However, the adverse roles of genotypic variants for this gene in oncogenesis of BCR-ABL-positive patients with CML have studied not enough.
Aims: Evaluation the role of A2425G polymorphism of CYP1A1 gene in the formation the mutant clone of tumor and the development of CML.
Methods: The work is performed on DNA samples isolated from the peripheral blood of the patients in the clinic of scientific research Institute of Hematology and blood transfusion in Uzbekistan. We studied 146 patients with CML. The control group was formed from 217 individuals of Uzbek nationality, without of any cancer disease. The diagnosis of CML verified in accordance with the International nomenclature ISCN. Standardized PCR with detection in real-time was carried out on a thermal cycler Rotor-Gene 6000 (Corbett Research, Australia), using a set of reagents "AmpliSens® Leucosis quantum M-bcr-FRT"(InterLabServis, Russia). Testing A2425 polymorphism of CYP1A1 gene was performed on a programmable thermal cycler of the company "Applied Biosystems" (USA) using test systems company "Litech" (Russia) according to the manufacturer's instructions. Statistical analysis of results was carried out using the statistical software package "2009 OpenEpi, Version 2.3".
Results: The frequencies of allele A and G are as follows: 87.7% and 12.3% in patients with CML, and 93.3% and 6.7% in the control group, respectively. The frequency distribution of genotypes A / A, A / G and G / G were as follows: 76.7%, 21.9% and 1.4% - in CML patients, and 86.6%, 13.4% and 0.0% - in the control group. Observed frequencies of genotypes in the studied groups was consistent with the theoretically expected and were in equilibrium with hardy-Weinberg equilibrium (P>0.05). There was a statistically significant decrease in carriage of the adverse alleles in the population sample comparison group patients (1.4% *vs* 6.7%, respectively; χ2=6.8; P=0.01; OR=2.0; 95% CI 1.17- 3.282). Also detected significant association of heterozygous genotype A/G in patients with CML, compared with the control group (21.9% *vs* 13.4%, respectively). The risk of mutant formation of the tumor clone in carriers of this genotype was 1.8 times significantly higher compared with patients not having it (χ2=4.6; P=0.03; OR=1.8; 95% CI 1.046-3.166). The homozygous genotype G/G in the group of patients was significantly more compared to the control group (1.4% *vs* 0.0%), but due to the small number of carriers of this genotype differences in the results did not reach statistical significance (P>0.05). Functionally favorable genotype A/A was found with high frequency in a population-based sample of 86.6% *vs* 76.7% cases of patients. At the same time, the differences reached the threshold level of significance (χ2=6.0; P=0.01; OR=0.5; 95% CI 0.29-0.88), that is evidence of a favorable protective effect of this genotype against the development of CML.
Summary/Conclusions: Our results suggest that the G allele and the heterozygous genotype A/G A2425G polymorphism of CYP 1A1 gene are important markers of increased risk in formation of malignant tumor cells and development of CML in Uzbekistan (P<0.05). In this case, homozygous genotype A/A of A2425G polymorphism of CYP 1A1 gene has a protective character in relation to risk of CML.

Chronic myeloid leukemia - Clinical

E1051

HEMATOLOGIC TOXCITIY GRADE III-IV IS ASSOCIATED WITH LOWER SURVIVAL IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA TREATED WITH TYROSINE KINASE INHIBITORS

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Background: TKIs introduction in the treatment of chronic myeloid leukemia (CML) has offered an outstanding improvement in survival outcomes. These results were obtained from clinical trials but little is known about long-term toxicity and their translation to real life. In addition, clinical trials results are mainly based on the analysis of the therapy of interest (experimental or control), but the descriptions of the subsequent treatment sequences due to failure or intolerance are normally lacking.
Aims: To analyze the long-term toxicity of patients outside clinical trials in clinical trials. The setting was a multicentric, hospital-based registry.
Methods: Toxicity grade III-IV and survival and their potentially associated variables were studied.

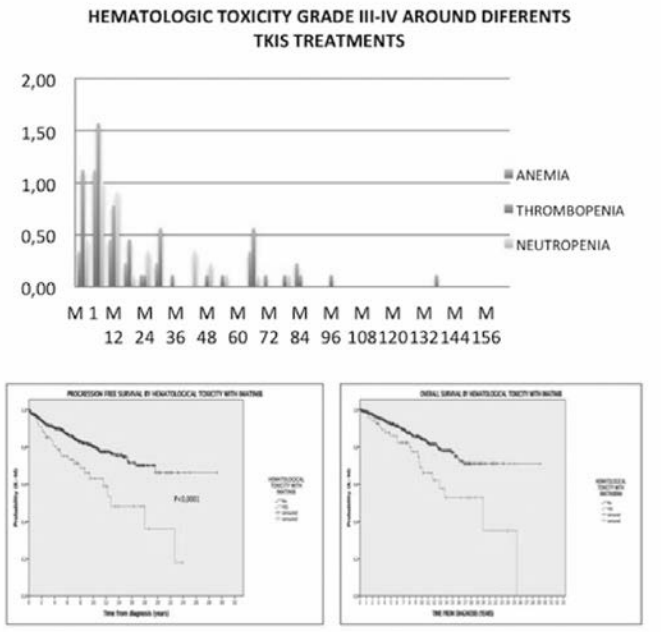

Figure 1. Evolution of hematologic toxicity grade 3-4 with time (all treatments sequences included).

Results: Demographics, risk and treatment distribution: 893 patients (533 men, 360 women) with a median age at diagnosis of 52 y (14-94y) were included with a follow up of 85±7 months (m) from diagnosis, 78±6.6 m from first treatment, and 69±6 m from first TKIs. 151 patients (16,9%) were over 70y. The risk distributions were as follows: Sokal: low (L) 48%, intermediate (I) 37% and high (H) 14%; Euro score: L 50%, I 45% and H 5%; EUTOS L: 92% and H 8%; EUTOS LT: L 70%, I 23% and H 7%. Treatment groups were the following: Group 1: IFN alpha and then imatinib or 2º GTKIs (221 patients); Group 2: imatinib only (404 patients); Group 3: imatinib and then nilotinib, dasatinib or both due to failure or intolerance (177 patients) and Group 4: 2ºGTKIs in first line (93 patients).Hematologic toxicity grade III-IV: Figure 1 shows the incidence through the years (all group of treatments). From 800 patients treated with imatinib (first o second line) 67 (8,3%) had grade III-IV toxicity, and 26 had to switch treatment due to toxicity. From 166 patients treated with dasatinib (29

in1st line, 114 in 2nd, 56 in 3rd) only 13 had hematologic toxicity and 6 had to switch, 14 had pleural effusion grade III-IV and 9 had to switch. From 115 patients treated with nilotinib (49 in 1st line and 66 in 2nd) only 10 had hematologic toxicity and 10 switched treatment. Survival: Estimated survival by 10 years was 80%. Variables associated with survival: In the univariate survival analyses (log rank test) either from diagnosis, first therapy or first TKIs, the Sokal, Eutos, Euro and EUTOS LT scores, as well as age over 70y were the only statistically significant variables associated with survival. Hematology toxicity grade III-IV was associated with lower PFS or OS (figure 1). In the multivariate analysis (Cox model), only hematology toxicity grade III-IV and age over 70y were independent variables.

Summary/Conclusions: 1.These results show that the probability of survival by 10 years is roughly 80%, and extend the findings of our previous work showing that this probability is not different across different sequential treatments (imatinib 1st line or post-IFN, or switched to 2ªGTKis due to intolerance o failure) (1). This fact emphasizes the rescue potential of available TKI therapies. 2. Hematologic toxicity grade III-IV in the first two years identified a group of patients with worse survival outcome. 3. Patients over 70 years have shorter survival due to reasons different than progression. 4 Second GTKIs showed better hematologic toxicity profile.

Reference

1. Casado LF, *et al.* Cancer Med. 2015 Mar 10.

E1052

5-YEAR EFFICACY OF DASATINIB AND IMATINIB IN NEWLY DIAGNOSED PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP) WITH DOSE MODIFICATIONS FROM DASISION

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Background: Multiple dosage strengths are approved for dasatinib (DAS), permitting dose-optimization strategies for patients who experience adverse events (AEs). In a 2-year retrospective analysis of DASISION, efficacy was maintained in DAS- and imatinib (IM)-treated patients with dose reductions or interruptions to manage AEs (Jabbour ASH 2011); cytogenetic and molecular response rates were higher for patients given DAS *vs* IM, even when daily doses were modified. Longer term follow-up is needed to fully understand the potential impact of dose reductions on efficacy.

Aims: To evaluate the effect of dose reduction for any AE and for pleural effusion on efficacy in DAS- or IM-treated patients from DASISION.

Methods: Treatment-naïve patients with CML-CP in DASISION (NCT00481247) were randomized to receive either DAS (100mg once/day; N=259) or IM (400mg once/day; N=260). Dose reductions for AEs (up to 2) were allowed: DAS: 80mg, then 50mg; IM: 300mg, then 200mg. Five-year molecular and cytogenetic response rates in all patients were assessed retrospectively.

Table 1.

Results: Patients on DAS maintained higher molecular response rates than patients on IM, whether or not they had dose reductions for an AE; these rates were similar in patients with and without dose reductions in each arm (table). 95 (37%) DAS- and 44 (17%) IM-treated patients had dose reductions at any time due to AEs. Median time to first DAS dose reduction was 289 days (range: 22-2123), and median time to first IM dose reduction was 160 days (range: 31-2052). For patients with reductions due to any cause, median average daily dose was DAS 83mg and IM 328mg; for DAS patients with reductions due to pleural effusion, median average daily dose was 82mg. Median duration of treatment (excluding interruptions) was 54 months (range: 3-70) for patients who had a DAS dose reduction and 57 months (range: 2-71) for patients who had an IM dose reduction. Changes in level of response were tracked for patients who achieved complete cytogenetic response (CCyR) or major molecular response (MMR) before or after the first dose reduction (table). Many patients maintained or increased to CCyR or MMR following dose reductions for any AE. Hematological toxicity (9%) was the most common AE resulting in dose reduction for IM, and pleural effusion (12%) was the most common for DAS.

Summary/Conclusions: Reducing DAS doses to 80mg or 50mg was a safe and effective means of managing patients who experienced AEs in this 5-year retrospective analysis of DASISION. These results were consistent with previous reports and continued to show that efficacy was not affected by dose reductions for any cause, including pleural effusion. Notably, there was no loss of CCyR following dasatinib dose reductions. Molecular responses remained higher for DAS *vs* IM irrespective of dose reductions due to AEs.

E1053

EFFECT OF PLASMA TROUGH CONCENTRATION OF NILOTINIB AND POLYMORPHISMS OF DRUG TRANSPORTER GENES ON THE FREQUENCY OF ADVERSE EVENTS IN CHRONIC PHASE OF CHRONIC MYELOID LEUKEMIA: STAT1 AND STAT2 TRIALS

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Background: STAT trials (STAT1 and STAT2) are multicenter, phase II, single-treatment arm, open-label clinical studies designed to evaluate the efficacy and safety of two-year consolidation by nilotinib (NIL) for achieving a deep molecular response (DMR) or successful treatment-free remission (TFR) in patients with chronic phase chronic myeloid leukemia (CML).

Aims: In this report, we focus on the adverse events (AEs), especially anemia and liver dysfunction observed in the STAT trials. Additionally, we analyzed the relationship between laboratory abnormalities and pharmacokinetics (PK)/ phramacogenetics (PGx) of NIL.

Methods: AEs were assessed according to the Common Terminology Criteria for Adverse Events (CTCAE) v4.03. Safety evaluations were conducted throughout the study. Plasma trough concentrations of NIL were determined with high-performance liquid chromatography (HPLC) at 1 month (1M), 3M, 6M, 12M, and 24M in the STAT trials. Genotyping of *CYP3A5*3* [*6986A>G* (rs776746)], *ABCB1* [*3435T>C* (rs1045642)], *ABCG2 421C>A* (rs2231142), and *UGT1A1*6*, **27,* and **28* was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). All genotype frequencies were tested for Hardy-Weinberg equilibrium.

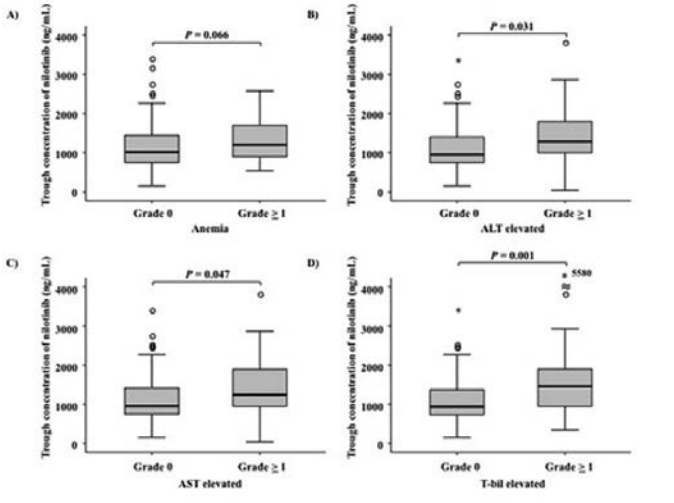

Figure 1.

Results: Between July 2011 and December 2012, CML patients were recruited in the STAT trials. NIL was administered twice daily (600mg/day) for 2 years according to the study protocol. A total of 76 and 96 patients were analyzed as a safety data set in STAT1 and STAT2, respectively. In STAT1, 18 patients who achieved a confirmed DMR were switched from STAT1 to STAT2. These patients entered both trials, but safety data had not been collected in STAT1 after entering STAT2 to avoid double counts. The PK/PGx data of 147 of 154 patients were available and were evaluated in this study. Median trough concentrations of NIL were 1265 ng/ml at 1M, 1154 ng/ml at 3M, 974 ng/ml at 6M, 735 ng/ml at 12M, and 781 ng/ml at 24M. Although any-grade AEs were reported in 43 patients in STAT1 and 55 patients in STAT2, the most common drug-related hematological and non-hematological AEs were elevated total bilirubin (28.6%), anemia (24.5%), elevated ALT (21.1%), and elevated AST (18.4%). The incidence of these AEs, except for anemia, was significantly associated

with high trough concentration of NIL (Figure1). There were statistically significant correlations between median concentrations of NIL and the grades of each AE. Based on the results of the analysis using Cox proportional-hazards model, the trough concentration of NIL [hazard ratio=1.001 (1.000-1.002), *P*=0.004] and *ABCG2* 421A/A [hazard ratio=3.044 (1.155-8.027), *P*=0.024] were independent factors for the elevated ALT. Similarly, the trough concentration of NIL [hazard ratio=1.001 (1.000-1.002), *P*<0.001] and *UGT1A1 *1/*1* [hazard ratio=0.475 (0.246-0.919), *P*=0.027] were independent factors for the elevated total bilirubin.
Summary/Conclusions: In this study, we identified the relationship between NIL trough concentration and liver dysfunction. Our finding suggests that therapeutic drug monitoring might help avoid drug interruption and discontinuation because of AEs, especially liver dysfunction.

E1054

VERY EARLY MOLECULAR RESPONSE (VEMR) WITH FRONTLINE DASATINIB TREATMENT IS A STRONG PREDICTOR OF LONG-TERM BCR-ABL1 TRANSCRIPT LEVELS IN CHRONIC MYELOID LEUKEMIA PATIENTS: PCR-DEPTH STUDY

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Background: In BCR-ABL1 tyrosine kinase inhibitor (TKI) treated chronic phase chronic myeloid leukemia (CP-CML), early molecular response (EMR) at 3 month is currently identified as being one of the most important prognostic factors. Sokal risk score and dose intensity during first 3 months were strongly associated with achievement of EMR. As dasatinib is a novel, oral tyrosine kinase inhibitor with improved potency, identification of very early molecular response (VEMR) would be beneficial.
Aims: We evaluated the possibility of the VEMR at 1 month predicting long-term outcomes in newly diagnosed CP-CML patients treated with dasatinib.
Methods: In this multi-center, observational, open-label study, 102 patients with CP-CML were enrolled to receive dasatinib at a dose of 100mg once daily. The primary end point was complete molecular response (CMR) by 18 months. Secondary end points including molecular response (MR) by 1, 3, 6, 12, 18, 24 month, time to and duration of MMR and CMR, and safety were tested. A receiver operating characteristic (ROC) curve from BCR-ABL1 transcript level on Day+28 was calculated to predict EMR and MMR at specific timepoints.
Results: Median age was 49 years (19-81 years) and 61 patients were male. With median follow-up duration of 28 months (0.9-33.8 months), 80 (78.4%) out of 102 patients were still on dasatinib treatment and 22 patients discontinued due to disease progression (n=2) or treatment failure (n=3) or adverse events (n=8) or other reasons (n=9). The BCR-ABL1 mutations, assessed in 10 patients after dasatinib discontinuation, were detected in 3 patients which were all T315I mutation. The cumulative CMR by 18 months and MMR by 24 months were 20.5% and 79.6% respectively. In safety analyses, grade 3/4 thrombocytopenia (30.3%) was most common. Pleural effusion occurred in sixteen (15.6%) patients which were mostly grade 1/2. The cut-off value of BCR-ABL1 transcript on Day+28 was 40% by ROC curve analysis. Among 95 patients who had available molecular data of both D+28 and 12 months, fifty nine (62.1%) patients had less than 40% of BCR-ABL1 transcript (VEMR) on Day+28. Among them, 49 (83.1%) patients achieved MMR at 12 months. However, only 27.8% (10 out of 36 patients) of patients without VEMR achieved MMR (p<0.0001). Among 85 patients who had available molecular data of both D+28 and 24 months, fifty two (61.2%) patients achieved VEMR. In 52 VEMR patients, 46 (88.5%) patients achieved MMR at 24 months. However, only 48.5% (16 out of 33 patients) of patients without VEMR achieved MMR (p=0.0001). Overall survival (OS) & progression-free survival (PFS) rates by 24 months were 98.0% and 95.1% respectively. PFS rates by 24 months for VEMR and no VEMR group were 98.4% *vs* 88.8% respectively (p=0.04).
Summary/Conclusions: Our study shows that VEMR at 1 month can be a strong predictor for further molecular responses as well as long-term outcome. Therefore it would be helpful to monitor BCR-ABL1 transcript level at 1 month in patients who treated with more potent TKIs.

E1055

SURVIVAL OUTCOMES IN PATIENTS WITH CHRONIC-PHASE CHRONIC MYELOID LEUKEMIA (CP-CML) RECEIVING THIRD- OR SUBSEQUENT LINE (3L) TREATMENT PRIOR TO THE AVAILABILITY OF PONATINIB

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Background: PACE was a phase 2 single-arm trial of ponatinib, a 3rd-generation tyrosine kinase inhibitor (TKI), in 449 highly-refractory patients with CML or Philadelphia-chromosome positive (Ph+) acute lymphocytic leukemia (ALL) or who had the BCR-ABL T315I mutation. Overall survival (OS) for 3L CP-CML patients in PACE at 1, 2, 3 and 4 years was estimated to be 91%, 83%, 80%, and 79%, respectively. Expected survival for 3L CP-CML patients prior to the availability of ponatinib has not been documented.
Aims: To estimate OS in patients with CP-CML receiving 3L treatment prior to ponatinib via a systematic literature review.
Methods: Studies were identified from a review by Lipton *et al.* (2015), updated with studies identified from searches of electronic databases (MEDLINE, EMBASE, Cochrane Libraries) and abstract databases of key conferences. Landmark and median survival were extracted from study reports. Pseudo-individual patient data (IPD) for survival outcomes were derived from digitized Kaplan-Meier (KM) survival curves then pooled and analyzed using KM methods.
Results: Fifteen studies (717 patients) were identified that reported median, landmark, or KM curves for survival outcomes for CP-CML patients receiving 3L treatment without ponatinib. KM curves for OS were obtained for 6 arms (3 nilotinib and/or dasatinib; 3 other TKIs). OS at 1, 2 and 3 years based on the pooled IPD is reported in the Table. To avoid confounding of OS from post-progression treatment with ponatinib, 1 study was excluded that included follow-up after the date of ponatinib's approval.

Table 1.

Table. Estimated OS in CP-CML patients receiving 3L treatment prior to ponatinib

Time (Years)	OS		
	Pts	Arms	Probability (95%CI)
0	327	5	100% (-- , --)
1	257	5	90% (86%, 93%)
2	179	5	77% (72%, 83%)
3	89	2	66% (59%, 72%)

Summary/Conclusions: Estimated OS in patients with CP-CML receiving 3L treatment prior to ponatinib appears to be shorter than that observed among ponatinib-treated patients in PACE: 4-year survival probability in PACE is higher than estimated 2-year survival probability prior to ponatinib. Further analyses are needed to identify and adjust for potentially confounding factors.

E1056

DETECTION AND MONITORING OF BCR-ABL1 KINASE DOMAIN MUTATIONS IN CML AND ALL PATIENTS BY NEXT GENERATION SEQUENCING AND DROPLET DIGITAL PCR, A BELGIAN PROSPECTIVE STUDY

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Background: Among myeloproliferative diseases, development of chronic myeloid leukaemia (CML) is associated with the emergence of the fusion oncogene *BCR-ABL1* resulting from a t(9,22) chromosomal translocation (Philadelphia chromosome). This chimeric transcript is also present in all acute lymphoblastic leukaemia (ALL) patients bearing a Philadelphia chromosome (Phi+ ALL). Mutations of the *BCR-ABL1* kinase domain constitute a major cause of treatment failure in CML and Phi+-ALL patients receiving first or second generation tyrosine kinase inhibitor (TKI) treatment. So far, the gold standard procedure to detect *BCR-ABL1* kinase domain (KD) mutations is the conventional Sanger Sequencing, endowed with an analytical sensitivity of 15-20%. Recent studies on the implementation of Next Generation Sequencing (NGS) for detection of BCR-ABL1 KD mutations showed a significant dropping down of the sensitivity level (1-5%), improving patient's treatment management.

Aims: Both NGS and droplet digital PCR (ddPCR) were used in this prospective study. NGS screened all known mutations in the *BCR-ABL1* KD and ddPCR targeted only the 3 most common mutations, T315I, E255K and Y253H, which represent approximatively 75% of the *ABL1* mutations. Patients eligible for the study were i) CML patients with failure or warning to all lines of TKI therapy according to the 2013 ELN-guidelines, with no suspected lack of adherence and ii) Phi+ ALL patients at diagnosis and/or molecular relapse. Monitoring was performed when clinically appropriate.
Methods: Total *BCR-ABL1* RNA was transcribed into a long range cDNA covering the kinase and the regulatory and the SH2/SH3 domains of either p190 or p210 *BCR-ABL1* transcripts (exons 4 to 10). For NGS, primers designed with the Ampliseq™ Designer Software generated a set of 10 amplicons. Barcoded libraries, constructed according to the AmpliSeq™ protocol, were sequenced on the Ion Torrent PGM platform (sensitivity of 2.5%). For ddPCR, cDNA was analysed for the presence of one of the 3 main mutations (T315I, E255K and Y253H) on the Bio-Rad QX200 platform (sensitivity of 1%).
Results: The participation of more than 20 Belgian centres allowed the inclusion of 60 samples on a 9 months period (54 CML and 6 Phi+ ALL). The overall number of *BCR-ABL1* mutated samples was 18 (15 CML and 3 Phi+ ALL), representing 30% of the cases. Among these samples, 27 mutations were found. 9 samples presented with one mutation: T315I (2), E255K (3), G250V (1), F359I (1), M237T(1) and E255A (1) and 9 harboured compound mutations: T315I + E255K (6) and T315I + Y253H (3). A high frequency (85%) of T315I, E255K and Y253H mutations was also observed (23/27). As far as these 3 mutations are concerned, reproducibility to determine mutational burden was found to be very high between NGS and ddPCR.
Summary/Conclusions: Advances in sequencing technologies and further lowering sensitivity levels contribute to optimal management of CML and Ph+ ALL patients and improve treatment outcome. The earlier a mutation in the kinase domain is detected, the earlier an informed choice can be made regarding optimal subsequent TKI treatment.

E1057

CLINICAL AND IMMUNOLOGICAL EFFECTS OF NILOTINIB IN COMBINATION WITH PEGYLATED INTERFERON-A2B IN PATIENTS WITH SUBOPTIMAL MOLECULAR RESPONSE ON IMATINIB (NORDDUTCHCML009)

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Background: The ability to "cure" a proportion of chronic myeloid leukemia patients (CML) makes treatment free remission (TFR) an important treatment goal which requires sustained deep molecular response on TKI therapy. Unfortunately, not all patients achieve deep molecular responses on their first line treatment. Novel treatment strategies to increase the proportion of CML patients eligible for a TKI stop attempt are therefore needed.
Aims: The primary objective of the NordDutchCML009 study was to assess if, in CML patients who did not achieve an $MR^{4.0}$ or better to imatinib could attain this $MR^{4.0}$after a switch to nilotinib with pegylated interferon-α2b (pegIFN-α2b) combination treatment for at least 12 months. Assessment of safety, tolerability and immunological effects of the combination treatment were additional study goals.
Methods: In a phase II, single arm, multicenter trial, patients with *BCR-ABL1* levels above 0.01% (IS) after at least two years of imatinib treatment were switched to nilotinib 300mg BD (Nilo). PegIFN-α2b (Peg) 25 µg/week was introduced after three months, increased to 40 µg/week at month 6 if tolerated and continued until month 12. Adverse events were assessed according to CTCAE version 3. Flow cytometry assessments of T-, NK- and B-cell populations, activated T-cells, myeloid-derived suppressor cells (MDSCs) and regulatory T-cells (Tregs), cytotoxicity assays of NK-cells against K562 target cells, IFN-γ production analysis of stimulated CD3+ cells (Elispot), and Granzyme B staining of T-cells were performed at baseline (imatinib), 3 months (nilotinib) and 6 months (nilotinib + pegIFN-α2b) after inclusion and compared using a repeated measures ANOVA with multiple comparisons.
Results: In total 16 patients were enrolled in the study. Recruitment of patients was difficult and the study was prematurely closed for this reason. Confirmed $MR^{4.0}$ at 12 months was achieved by 50% (7/14) of the patients. Two patients discontinued nilotinib treatment within the first 3 months due to hepatotoxicity and lipase elevation (grade 4). Five patients did not tolerate PegIFN-α2b combination treatment, mainly due to mood disturbances. Finally, only 56% of patients completed the scheduled Nilo/Peg combination treatment for the first 12 months. We found a reduction in the proportion of CD56+ NK-cells after addition of PegIFN-α2b to the nilotinib treatment (*P*=.046), but no significant changes in the other immunological parameters. In the subgroup analysis this finding only demonstrated as a trend for a decreased NK-cell count after PegIFN—α2b administration in patients who attained confirmed $MR^{4.0}$ at 12 months (p=0.063), and was not established in patients who did not attain confirmed $MR^{4.0}$ at 12 months. In those patients who did not attain confirmed $MR^{4.0}$ at 12 months, we observed an increased number of stimulated CD3+ cells producing IFN-γ after introduction of PegIFN-α2b (p=0.026). These results from the subgroup analysis need to be interpreted with caution due to low statistical power.
Summary/Conclusions: Despite relatively poor tolerability of the scheduled Nilo/Peg combination treatment in the current study, CD56+ NK cells were significantly modulated and more than half of the patients achieved a sustained $MR^{4.0}$, which would allow for a TKI stop attempt. The discontinuation rate suggests that the PegIFN dose was too high in combination with nilotinib treatment in our study population.

E1058

ANALYSIS OF VASCULAR ADVERSE EVENTS IN TKI TREATED JAPANESE CML PATIENTS: RETROSPECTIVE LARGE COHORT STUDY OF CML COOPERATIVE STUDY GROUP

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Background: Chronic myeloid leukemia (CML) is a disease of hematopoietic stem cells resulting from oncogenic chromosome translocation that leads to the formation of the *BCR-ABL1* fusion gene.Treatment of chronic phase (CP) CML has dramatically changed since the emergence of the first-in class tyrosine kinase inhibitor (TKI) imatinib, and treatment based on TKI has improved the outcome in the majority of CP-CML patients. Nowadays, second generation TKIs are available and brought about faster and deeper clinical responses, and lower disease progression rate than imatinib. On the other hand, longer treatment duration and the increased types of TKIs gave rise to various kinds of unexpected adverse events (AEs). In 2011, drug-induced peripheral artery occlusive disease (PAOD) was first reported, followed by vascular AEs (VAEs) including ischemic heart disease (IHD) and cerebral infarction (CI). Furthermore, it became clear that the incidence of VAEs increased with the dose and treatment duration, therefore VAEs are considered a more fatal complication of TKI treatment. However, there is no available data about the incidence of VAEs in Japanese patients.
Aims: We investigated the vascular safety issue and estimated the 1000 person-years risk of developing VAEs during TKI treatment, including imatinib, nilotinib, and dasatinib, using 3 risk assessment tools among 320 Japanese patients who were enrolled in the CML Cooperative Study Group.
Methods: A surveillance data of 320 patients enrolled in the CML Cooperative Study Group was conducted in this analysis. Briefly, the study included patients who were diagnosed with CML-CP from April 2001 to January 2016, whose median age was 57 years old (15-80) and median time of follow up was 64.2 months. Patients in the accelerated or blastic phase (AP/BP) were excluded. The study was approved by the research ethics boards of each institutions and was conducted in accordance with the Declaration of Helsinki. All patients who developed VAEs were analyzed using 3 risk assessment tools (SCORE chart, Framingham risk score, Suita-score) to estimate the patients' 10-year risk of VAEs.
Results: Among the 320 newly diagnosed CML-CP patients, 16 (5.0%) cases of VAEs were reported during the study period. Seven cases were treated by imatinib, 3 cases by nilotinib, 1 case by dasatinib, 4 cases were a switch from imatinib to nilotinib, and 1 case was a switch from dasatinib to nilotinib. The VAEs were 9 IHD, 5 CI, and 2 PAOD cases. Results from the 3 risk assessment tools are as follows: SCORE (2 low, 9 moderate, 1 high, 4 very high risk), Framingham score (3 low, 5 moderate, 7 high risk), and Suita-score (13 low, 1 intermediate, 1 high risk). The incidence rate of IHD and CI per 1000 person-years were 5.26 and 2.92 in the enrolled CML-CP patients, 4.65 and 2.33 in the imatinib-treated patients, 14.34 and 10.75 in the nilotinib-treated patients, 4.08 and no case in the dasatinib-treated patients, and 1.787 and 3.342 in the age-

matched general population, respectively. Among the 320 newly diagnosed CML-CP patients, 16 (5.0%) cases of VAEs were reported during the study period. Seven cases were treated by imatinib, 3 cases by nilotinib, 1 case by dasatinib, 4 cases were a switch from imatinib to nilotinib, and 1 case was a switch from dasatinib to nilotinib. The VAEs were 9 IHD, 5 CI, and 2 PAOD cases. Results from the 3 risk assessment tools are as follows: SCORE (2 low, 9 moderate, 1 high, 4 very high risk), Framingham score (3 low, 5 moderate, 7 high risk), and Suita-score (13 low, 1 intermediate, 1 high risk). The incidence rate of IHD and CI per 1000 person-years were 5.26 and 2.92 in the enrolled CML-CP patients, 4.65 and 2.33 in the imatinib-treated patients, 14.34 and 10.75 in the nilotinib-treated patients, 4.08 and no case in the dasatinib-treated patients, and 1.787 and 3.342 in the age-matched general population, respectively.

Table 1.

Incidence rate of VAEs

	Control	CML patient (N = 320)			Imatinib (N = 155)			Nilotinib (N = 105)			Dasatinib (N = 127)		
	Incidence rate *1	Events	Exposure Time (yr)	Incidence rate *1	Events	Exposure time (yr)	Incidence rate *1	Events	Exposure Time (yr)	Incidence rate *1	Events	Exposure Time (yr)	Incidence rate *1
All thrombotic events		16	1711	9.35	7	860	8.14	8	279	28.67	1	245	4.08
IHD	1.787*2	9	1711	5.26	4	860	4.65	4	279	14.34	1	245	4.08
CI	3.342*3	5	1711	2.92	2	860	2.33	3	279	10.75	0	245	0
PAOD	N/A	2	1711	1.17	1	860	1.16	1	279	3.58	0	245	0

*1 incidence rate per 1000 person-year
*2 TAKASHIMA AMI registry 1990-2001 Am J Epidemiol 2008;167:1358-1364
*3 TAKASHIMA stroke registry 1988-2004 Stroke 2010;41:1871-1876
N/A: no available data
yr: years

Summary/Conclusions: The incidence rate of IHD per 1000 person-years were higher in the nilotinib- and lower in imatinib- and dasatinib-treated CML patients, and the patients showed almost the same rate of CI as compared with the age-matched general population, even though the incidence of VAEs were lower in Japanese as compared to the European cohort. More patients were estimated to have very-high and high risk of VAEs in the SCORE and Framingham risk score assessment tools as compared with the Suita-score tool.

E1059

UPDATE OF CMREGISTRY: AN OBSERVATIONAL, MULTI CENTER, PROSPECTIVE FOLLOW-UP REGISTRY OF PATIENTS WITH CHRONIC PHASE CML WITH A HIGH PROBABILITY OF OBTAINING A DEEP MOLECULAR RESPONSE >CMR4 (IS)

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Background: Since the introduction of Tyrosine Kinase Inhibitors (TKI), many patients diagnosed of Chronic Myeloid Leukemia (CML) in chronic phase achieve a deep molecular response. Around 50% of these patients are expected to maintain their molecular responses even after discontinuation of their TKI treatment. Several clinical trials are exploring the best way of stopping TKI therapy and evaluating patient and disease characteristics that could predict relapse after treatment discontinuation.
Aims: This is an update of the CMRegistry study aimed at collecting clinical data and molecular information from spanish CML patients that have achieved a series of molecular milestones to any of the tyrosine kinase inhibitors in order to monitor their progress and the achievement of a stable deep molecular response: >MR4 (IS).
Methods: CMRegistry is an observational, multi-center and prospective study. CML patients treated with any of the tyrosine kinase inhibitors who are likely to achieve, or have already achieved, a deep molecular response (>MR4.0 (IS)) are included. This likelihood of achieving >MR4 is defined, for the purposes of the study, as a bcrabl/abl ratio of: 1) ≤1% at 3 months from start of TKI therapy; 2) ≤0.1% at 6 months from start of TKI therapy; or 3) ≤0.01% any time point during treatment. Clinical data have been collected using a specific CRF. All data were registered in an anonymous manner. The BCR-ABL ratios in the IS have been provided by standardized labs in Spain.
Results: From June 2014 to Februay 2017, 976 patients were registered in the study. Median age was 51 years (15-88). The Sokal risk groups were as follows: 345 patients low risk, 307 intermediate risk and 129 high risk. Eutos classification yielded 714 patients in the low risk and 79 in the high risk categories. The majority of patients received first-line treatment with imatinib (626 patients), dasatinib (39 patients) or nilotinib (87 patients). Of note, 5 patients received bosutinib, 1 patient ponatinib and 74 patients were treated with Interferon previous to TKI administration. So far 14 patients have died of non-CML related conditions such as carcinoma (2 patients), ischemic heart disease, respiratory failure and sepsis. Interestingly, 2 patients developed progression of their CML to accelerated phase and blast crisis (1 patient each) with no deaths. At present, 104 patients (11%) have achieved a MR4.0, 174 patients (18%) a MR4.5 and 123 patients (13%) have obtained a complete molecular remission (undetectable bcr-abl transcripts with a sensitivity of at least 10^{-5}).
Summary/Conclusions: Almost one thousand CML patients have been included in this spanish prospective study owing to their promising molecular response that would predict for a sustained deep molecular remission. Four hundred and one patients have already achieved a deep molecular response (>MR4 (IS)) and could be enrolled in prospective discontinuation studies.

E1060

ANALYSIS OF DASATINIB AND IMATINIB 5-YEAR EFFICACY AND SAFETY BASED ON BASELINE COMORBIDITY AND AGE IN PATIENTS WITH NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP) IN DASISION

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Background: Patients with CML often have comorbidities, which may influence treatment-related decisions and impact response and survival. In a retrospective analysis of 1-year data from the phase 3 DASISION study, the overall safety or response in dasatinib- or imatinib-treated patients was not substantially impacted by baseline comorbidities, although certain adverse events (AEs) trended higher in patients with ≥1 *vs* 0 comorbidities (Khoury ASH 2010). Further analysis is warranted to determine how comorbidities may impact long-term outcomes.
Aims: To evaluate the impact of baseline comorbidities and patient age on 5-year safety and efficacy in dasatinib- or imatinib-treated patients from DASISION.
Methods: In DASISION (NCT00481247), patients were randomized to receive dasatinib 100mg/day (N=259) or imatinib 400mg/day (N=260). For this retrospective analysis, patients were grouped as having 0 or ≥1 baseline comorbidity, by baseline disorder (diabetes mellitus, hepatobiliary disease, hyperlipidemia, cardiovascular disorder, or pulmonary condition), or by age group (<46 years, 46–65 years, >65 years). Safety (treatment-related AEs in ≥10% of patients) and efficacy (response rates by 5 years and median times to response) were assessed for each group and treatment.

Table 1.

	DASATINIB				IMATINIB			
	Comorbidities							
	≥1 (n = 192)		0 (n = 66)		≥1 (n = 192)		0 (n = 66)	
Median average dose, mg/day	99		100		400		400	
Discontinuation, %	39		38		36		39	
Responses	≥1 (n = 193)		0 (n = 66)		≥1 (n = 193)		0 (n = 67)	
Response by 5 years, n (%)								
CCyR	171 (89)		57 (86)		165 (85)		53 (79)	
MMR	148 (77)		49 (74)		130 (67)		36 (54)	
$MR^{4.5}$	88 (46)		21 (32)		70 (36)		15 (22)	
Median time to response, months								
CCyR	3		3		6		6	
MMR	9		9		15		20	
$MR^{4.5}$	35		36		47		42	
AEs	≥1 (n = 192)		0 (n = 66)		≥1 (n = 192)		0 (n = 66)	
AE, n (%)	All grade	Grade 3/4	All grade	Grade 3/4	All grade	Grade 3/4	All grade	Grade 3/4
Pleural effusion	64 (33)	5 (3)	9 (14)	2 (3)	2 (1)	0	0	0
Rash	27 (14)	0	3 (4)	0	25 (13)	3 (2)	12 (18)	0
Peripheral edema	19 (10)	0	2 (3)	0	21 (11)	1 (1)	5 (8)	0
Muscle spasms	14 (7)	0	0	0	46 (24)	1 (1)	9 (14)	0
Face edema	12 (6)	0	1 (1)	0	24 (13)	0	2 (3)	0
Eyelid edema	5 (3)	0	1 (1)	0	34 (18)	0	4 (6)	0

CCyR=complete cytogenetic response; MMR=major molecular response (*BCR-ABL1* ≤0.1% International Scale [IS]); $MR^{4.5}$=*BCR-ABL1* ≤0.0032% IS.

Results: The number of patients with 0 or ≥1 comorbidity was similar in the dasatinib (66 [25%]; 193 [75%]) and imatinib (67 [26%]; 193 [74%]) arms, respectively; most (>90%) patients were <65 years old. In patients with 0 or ≥1 baseline comorbidity, the median average daily dose was comparable within arms and discontinuation rates (36%–39%) were similar within and across arms (table). The overall safety profiles were comparable in the 0 and ≥1 comorbidity groups in both arms, other than specific AEs, which had a ≥2 times higher frequency in patients with ≥1 *vs* 0 comorbidities; the majority of these were grade 1/2 AEs (table). The incidence of peripheral edema increased with patient age for both imatinib and dasatinib (<46 years: 5% each; 46–65 years: 12% and 10%; ≥65 years: 21% and 20%). In this analysis, the increased incidence of pleural effusion (PE) in dasatinib-treated patients was most highly associated with increased age: <46 years (16%) *vs* 46–65 years (37%) *vs* >65 years (60%). PE incidence did not appear to be related to baseline pulmonary comorbidity and was similar in dasatinib-treated smokers (33%) *vs* nonsmokers (27%). Within each arm, patients with 0 or ≥1 comorbidity (table) and across age groups had similar response by 5 years, although rates were numerically higher for patients with ≥1 *vs* 0 comorbidities in both arms ($MR^{4.5}$ on dasatinib: 46% *vs* 32%; $MR^{4.5}$on imatinib: 36% *vs* 22%). Median time to response (months) for patients with 0 or ≥1 comorbidity did not differ within each arm, but was numerically shorter for dasatinib ($MR^{4.5}$; 36 or 35) *vs* imatinib ($MR^{4.5}$; 42 or 47).
Summary/Conclusions: The superior efficacy of dasatinib over imatinib was shown in previous studies. Response rates and times to response were comparable in patients with 0 or ≥1 comorbidity and trended in favor of dasatinib *vs* imatinib. Although a few AEs (most grade 1/2) appeared to occur at a higher frequency in patients with ≥1 *vs* 0 comorbidities in either treatment arm, the overall incidence of AEs and discontinuation rates at 5 years in patients who were treated with first-line dasatinib or imatinib did not appear to be substantially affected by baseline comorbidities.

E1061

ADHERENCE TO SECOND LINE THERAPY WITH NILOTINIB AND QUALITY OF LIFE OF PATIENTS WITH CHRONIC MYELOID LEUKEMIA: A MULTICENTER PROSPECTIVE OBSERVATIONAL STUDY

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Background: Introduction of second-generation TKIs (2GTKIs) provided additional options to treat CML patients effectively. Patient compliance is crucial to achieve good outcomes of TKI therapy. The recommended dose of nilotinib – potent 2GTKI in a second-line treatment is 400mg twice daily. The drug's administration might be more challenging for patients as compared to other TKIs. Nilotinib should be taken twice daily approximately 12 hours apart, and must not be taken with food. No food should be consumed two hours before and at least one hour after the drug is administered. Recent studies comparing adherence to the second-line CML treatment with nilotinib and dasatinib reported conflicting results. It has been reported that the therapy with TKIs might have an adverse effect on the quality-of-life (QOL). To date the majority of the research on QOL among patients treated with TKIs has been focused on imatinib.
Aims: The aim of this study was to assess the adherence to nilotinib used as a second line therapy and to evaluate the quality-of-life (QOL) in patients with chronic myeloid leukemia in a chronic phase (CML-CP), as well as to analyze the correlation between QOL and drug compliance, the correlation between patient's and physician's assessment of drug compliance and to evaluate the relationship between drug compliance and dosing schedule (twice daily, once daily), patient's age, educational and marital status, satisfaction with medical care and the QOL.
Methods: The study was designed as a multicenter prospective observational trial. The enrollment period lasted from June 2010 to June 2012. The duration of the observation of an individual patient was 12 months. 177 patients were recruited in 23 centers in Poland and evaluated during the study at six time points. Nilotinib is not reimbursed in Poland as a first-line therapy. Therefore, eligible were patients suffering from CML-CP, treated with nilotinib as a second line therapy due to the ineffectiveness or intolerance of first line therapy. The adherence to the therapy was assessed using the 4-item Morisky Medication Adherence Scale (MMAS) and reported by patients and their physicians at 1, 3, 6, 9 months and at the completion of the observation. The QOL was evaluated with the standard Core Quality of Life (EORTC QLQ-C30) questionnaire. Basic descriptive statistics were used to present results of the study.
Results: 83.2% of patients assessed themselves as highly compliant at their first visit and 93.4% at the 5th visit. Males were less compliant to nilotinib than females. Patients who live with families were more compliant than those who live with a partner or live alone. Low compliant patients represented 1.7% of total during visit 1; none of the patients assessed themselves as low compliant since the 4th visit. At the first visit 85.3% of patients were categorized by their physicians as highly compliant and 96.0% during the last three visits. Patients and physicians assessments were significantly correlated. No significant differences in drug compliance in patients treated once daily *vs* twice daily were found in all groups of patients. The average QOL expressed as QL2 parameter in patients that have completed the study was significantly higher during the last visit (69.4±17.4) than at the start of the study (59.1±18.8; p<0.001).
Summary/Conclusions: The adherence to the treatment was high and the QOL among patients on nilotinib administered as a second-line therapy was very good. Both have been improved during the study. The efficacy and safety of the drug were confirmed in the real-life setting.

E1062

RADOTINIB TREATMENT IN CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS WITH RESISTANCE OR INTOLERANCE TO BCR-ABL1 TKIS: 36 MONTHS UPDATE OF RADOTINIB PHASE 2 STUDY

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Background: Radotinib is an orally active, selective BCR-ABL1 tyrosine kinase inhibitor (TKI), approved for the first-line and second-line treatment of chronic phase chronic myeloid leukemia (CP-CML) patients in Korea. Earlier 12 and 24 month results demonstrated that radotinib is effective and well tolerated in CP-CML patients with resistance and/or intolerance to BCR-ABL1 TKIs.
Aims: We update the long-term outcome of radotinib treatment in patients failed to BCR-ABL1 TKIs with a minimum follow-up of 36 months.
Methods: Ph^+ CP-CML patients who failed prior TKI therapy were enrolled between July 2009 and November 2011. All patients were treated with radotinib 400mg twice daily. Cytogenetic and molecular assays were performed at baseline, every 3 months, and at treatment failure. Safety parameters were also analyzed. Probabilities of overall survival (OS) and progression free survival (PFS) were calculated using Kaplan-Meier method.
Results: A total of 77 CP-CML patients (18 years of age or over) were enrolled. This analysis includes data from last enrolled patient who received at least 36 months of radotinib therapy. With a median follow-up of 45.7 (range 0.9-65.7) months, 31 patients (40.3%) completed 36 months treatment, and 46 patients (59.7%) discontinued the treatment before 36 months. Main reasons of discontinuation were abnormal laboratory test (n=18), adverse events (n=4), treatment failure including disease progression and lack of response (n=18), death (n=2), and other reasons (n=4). Median duration of radotinib exposure was 19.5 (0.3-60.9) months. Cumulative incidence of complete cytogenetic response (CCyR) by 36 months was 90.0% and of patients achieving CCyR, 45.0% (18/40) achieved MMR. The drug-related safety profiles were consistent with those previously reported and new safety issues have not been observed after 12 months. Most drug-related AEs have developed within 12 months, and have shown minimal increase compared with rates at 12 months follow-up. Estimated OS and PFS at 36 months were 87.6% and 85.7%, respectively.

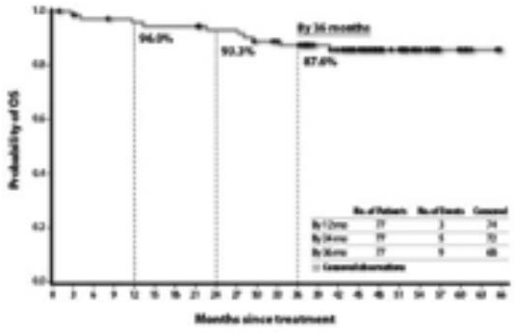

Figure 1.

Summary/Conclusions: The 36 months data supports radotinib treatment in TKI failed CML-CP patients maintains the effective response and high rates of OS & PFS rate. Thus, radotinib demonstrated a promising alternative treatment for patients with TKIs failure.

E1063

100 YEARS OF CHRONIC MYELOID LEUKEMIA PREVALENCE IN FRANCE

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Background: The success of imatinib and other rationally designed tyrosine kinase inhibitors (TKI) in the treatment of chronic myeloid leukemia has drastically improved the fate of CML patients. From a couple of years in median survival in the 70's, a majority of patient now achieve a near normal life expectancy with lifelong treatment though. This new paradigm where CML can be regarded as a chronic disease has mechanical consequences on the disease prevalence and overall costs for health care systems. The success of imatinib and other rationally designed tyrosine kinase inhibitors (TKI) in the treatment of chronic myeloid leukemia has drastically improved the fate of CML patients. From a couple of years in median survival in the 70's, a majority of patient now achieve a near normal life expectancy with lifelong treatment though. This new paradigm where CML can be regarded as a chronic disease has mechanical consequences on the disease prevalence and overall costs for health care systems.
Aims: We present here a fully detailed and comprehensive analysis of the French CML prevalence over a century from 1960 to 2060.
Methods: Prevalence of CML was estimated using a cohort component-based model fromestimates of incidence rate of the disease by age and sex, population and demo-graphic projection from the National Institute of statistics and Economics Studies, and various hypotheses on the relative survival of CML patients. Prevalence of CML was estimated using a cohort component-based model fromestimates of incidence rate of the disease by age and sex, population and demo-graphic projection from the National Institute of statistics and Economics Studies, and various hypotheses on the relative survival of CML patients. The number of CML patients is estimated over time and the resulting CML prevalence expressed as a number of CML patients per 100,000 inhabitants.
Results: The CML prevalence in France, expressed in cases per 100,000 inhabitants, was estimated to be around 3 before the 80's, 6 before the 2002, 17 in 2016, 25 in 2030 where the tendency inflects, and 30 after 2040. Considering the 100% relative survival hypothesis, a target CML prevalence were defined, the level of which will be nearly reached by 2060. By simulation, we showed that given constant incidence rates and high relative survival hypotheses, the CML prevalence will be driven by population aging, and that the target prevalence, defined as the maximum CML prevalence, should be nearly reached by 2050 to levels above 30 per 100,000 in-habitants. By simulation, we showed that given constant incidencerates and high relative survival hypotheses, the CML prevalence will be driven bypopulation aging, and that the target prevalence, defined as the maximum CMLprevalence, should be nearly reached by 2050 to levels above 30 per 100,000 inhabitants.

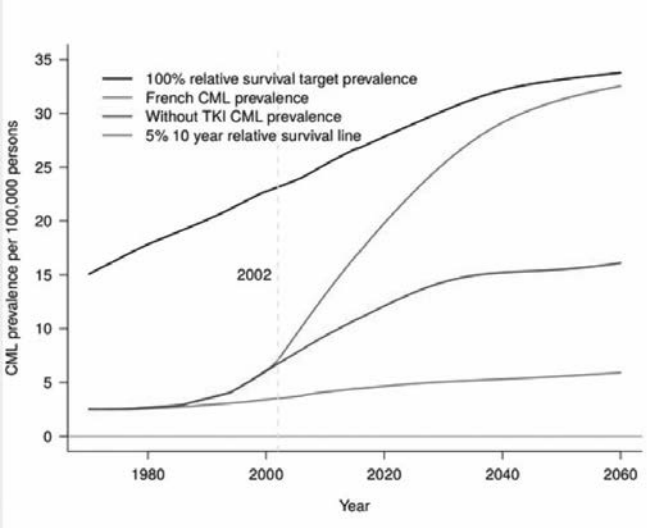

Figure 1.

Summary/Conclusions: Due to high rates of relative survival observed after introduction of imatinib, the trajectory of the CML prevalence in France, as in other western countries, has changed. Given particular hypothesis on the CML incidence rates, this trajectory will bring the CML prevalence by the mid century to levels fully determined by population aging. For France, we have estimated this level above 30 cases per 100,000 inhabitant. Due to high rates of relative survival observed after introduction ofimatinib, the trajectory of the CML prevalence in France, as in other westerncountries, has changed. Given particular hypothesis on the CML incidence rates,this trajectory will bring the CML prevalence by the mid century to levels fullydetermined by population aging. For France, we have estimated this level above30 cases per 100,000 inhabitant.

E1064

THE ROLE OF MICRORNAS IN CHRONIC MYELOID LEUKEMIA THERAPEUTIC SELECTION

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Background: Chronic myeloid leukemia (CML) is characterized by the presence of *BCR-ABL* fusion gene. This molecular event becomes the main therapeutic target with Imatinib as first-line treatment. In spite of the continued clinical success of Imatinib on CML treatment, the emergence of resistance to tyrosine kinase inhibitors (TKIs) had stimulated the research of the mechanisms involved. These included those related with target changing (e.g. the presence BCR-ABL gene mutations and amplifications) and with intracellular drug concentrations (e.g. the abnormal levels of influx and efflux transporters such as OCT1/OCNT2 and PgP/BCRP, respectively). MicroRNAs (miRNA) are important regulators of both mechanisms, and so, could influence TKIs response.
Aims: In this context, we investigated the role of miR-203, miR-21, miR-519c, miR-451 and miR-26 expression levels in TKI response in CML patients, and correlated them with TKI sensitivity, BCR-ABL levels, and disease progression, among other clinical and laboratory data.
Methods: To this end, we assessed the expression levels of miR-203, miR-21, miR-519c, miR-451, miR-26 and miR-16 (endogenous control) by TaqMan MicroRNA Assays in peripheral blood cells from 31 patients with CML at follow-up examinations. We also studied 4 CML cell lines, K562 a cell line sensitive to Imatinib, LAMA-84 a cell line with 4 copies of chromosome Philadelphia (Ph), and 2 Imatinib resistant cell lines models created in our lab (K562-RC and K562-RD). K562-RC cells, generated by continuous exposure to Imatinib, present an IC50 8x times higher than the parental cell line (K562); in K56-RD cells (created by discontinuous exposure), the degree of resistance is 18x. Statistical analysis was performed with ANOVA and multiple comparison tests, with significance levels of 95% ($p<0.05$).
Results: The miR-203 and miR-519c expression was not detected in any cell line or CML patient. First, we correlated miRs expression with *BCR-ABL* levels. Higher levels of tumor suppressor miR-451 were associated with a higher reduction of *BCR-ABL* levels (lower than 0.01%) in CML patients and patients with higher *BCR-ABL* present lower levels of expression of miR-451. This miR was also down-regulated in LAMA-84, K562-RC and K562-RD comparing with sensitive cell line (K562; $p<0.05$). On the other hand, patients with more BCR-ABL content (between 1.0% and 0.1%) present higher expression of the oncomiRs, mir-21 and miR-26. These miRs were also up-regulated in resistant cell lines. MiR-21 was more relevant for K562-RC cells (4-fold higher than K562). LAMA-84 and K562-RD cell lines showed almost 2 times more expression of miR-26. Next we analyzed if treatment options affected miRs expression. CML patients under Imatinib treatment showed higher levels of miR-451 associated with less expression levels of miR-21 and miR-26. Imatinib had been described to be able to block the BCR-ABL negative feedback on miR-451, increasing miR function. Since miR-21 and miR-26 were also lower expressed, more PTEN is available to block PI3K-AKT-mTOR pathways, decreasing this survival signaling. Opposite profile was observed in patients that changed treatment to a second generation TKI suggesting a different effect of this TKI on microRNA expression.
Summary/Conclusions: Our preliminary results suggested the involvement of miRNAs in *BCR-ABL* levels regulation and in TKI response, supporting the search of a miRNAs TKI response profile that could predicts the response in CML patients. This information could act as powerful tool for the stratification and selection of the best therapeutic approach (lower toxicity and cost effective), contributing to higher survival rates and better quality of life in CML patients.
Work supported by the Faculty of Medicine of the University of Coimbra and Santander Totta Bank, grant reference FMUC-BST-2016-214.

E1065

IMPACT OF ABCB1 AND ABCG2 POLYMORPHISMS ON RESPONSE TO IMATINIB AND 2G-TKIS THERAPY IN PATIENTS WITH CHRONIC PHASE CML

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Background: Overexpression of multidrug resistance proteins ABCB1 and ABCG2 confers resistance to anticancer drugs, including tyrosine kinase inhibitors (TKIs). Various ABCB1 and ABCG2 single nucleotide polymorphisms

(SNPs) affect the transporter activity, but their impact on clinical response to imatinib in chronic myeloid leukemia (CML) is discordant; even less is known on their role in patients treated with second generation (2G) TKIs dasatinib and nilotinib.

Aims: To investigate the role of the most common ABCB1 and ABCG2 genetic polymorphism in chronic phase CML patients treated with imatinib and 2G-TKIs.

Methods: We analysed four polymorphisms of ABCB1 (129T>C, 1236C>T, 2677G>T/A and 3435C>T) and two polymorphisms of ABCG2 (34G>A and 421C>A) in 196 CP-CML patients, of whom 139 treated with imatinib (114 in first line and 25 after interferon failure) and 57 treated with dasatinib or nilotinib (22 in first line and 35 after imatinib failure), We compared the rates of optimal response at 3 months (defined as BCR/ABL <10%), at 6 months (BCR/ABL<1%) and at 12 months (BCR/ABL<0.1%), progression-free survival (PFS) and time to treatment failure (TTF) according to the different protein genotypes. TTF was calculated from the start of therapy to any of the followings: progression to accelerated or blastic phase (ABP), death for any cause at any time, treatment discontinuation for primary or secondary resistance or intolerance. PFS was calculated from the start of TKI to ABP or death.

Results: A total of 196 patients with CP-CML (median age 57 years, range 21-84) were included in the analysis. Frequency of ABCB1 and ABCG2 SNPs expression is summarized in Table 1. Considering response to therapy, either in imatinib-treated patients and in those receiving a 2G-TKI, we did not find any significant difference in terms of optimal response at the various timepoints, TTF or PFS for ABCB1 C1236T, G2677T and C3435T and of ABCG2 G34A and C412A polymorphism, even if there was a trend for a worse PFS in the few patients (n=3) with 1236 allele A treated with imatinib. Conversely, we found a lower rate of optimal response at 3 (p=0.1), 6 (P=0.05) and 12 (p=0.2) months in imatinib-treated patients with TC genotype of ABCB1 T129 SNP, though the small number of patients (7) had probably and impact on statistical significance. However, TTF was shorter for ABCB1 129T>C patients, both receiving imatinib (P=0.05) and 2G-TKIs (P=0.07), and also PFS was significantly shorter in this cohort (P=0.003).

Table 1.

MDR protein	SNP	Genotype	Frequency
ABCB1	C1236T	CC	32%
		CT	49%
		TT	19%
	G2677T	GG	33%
		CT	46%
		TT	18%
		A	3%
	C3435T	CC	26%
		CT	50%
		TT	24%
	T129C	TT	94%
		TC	6%
		CC	-
ABCG2	G34A	GG	92%
		GA	8%
		AA	-
	C421A	CC	83%
		CA	15%
		AA	2%

Summary/Conclusions: With the limits of the low expression rates of some SNPs, our data suggest a lower response in patients harboring 129T>C polymorphism, at least in those receiving imatinib. Other ABCB1 and ABCG2 genotypes do not seem to impact on response to TKI treatment.

E1066

THE INTRODUCTION OF SECOND-GENERATION TYROSINE KINASE INHIBITORS MAY REDUCE THE PROGNOSTIC IMPACT OF HIGH-RISK PATIENTS ACCORDING TO EUROPEAN TREATMENT AND OUTCOME STUDY (EUTOS) SCORE

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Background: The discovery of tyrosine kinase inhibitor (TKI) imatinib has revolutionized the conception of chronic myeloid leukemia (CML) as a mortal disease to a long-term controllable disease. The European Treatment and Outcome Study (EUTOS) score is a clinical tool that utilizes imatinib-based objectives to predict treatment response and progression free survival (PFS) in patients with CML in chronic phase (CP). However, it is currently unknown whether the introduction of second generation TKIs (2nd TKIs) affects prognostic score of patients with CML-CP, particularly among those considered high-risk according to EUTOS score.

Aims: Our study aims to highlight the critical role of the introduction of 2nd TKIs on patient prognosis as determined by EUTOS score.

Methods: Patients data was obtained retrospectively from patients enrolled in the CML Cooperative Study Group. Patients with CML-CP who were treated with any TKIs as first line therapy between April 2001 and January 2016 were enrolled to the study and were classified according to date of diagnosis. Those who were diagnosed with CML-CP before March 2009 were classified into the imatinib group, and those diagnosed after April 2009 were classified into the 2nd TKI group, as these patients were able to be treated with 2nd TKIs. The study was approved by the research ethics boards of each institution and was conducted in accordance with the Declaration of Helsinki.

Results: There were 308 patients newly diagnosed with CML-CP during the study period. Of these patients, 104 (34%) were assigned to the imatinib group and 204 (67%) were assigned to the 2nd TKI group. With respect to EUTOS score, 223 patients were classified as low-risk, of which 69 were in the imatinib group and 154 were in the 2nd TKI group. Forty-six patients were considered high-risk, of which 19 were in the imatinib group and 27 were in the 2nd TKI group. EUTOS score was unavailable in 39 patients. With regard to initial TKI, all patients were treated with imatinib in the imatinib group. Among patients assigned to the 2nd TKI group, 149 (73%) were initially treated with any TKI. The median follow-up period for all patients was 48 months (range: 1–185 months). Among patients in the 2nd TKI group, CML-associated death rates were significantly lower than those in the imatinib group. EUTOS high-risk patients score exhibited significantly worse outcomes in EFS, PFS, and CML-associated death compared to those considered low-risk. Most importantly, risk stratification by EUTOS score was predictive of risk-associated clinical outcomes in patients assigned to the imatinib group; however, EUTOS score failed to predict risk-associated clinical outcomes of patients assigned to the 2nd TKI group (see Figure). The EUTOS high-risk patients in the imatinib group showed worse clinical outcomes than those in the 2nd TKI group (hazard ratio [HR] 6.35, 95% confidence interval [CI] 1.79 – 22.6, *p*=0.0042). However, prognostic effect was less in the 2nd TKI group (HR 3.21, 95% CI 0.59 – 17.6, *p*=0.18). Out of 308 patients, 9 progressed to AP/BP, of which 8 transformed during imatinib therapy and 1 transformed during dasatinib therapy.

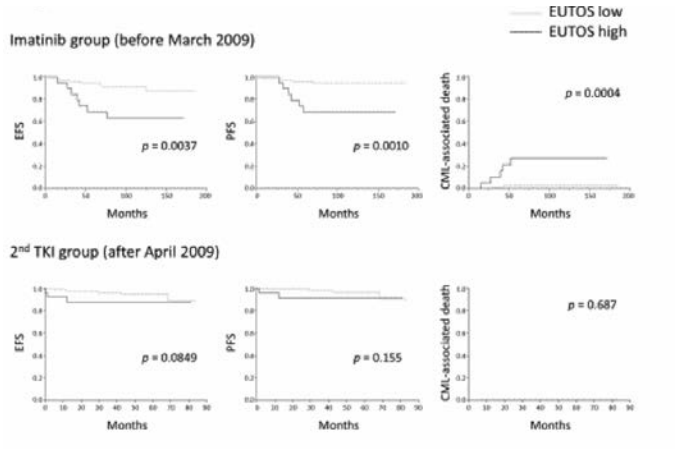

Figure 1.

Summary/Conclusions: Among patients assigned to the imatinib group, risk stratification by EUTOS score was predictive of clinical outcomes in that those considered high-risk experienced considerably more adverse events (EFS, PFS, or CML-associated death) than those considered low-risk. Our results support the use of 2nd TKIs in treating high-risk patients with CML-CP in order to avoid disease progression. Future large-scale studies are necessary to evaluate the clinical significance of EUTOS scoring in the accurate prediction of prognosis among patients with CML-CP treated with 2nd TKIs.

E1067

CHRONIC MYELOID LEUKEMIA DIAGNOSED DURING PREGNANCYI: THERAPY TACTICS AND OUTCOMES

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Background: Chronic myeloid leukemia (CML) diagnosed at pregnancy is a serious challenge. Treatment by tyrosine kinase inhibitors (TKI) today is considered harmful for fetus due to possible teratogenicity. On the other hand TKI delay is dangerous for disease progression as no other options have comparable to TKI effectiveness. Pregnancy termination by abortion may be crucial for desired pregnancies as further childbirth is postponed for years until stable deep molecular response (DMR). Due to limited number of cases and ethical issues there is no consensus on how to behave in such delicate cases.
Aims: To describe pregnancy outcomes and therapy tactics for CML diagnosed at pregnancy.
Methods: Information regarding CML diagnosed at pregnancy was collected within the database of countries participating in the observational study of European LeukemiaNet (ELN Pregnancy Registry). The data included CML clinical characteristics at diagnosis, cytogenetic and molecular parameters, information of therapy, pregnancy outcomes and data of newborns.

Table 1.

Table 1. Number of pregnancy cases in CML by countries and outcomes of pregnancies diagnosed simultaneously with CML

Country	number of CML females with pregnancies in database	number of pregnancy cases (including subsequent) in CML females	number of CML females diagnosed at pregnancy	Outcomes of 31 pregnancies diagnosed simultaneously with CML			
				Induced abortion	Spontaneu os abortion	Labor	Ongoing pregnancy
Russian Federation	98	135	21	7	1	11	2
Italy	ND*	100	2	2	-	-	-
Uzbekistan	16	19	3	-	-	3	-
South Korea	9	12	1	-	-	1	-
Ukraine	8	13	4	1	-	3	-
Armenia	2	3	-	-	-	-	-
Total number	133 (not counting Italian)	282	31	10	1	18	2

*the data are in progress of database input

Results: Thirty one women with median age 26 years (range 20-39) were diagnosed with Ph-positive chronic phase CML during pregnancy. That was 11% of all 282 pregnancy cases. In certain countries (Russia) up to 21% of women with CML and pregnancy had the synchronistic onset of these events (table 1). Sokal low/intermediate/high and EUTOS low/high risk score was in 22/5/3 and 28/2 patients correspondingly, no data for risk score was in 1 patient. CML was diagnosed in 1st/2nd/3rd trimester in 18/7/6 females correspondingly. Induced abortion was done before CML therapy was started in 10 (32%) around 10 obstetrician week (range 6-17), spontaneous abortion happened in 1 woman (at week 8th). Pregnancy was prolonged by consistent desire or religious reasons in 20 females: 2 pregnancies are ongoing now and 18 have ended in childbirth. No treatment till labor was in 5 women. Treatment of CML during pregnancy was initiated in 15 of 20 patients. Interferon alpha (IFN) was given to 5 women and 3 of them were switched to imatinib (IM) 400mg daily in 2nd-3rd trimester due to insufficient control of complete blood count. IM 400mg daily in 1st line was also taken by 9 women since 2nd-3rd trimester. In summary 12 women got IM from Me 22nd week (range 16-36). One woman got hydroxyurea (HU) from 3rd trimester till labor and 3 got HU shortly for 5-7 days before any other treatment in 1st-2nd trimester. Nineteen healthy newborns (including twins in 1 case) were born: 14 at term, 5 by preterm delivery at week 35-37. There were no birth abnormalities in 10 newborns exposured to IM in 2nd-3rd trimester. Two pregnancies under IM exposure are currently at week 17 and 31, developing normally. Four infants had low birth weight (<2500 g): 3 of them were exposed to IM and 1 for HU at pregnancy. Follow-up of children was uneventful with Me observation time 47 months (range 9-192) and Me observation time 38 months (range 9-63) for 10 infants exposed to IM in 2nd-3rd trimester. Twenty nine of 31 women with CML diagnosed at pregnancy are alive and continue TKI treatment, 2 women unfortunately died: 1 patient with postponed switch from IFN to IM during pregnancy progressed to blast crisis after labor and had furher bone marrow transplant failure while 1 patient after induced abortion developed rapid blast crisis with *BCR-ABL* compound mutations including T315I.
Summary/Conclusions: This is the first report of a large database of women diagnosed with CML during pregnancy. Management of this very delicate subset of patients is a challenge especially when a woman refuses from abortion. Individual treatment approach may differ considering pregnancy terms and clinical status. Although normal childbirth is possible using IM after 2nd-3rd trimester, the risks of pregnancy prolongation remain still not well defined. To get the most safe prognosis for mother and child pregnancy in CML should be planned in a stable DMR.

E1068

IMPACT OF KIR3DL1*00501 IN TYROSINE KINASE INHIBITOR-TREATED CML

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Background: The BCR-ABL1 tyrosine kinase inhibitors (TKIs) dramatically improved long-term survival of the patients with chronic myeloid leukemia (CML). As increased NK cells during TKI therapy positively correlate with better outcome, antitumor immunity by NK cells may contribute to the effects of TKIs. However, the response to TKIs depends on each case, and the determinants of it remain to be elucidated.
Aims: Given that NK cell function is regulated depending on the interaction between killer immunoglobulin-like receptor (KIR) and human leukocyte antigen (HLA) class I molecules, we hypothesized that polymorphisms of KIR and HLA play important roles on the responses to TKIs. Then we performed allele genotyping of KIR and HLA with deep sequencing in CML patients, and here report their clinical impacts.
Methods: KIR and HLA high resolution typing were performed on peripheral blood DNA from 76 CML patients in chronic phase (CML-CP) using Scisco Genetics typing kit (Scisco Genetics Inc., Seattle WA) and MiSeq as platform by NGS. Therapeutic effects of TKIs were evaluated based on *bcr-abl* mRNA levels measured by real-time quantitative (RQ)-PCR compensated according to international scale (IS) and/or transcription mediated amplification (TMA) method. Major molecular response (MMR) was defined as 3-log reduction (MR^3) in RQ-PCR (IS) or BCR-ABL transcript level of less than 50 copy/0.5 μg RNA in TMA method. We also defined DMR as 4-log reduction ($MR^{4.0}$) in RQ-PCR (IS), which is similar to undetectable of BCR-ABL transcript level in TMA method. The Cox proportional hazards model was used in the time-to-event analysis. A p value<0.05 was considered statistically significant.
Results: Second generation TKIs as first-line therapy (n=46) and female (n=29) sex were strongly associated with superior DMR at the 2-year of therapy (second generation TKIs as first-line treatment, HR 7.305, 95% CI, 3.377 to 15.803; p<0.001; female sex, HR, 1.709; 95% CI, 1.028 to 2.842; p=0.039). After adjustment with these two factors, several KIR alleles positively correlates with superior DMR at the 2-year; KIR2DL4*008 or 011/00501 (HR 1.942, 95%CI 1.160 to 3.250, p=0.012); KIR2DS4*00301 or 007/010 or 015 (HR 2.811, 95% CI, 1.590 to 4.968, p<0.001); KIR3DL1*00501 (HR 3.634, 95% CI 1.884 to 7.013, p<0.001). Interestingly, KIR3DL1*00501 for the patients has more strong linkage to KIR2DL4*008 or 011/00501, and 2DS4*00301 or 007/010 or 015 than other KIR3DL1 alleles. (Fisher's exact test, p<0.001).
Summary/Conclusions: KIR3DL1*00501 and several KIR2DL4 and 2DS4 alleles positively correlate with better therapeutic effects of TKIs, and they may be form the same KIR haplotype. Our data indicate that these KIR alleles represent strong anti-CML immunity by NK cells, and consequently may associate with long-term outcome and treatment-free remission in CML.

E1069

COMPARISON OF MOLECULAR KINETICS AFTER THE FIRST AND SECOND IMATINIB DISCONTINUATION: RESULTS FROM THE KID STUDY

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Background: Recent reports have demonstrated that tyrosine kinase inhibitors (TKIs) discontinuation can be employed in chronic phase chronic myeloid leukemia (CP CML) patients with sustained deep molecular responses after enough TKI therapy. Consequently, treatment-free remission (TFR) has been a new therapeutic goal. Although 50-70% of patients experienced molecular relapse by several imatinib (IM) discontinuation studies, the most of patients resumed molecular responses (MR) following restart of IM.
Aims: Though the Korean multicenter prospective study (Korean Imatinib Discontinuation Study; KID Study), we have explored molecular kinetics after the first IM discontinuation and after IM resumption for molecular relapse. In patients regaining durable UMRD with IM resumption, we tried second IM discontinuation and compared molecular kinetics between the first IM stop and second IM stop.
Methods: CP CML patients who were treated with IM for more than 3 years and had undetectable levels of BCR-ABL1 transcripts determined by quantitative reverse transcriptase polymerase chain reaction (PCR) for at least 2 years were eligible for KID study and in cases of MMR loss after 2 consecutive assessments, IM treatment was re-introduced. After IM resumption for MMR loss, the molecular response was evaluated every month until MMR was re-achieved and every 3 months thereafter. The second stop was permitted in the patients who were in second UMRD for at least 2 years.
Results: Among patients who lost MMR in 2 consecutive analyses and resumed IM in the KID study, 12 patients (6 men and 6 women) with a median age of 45 years (range, 25-59 years) entered into a second IM discontinuation after maintaining UMRD at least 2 years. Prior to first discontinuation, the median duration of IM therapy was 68.9 months (range, 38.5-115.1 months) and the duration of sustained UMRD was 32.9 months (range, 24.8-64.5 months). After first attempt of IM discontinuation, they relapsed after a median duration of 3.7 months (range, 1.8-13.1 months) and re-achieved UMRD at a median of 6.7 months (range, 3.3-13.6 months) after IM resumption. After sustaining a second UMRD for a median of 25.5 months, IM therapy discontinued for a second time. After a median follow-up of 8.8 months (range, 0.3-38.1 months) after second IM discontinuation, 10/12 patients (83%) and 8/12 patients (67%) lost UMRD and MMR, respectively. Among two patients who lost UMRD but not MMR, one patient showed fluctuation of *BCR-ABL1* transcript under the level of 0.1% on IS for 9.4 months and another patient have shown gradually increasing BCR-ABL1 transcripts under the level of 0.1%. Eight patients who experienced second relapse (MMR loss) after a median 2.9 months (range, 1.9-30.7 months). The patients who lost MMR, except one patient, were retreated with IM for a median of 7.1 months (range, 0.8-24.8 months); five patients re-achieved MMR at a median of 1.8 months (range, 1.0-10.2 months) and one re-achieved UMRD at 5.5 months.
Summary/Conclusions: Our data demonstrated that a second attempt might be possible and the median time to MMR loss after second discontinuation was similar to those of the first discontinuation. But the molecular kinetics after second IM resumption needs longer follow-up with more patients. Further studies on the predictors to select patients for a trial of second TFR and novel strategies such as intermittent therapy will be warranted.

E1070

CLINICAL IMPACT BY 24 MONTHS ACCORDING TO BCR/ABL1 TRANSCRIPT LEVEL AT 3 AND 6 MONTHS IN NEWLY DIAGNOSED CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH RADOTINIB 300MG BID OR IMATINIB

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Background: BCR-ABL1 transcripts ≤1% after 6 months was an effective predictor. Also, radotinib showed that optimal molecular responses at landmark time point 3, 6, and 12 month were significantly higher than imatinib group.
Aims: We evaluated the impact of molecular response by 24 months for molecular level after 3 months (BCR-ABL1 transcript level ≤10%) and 6 months (BCR-ABL1 transcript level ≤1%) of radotinib 300mg twice daily (bid) approved for first-line use in Korea or imatinib treatment in newly diagnosed CML-CP.
Methods: Based on baseline demographics and Sokal risk score, 241 patients were randomized 1:1:1 to radotinib 300mg bid (n=79), radotinib 400mg bid (n=81), or imatinib 400mg once daily (qd) (n=81). 157 patients with available 3 months qRT-PCR on study therapy [radotinib 300mg bid (n=79), and imatinib 400mg qd (n=78)] were evaluated. And, total of 151 patients who received radotinib 300mg bid (n=77), and imatinib 400mg qd (n=74) were evaluable at 6 months. Molecular response was assessed by RQ-PCR at baseline and every 3 months. BCR-ABL1 transcript level was measured by RQ-PCR, standardized according to international scale (BCR-ABL^{IS}). Major molecular response (MMR) was defined by BCR-ABL1/ABL1 ratio ≤0.1%^{IS} and MR$^{4.5}$ was defined as ≥4.5 log reduction in BCR-ABL1 transcript levels from standardized baseline or BCR-ABL1/ABL1 ratio ≤0.0032%^{IS}.
Results: In two study groups, early molecular response (EMR) at 3 months were observed in 86.1% of patients in the radotinib 300mg bid group and 67.9% in the imatinib group *(P=0.0179)*. More patients treated with radotinib 300mg bid who had EMR at 3 months achieved MMR and MR$^{4.5}$ by 24 months: 73.5% and 38.2% in the radotinib 300mg bid group and 63.6% and 29.1% in the imatinib group, respectively. At 6 months, 73.4% of patients in the radotinib 300mg and 53.1% patients in imatinib group (*P=0.0246*) achieved 6 months EMR. The patients who had EMR at 6 months in radotinib 300mg bid group were significantly higher MMR and MR$^{4.5}$ compared with the patients who were not achieved EMR (*P<0.0001*), which they achieved MMR and MR$^{4.5}$ by 24 months: 86.2% and 44.8%, respectively and imatinib group achieved 81.4% and 39.5%, respectively. By 24 months, overall survival (OS) and progression-free survival (PFS) according to 3 months or 6 months were not significantly different in two groups.
Summary/Conclusions: With minimum 24 months follow-up, early responses at 3 months or 6 months can predict better outcomes in newly diagnosed chronic myeloid leukemia patients treated with radotinib or imatinib. But, to evaluate the significant long-term prognostic value such as overall survival and progression-free survival by EMR, longer follow-up are needed.

E1071

HYDROXYUREA SUPPRESSES BCR-ABL1 T315I+ CML CLONES *IN VIVO* AND *IN VITRO* AND SYNERGIZES WITH PONATINIB IN ELIMINATING TKI-RESISTANT CML CELLS

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Background: In chronic myeloid leukemia (CML), *BCR-ABL1*^{T315I} leads to resistance against most BCR-ABL1 tyrosine kinase inhibitors (TKI). Long-term therapy with ponatinib, which suppresses BCR-ABL1^{T315I}, is problematic because of side effects. In addition, resistance against ponatinib may develop due to occurrence of compound mutations in *BCR-ABL1*. Therefore, alternative therapies have to be considered. Hydroxyurea (HU) has been used for (palliative) treatment of CML over decades. However, the effects of HU on TKI-resistant sub-clones have not been examined so far.
Aims: The aim of this study was to evaluate the effects of HU on CML subclones carrying *BCR-ABL1*^{T315I} mutations (isolated or in compound-configuration) *in vitro* and *in vivo* and to explore cooperative effects between HU and ponatinib.
Methods: Four *BCR-ABL1*^{T315I}+ CML patients were treated with HU (1-3 g/day) for 2 to 18 months. White blood counts (WBC), differential counts, and *BCR-ABL1* transcript levels were reported. The *BCR-ABL1*^{T315I}/*BCR-ABL1* ratio was determined by mutation-specific, ligation-dependent, PCR and next generation sequencing. In *in vitro* studies, primary CML cells, the CML cell lines K562, KU812, KCL-22, and KCL-22^{T315I} as well as Ba/F3 cells expressing *BCR-ABL1*^{WT} (Ba/F3p210^{WT}), or mutant *BCR-ABL1* (Ba/F3p210^{T315I}, Ba/F3p210$^{T315I/E255K}$, Ba/F3p210$^{T315I/F311L}$, Ba/F3p210$^{T315I/F359V}$, Ba/F3p210$^{T315I/G250E}$) were examined. Cell proliferation was quantified by ^{3}H-thymidine uptake. Apoptosis was determined by flow cytometry. Drug effects on competitive clonal growth were analyzed by mixing Ba/F3p210^{WT} (labeled with GFP) with Ba/F3p210$^{T315I/F359V}$ or Ba/F3p210$^{T315I/E255V}$ (labeled with tdTomato) at a ratio of 1:1. Then, cells were exposed to HU, pontinib, or

HU+ponatinib for 72 hours, and the percentage of viable cells in each sub-clone was analyzed by flow cytometry.
Results: HU treatment resulted in WBC stabilization in 3 of 4 patients, but failed to induce a molecular response. However, surprisingly, the percentage of *BCR-ABL1*^{T315I}decreased significantly in all 4 patients during HU treatment and was no longer detectable in 3 of 4 cases. Stem cell transplantation could be performed in 2 patients after 2-3 months. In one patient, stable disease over 18 months was obtained with HU-therapy. In one patient, the disease progressed rapidly despite temporary suppression of *BCR-ABL1*^{T315I}. In *in vitro* studies, HU was found to block the growth in all cell lines tested and in all primary cell samples (n=7) examined, with IC_{50} values ranging between 50 and 250 μM. Interestingly, cell lines exhibiting mutant *BCR-ABL1* were more sensitive against HU than cell lines expressing *BCR-ABL1*^{WT}. HU and ponatinib were found to synergize in inhibiting growth of all cell lines tested, including cells expressing *BCR-ABL1*^{T315I} or T315I-including compound mutations. Cooperative drug effects were also confirmed in primary CML cells (n=4). In cell mixing experiments, ponatinib was found to supress Ba/F3p210^{WT} cells but not Ba/F3p210$^{T315I/F359V}$ or Ba/F3p210$^{T315I/E255V}$ cells, whereas HU was found to exert stronger effects on cells expressing mutant *BCR-ABL1*, and the drug combination resulted in complete suppression of all sub-clones.
Summary/Conclusions: Our data show that HU exerts strong, sub-clone specific, anti-neoplastic effects in TKI-resistant CML cells *in vivo* and *in vitro*. In addition, HU and ponatinib produce synergistic growth-inhibitory effects on TKI resistant CML cells. Clinical studies are now warranted to define the exact value of the drug combination ponatinib+HU in TKI resistant CML.

E1072

ASSOCIATION OF BCL2L11 (BIM) DELETION POLYMORPHISM WITH MOLECULAR RELAPSE AFTER TYROSINE KINASE INHIBITOR CESSATION IN CHRONIC MYELOID LEUKEMIA PATIENTS WITH DEEP MOLECULAR RESPONSE

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Background: The inhibition of BCR-ABL1 kinase with tyrosine kinase inhibitors (TKIs) has markedly improved the prognosis of chronic myeloid leukemia (CML). Recently, it has been recognized that some CML patients with deep molecular response (DMR) can maintain treatment-free remission (TFR) after TKI cessation. However, no predictive prognostic factor for successful treatment cessation has yet been identified. *BCL2L11 (BIM)* deletion polymorphism (intron 2) has been reported to be associated with an inferior response to TKI (Ng *et al.* Nature Medicine, 2012). We have previously reported that *BIM* deletion polymorphism may predict relapse after TKI discontinuation (Katagiri *et al.* Br J Haematol, 2013).
Aims: To further clarify the role of predictive biomarkers in molecular relapse after TKI cessation, we performed a long-term follow-up of CML patients with DMR after TKI cessation.
Methods: Patients with DMR receiving TKI treatment were included. Molecular relapse was defined as a loss of the major molecular response (MMR). The genomic DNA of patients was obtained from their whole blood samples using the EZ1 DNA Blood 350 l kit (Qiagen, Valencia, USA). Deletion polymorphism was detected by Q-Invader assay using primers designed to detect a deletion site (2,903 bp) (Ohyashiki *et al.* J Hematol Transfus, 2014).
Results: Forty-six CML patients (29 men;17 women; median age, 58.5 years) were included in this study (Sokal category; low: 32, intermediate: 10, and high: 2). Thirty-three patients discontinued imatinib, five discontinued nilotinib, and eight discontinued dasatinib. Ten patients were treated with IFNα before TKI treatment. The median duration from TKI initiation to cessation was 85.0 (range: 22–177) months; the median duration of DMR before TKI cessation was 43.0 (range: 5–131) months. Treatment-free remission was estimated to be 66.5% at 12 months, 61.5% at 24 months, and 58.5% at 36 months. Thirty-six CML patients were analyzed for the presence of *BIM* deletion polymorphism of which six exhibited *BIM* deletion polymorphism. All patients with *BIM* deletion polymorphism relapsed within 12 months after TKI cessation. A significant difference was observed only in *BIM* deletion polymorphism between the patients who maintained and those who lost MMR (p=0.0000528). No patient died during the follow-up period. No significant difference was observed in the second TKI therapy, prior IFNα therapy, and time to DMR between relapsing and non-relapsing patients.
Summary/Conclusions: The analysis of *BIM* deletion polymorphism in CML patients is expected to be useful for predicting their early molecular relapse after TKI treatment discontinuation.

E1073

XPERT® BCR-ABL ULTRA, A HIGH SENSITIVITY ASSAY WITH A LIMIT OF DETECTION REACHING MR4.5 AND BELOW ON AN INTERNATIONAL REPORTING SCALE

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Background: The ability to consistently detect low levels of BCR-ABL transcripts in patients with chronic myeloid leukemia (CML) is important in the assessment of treatment outcomes in patients on tyrosine kinase inhibitor (TKI) therapy. Particularly, BCR-ABL assays that are sensitive in the measurement of deep level response may aid in the identification of potential candidates for treatment discontinuation. Xpert® BCR-ABL Ultra detects the most common BCR-ABL transcripts below MR$^{4.5}$ (Molecular Response at 4.5-log reduction) or 0.0032%, which is widely accepted as the clinical threshold that defines candidates who can safely discontinue TKI therapy.
Aims: The present studies were designed to verify the limit of detection (LoD) for the Xpert® BCR-ABL Ultra assay below MR$^{4.5}$ on the international Scale (*IS*) in clinical samples for both the b3a2 and b2a2 transcripts.
Methods: To overcome the challenge of testing numerous replicates requiring large volumes of patient samples,, serial dilutions ranging from BCR-ABL/ABL levels of 10% to <0.001% (*IS*) were prepared as contrived samples using CML patient blood with initial BCR-ABL level >10% (*IS*) and pooled blood from CML negative patients, ranged from 10% to <0.001% (*IS*). Twenty-one replicates of each dilution were measured for%BCR-ABL/ABL (*IS*). Determination of the LoD was performed by the statistical analysis to identify the lowest concentration of%BCR-ABL/ABL (*IS*) per test that can be reproducibly distinguished from negative samples with 95% confidence. The acceptable precision for%BCR-ABL/ABL (*IS*) is defined as the ability to detect at least a 3-fold difference for all concentrations tested.
In addition, analytical LoD studies were performed using spike-in CML cell lines and cell-line derived RNAs, carrying either b3a2 or b2a2 transcripts. Furthermore, the clinical sensitivity study was conducted using blood from twelve low BCR-ABL transcripts level CML patients on TKI therapy, who had achieved and maintained MMR (Major Molecular Response) [0.1% (*IS*)] with reporting below 0.05% (*IS*).
Results: Consistent results were observed in the both the diluted CML patient blood and spike-in CML cell lines or cell-line derived RNA studies for both the b3a2 and b2a2 transcripts, demonstrating an assay LoD of MR$^{4.5}$ and below with a less than 2-fold difference at the LoD levels. With the clinical sensitivity study, eleven out of twelve low CML subjects were detected in at least 19 out of 20 replicates tested per subject over a range of 0.038% (*IS*) (SD=~0.17 Log) to 0.0011% (*IS*) (SD=~0.4 Log). The overall ABL copy number present in clinical samples in each study was at least 5-10 times the required minimal ABL copy number of ≥32,000 to support a claim of MR4.5 and ≥100,000 for MR5.0.
Summary/Conclusions: These LoD evaluations demonstrate that the Xpert® BCR-ABL Ultra assay complies with the international guidelines for assay sensitivity achieving MR$^{4.5}$ with 5-10 times more than the required ABL copies to confidently identify candidate patients that may benefit from the discontinuation of TKI therapy.

Enzymopathies, membranopathies and other anemias

E1074

IDENTIFICATION OF INCIDENTS CASES OF GAUCHER DISEASE IN SPLENOMEGALY AND/OR THROMBOCYTOPENIA PATIENTS IN SPECIALIZED MEDICAL SERVICES IN COLOMBIA THROUGH THE USE OF A SELECTION ALGORITHM

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Background: Gaucher disease (GD) varies greatly in severity and organ involvement. Clinical characteristics are usually nonspecific and lead to late diagnosis with irreversible complications. Splenomegaly and thrombocytopenia are the two most common manifestations (Gaucher Registry, 2008), which reported 86% cases with moderate to severe splenomegaly and 60% thrombocytopenia at the time of diagnosis, thus demonstrating why patients are referred to hematology. A diagnosis of GD is considered after other diagnostic hypotheses have been ruled out. The consensus of international experts on the management of patients with GD established a diagnostic algorithm that is particularly intended for specialists (Mistry, 2010). Straightforward implementation of diagnostic algorithms to support medical specialties in Latin America for early diagnostic testing of GD is required.
Aims: To identify new cases of GD in a selected population with splenomegaly and/or thrombocytopenia referred to Hematology, Pediatric Hematology, Pediatrics, Genetics and Internal Medicine, using a selection algorithm for the general population (Mistry, 2010).

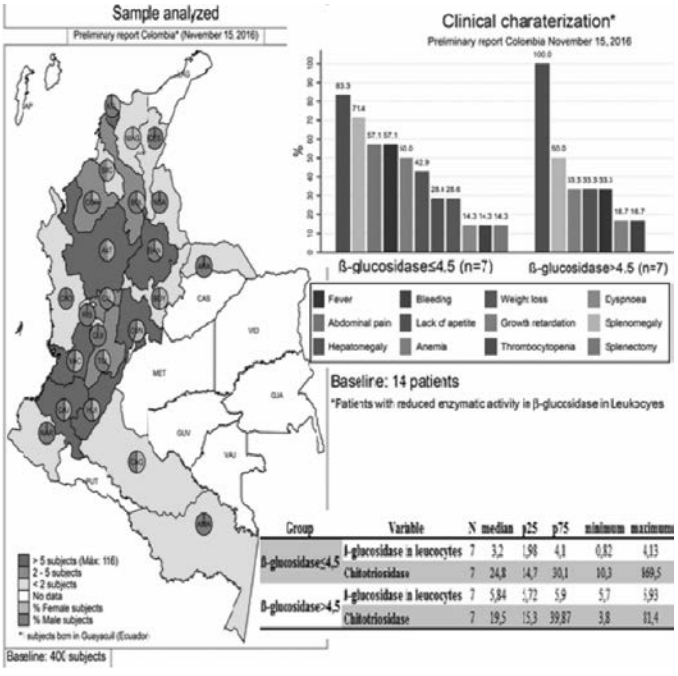

Figure 1.

Methods: Multicenter, descriptive study, in active recruitment process with non-probabilistic sampling by convenience. Currently, the study has 51 specialized medical centers in Hematology, Pediatrics and Internal Medicine in Colombia, approved by Ethics Committee (EC). The study has an expected duration of 24 months since EC approval for each center. Eligible subjects are those with three documented criteria: thrombocytopenia <150,000/cc plus anemia (hemoglobin <12g/dL in men and <11 g/dL in women) plus/or bone pain plus/or Monoclonal Gammapathy of Unknown Significance plus/or Polyclonal Gammapathy in subjects aged 30 years and older; and/or splenomegaly defined as palpable spleen ≥1cm below the costal rib or diagnosed by imaging, and/or Splenectomy by splenomegaly with no known cause. Subjects with prior diagnosis of GD, splenomegaly due to portal hypertension, hematologic malignancy, hemolytic anemia and thalassemia were excluded. Informed consent was obtained for all included subjects. Clinical information was collected from their medical history. The enzymatic activity of the β-glucocerebrosidase was performed in peripheral blood, using dried blood spots (DBS) and/or leukocytes. In subjects with reduced enzymatic activity in DBS, confirmatory β-glucocerebrosidase enzymatic activity in leucocytes was determined. GBA1 gene was analyzed.
Results: Since Feb/14 to Nov/16, 400 subjects have been included (51.3% men) with a median age of 28.79 years (range, 0.01 to 91.67). Reduced enzymatic activity of β-glucocerebrosidase was identified in 14 subjects (50% women) with a median age of 12.68 years (range, 0.9 to 74.85). All subjects were non-Ashkenazi origin, with 82.8% thrombocytopenia, 49.5% splenomegaly and 4.33% splenectomy. Detailed population description is on Figure 1.
Summary/Conclusions: This study suggests that selection algorithm could be implemented in Colombia, supporting specialists in making decisions on diagnosis of Gaucher Disease. Further characterization of the population is ongoing.

Acknowledgements: This study was funded by Sanofi Genzyme Colombia and coordinated by Caimed Colombia.

E1075

IMPACT OF PEROXIREDOXIN 2, GLUTATHIONE PEROXIDASE AND CATALASE INHIBITION ON OXIDATIVE STRESS MODIFICATIONS OF RED BLOOD CELL MEMBRANE AND CYTOSOL

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Background: Several anemias are associated with oxidative stress, namely, sickle cell anemia, β-thalassemia, glucose-6-phosphate dehydrogenase deficiency and hereditary spherocytosis. Red blood cells (RBC) are continuously exposed to oxidative stress, mainly due to their primary function as oxygen carriers; therefore, the erythrocytes are equipped with an efficient antioxidant system, however, when its capacity is overwhelmed, the cell is exposed to reactive oxygen species (ROS), triggering oxidative modifications. The antioxidant system includes several enzymes, such as peroxiredoxin 2 (Prx2), glutathione peroxidase (GPx) and catalase (CAT); in spite of their roles in cell defense being known, the interplay between these peroxidases is still unclear. The recent report of conoidin A, as a specific Prx2 inhibitor, offers the possibility to further explore the roles and contribution of these enzymes to antioxidant defense.
Aims: We aimed to study the importance of Prx2, GPx and CAT inhibition on defense against oxidative stress in normal erythrocytes.
Methods: We performed *in vitro* assays (*n*=3) with RBCs from healthy volunteers, inhibiting Prx2, GPx and CAT, either individually, two-by-two or all three; conoidin A, mercaptosuccinic acid and sodium azide were used as specific inhibitors of Prx2, GPx and CAT, respectively. Since the RBC membrane is a major target of ROS, we evaluated membrane lipoperoxidation (LPO) and membrane bound haemoglobin (MBH), as well as, cytosol's total antioxidant status (TAS), by spectrophotometric methods.
Results: Concerning TAS we found a trend towards decreasing values with enzyme inhibition (one or more); the lowest value of TAS was observed when all three enzymes were inhibited and, when only two enzymes were inhibited, the lower values were obtained for pairs that included CAT inhibition; when only one enzyme was inhibited, GPx inhibition showed the lowest TAS. Regarding LPO, a trend towards increasing values with enzyme inhibition was observed; the lowest value was obtained when all enzymes were active, and the highest when all of them were inhibited; when only one enzyme was inhibited, CAT inhibition showed the highest LPO value and when two enzymes were inhibited, LPO was highest for the pairs that included GPx. MBH was increased for all enzyme inhibitory conditions, when compared to the condition with all enzymes active, excepting when CAT was inhibited.
Summary/Conclusions: In conclusion, inhibition of these antioxidant enzymes, either alone or simultaneously, leads to oxidative stress modifications within the RBC, as showed by the increase in MBH and membrane LPO, and by the decrease in cytosolic TAS. Moreover, the inhibition of CAT or GPx (either alone or with other enzymes) presented more impact on oxidative modifications than Prx2 inhibition. Our data strengthens the importance of these enzymes in RBC's

antioxidant homeostasis, and suggests that inhibition or injury to one (or more) compromises erythrocytes, which might influence clinical presentation in oxidative stress associated anemias.

Acknowledgments: Financial support from FCT/MEC through national funds and co-financed by FEDER, under the Partnership Agreement PT2020 (UID/MULTI/04378/2013 – POCI/01/0145/FERDER/007728) and Norte Portugal Regional Coordination and Development Commission (CCDR-N)/NORTE2020/Portugal 2020 (Norte-01-0145-FEDER-000024).

E1076

MOLECULAR BASIS OF PKLR MUTATIONS IN PATIENTS WITH PYRUVATE KINASE (PK) DEFICIENCY: THE FIRST REPORT FROM SOUTHEAST ASIAN POPULATION

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Background: Recently we have identified a new form of transfusion dependent hemolytic anemia due to *KLF*1 mutations causing a *trans*-acting deactivation of pyruvate kinase genes (*PKLR*). Mutations of *PKLR per se* can affect red blood cells metabolism and cause a wide range of clinical manifestation from fetal anemia leading to hydroptic fetus, severe neonatal jaundice requiring multiple exchange blood transfusions, chronic to fully compensated hemolytic anemia. Understanding of the molecular basis of pyruvate kinase deficiency (PK def.) might be useful to predict clinical phenotypes and suggest appropriate clinical management of future patients. Moreover, an interaction of *PKLR* and *KLF1* mutations in such patient has not been explored.
Aims: This study aim to identify the mutation of patients with PK def. for the first time in Southeast Asian populations.
Methods: Seven unrelated patients; 6 from Thailand and 1 from Indonesia have been enrolled after inform consent. We have measured the PK activity of all patients and their parents and siblings using a standard biochemical technique as we have described earlier. A complete genomic analysis of all *PKLR*'s exons (NM_000298.5) including exon-intron boundaries were selectively amplified and followed by direct Sanger sequencing.

Table 1.

Table 1 Summary of baseline hematology, clinical phenotypes, PK activities and PKLR mutations in 7 Southeast Asian families with PK deficiency

Cases	sex	Hct (%)	Hb (g/dl)	Rbc (x10⁶/ml)	MCV (fL)	MCH (pg)	MCHC (g/dl)	RDW (%)	Retic (%)	Phenotype	PK activity (U/gHb (normal >18.5±4.0))	PK activity (% of normal range)	PKLR genotypes (NM_000298.5) allele 1	allele 2
PK-1 at 3 months	M	16	5.5	4.32	92	27.3	33.1	13.4	7	Severe HA	NA	NA	c.1269+3A>G	c.1483C>G + p.A495G
after BMT		41.3	13.6	5.09	81	26.7	32.9	13.9	4	normal	19.6	106		
Father	M	41.4	13.4	5.32	78	25.2	32.4	14.3	0.5	normal	7.2	38.9	c.1483 C>G = p.A495G	WT
Mother	F	36.1	11.4	5.47	66	20.9	31.7	16.3	2	normal	12.1	65.4	c.1269+3A>G	WT
PK-2	M	27	9	2.83	100	34.5	33.5	14.3	2	Severe HA	8.3	44.9	c.1541_1542delA p.V548fs	c.2570>C =p.R86P
Father	M	43.4	14.5	4.78	91	30.4	33.5	13.9	2	normal	14.4	77.8	c.2570>C =p.R86P	WT
Mother	F	39.2	12.5	4.81	82	27.1	32.7	13.9	2	normal	8.4	34.8	c.1541_1542delA p.V548fs	WT
PK-4	F	27.7	9.2	3.46	80	26.7	33.3	16.3	6	Severe HA	12.6	34.8	c.1483G>A = p.R488Q	??
Father	M	42.8	13.4	5.79	74	23.1	31.3	13.5	2	normal	10.5	56.8	c.1483G>A = p.R488Q	WT
Mother	F	37.1	12	4.76	78	25.2	32.4	13.6	3	normal	19.9	107.6	??	WT
Sister	F	36.7	8.6	3.84	80	22.3	27.9	16	20	normal	36.9	59.8	c.1483G>A = p.R488Q	WT
PK-5	F	20.2	6.8	1.74	115.8	38.8	33.6	9.5	70	normal	4.8	2	c.353A>G =p.N118S	c.941T>C =p.I314T
Father	M	36.8	12.6	4.1	89.7	30.6	34.1	13.4	3	Moderately sever HA	8.7	47.1	c.353A>G =p.N118S	WT
Mother	F	39.8	13.1	4.51	88.2	29.1	33	14.9	2	normal	11.3	61.2	c.941T>C =p.I314T	WT
PK-6	F	24.6	7.7	2.2	113	35	31.4	18	40	Moderately sever HA	11.5	8.6	c.941T>C =p.I314T	c.941T>C =p.I314T
Father	M	33	10	3.64	94	32.1	34.2	13	1	normal	6.3	34.1	c.941T>C =p.I314T	WT
Mother	F	36.9	12.1	4	93	30.3	32.9	11.6	2	normal	9.4	50.8	c.941T>C =p.I314T	WT
PK-7	M	30.9	10.4	3.39	90.9	30.5	33.6	12.8	1	Compensated HA	6.3	28.6	c.353+1914_1434delG GG	c.1468C>T =p.R490W
Father	M	44	14.8	4.85	94.5	31.9	33.8	10.5	1	normal	17.2	93	c.1468C>T =p.R490W	WT
Mother	F	36.4	12.1	3.9	93.3	31.1	33.3	10.8	1	normal	14.1	76	c.353+1914_1434delG GG	WT
PK-8	F	20.5	6.3	1.75	117	36	31	14.2	13.5	Moderately sever HA	15	26	c.865C>T =p.R289W	c.1618G>T =p.G540X

NA; not available, HA; hemolytic anemia, all mutation are described in HGVS nomenclature, In PK-1, the PK activity was performed after bone marrow transplantation (BMT)

Results: Seven index PK def. patients as confirmed by enzyme activities, age range 9-35 yrs old, were identified (Table 1). Three patients presented with severe hemolytic anemia and required regular blood transfusion; every 3-4 weeks in two (PK-1 and PK-3) and every 10-12 weeks (PK-2) in which one patient (PK-1) has been successfully treated with bone marrow transplantation and became transfusion-free. Three patients (PK-5, -6 and -8) had moderately severe hemolytic anemia and required blood transfusion occasionally. Only one patient (PK-7) from Indonesia had well-compensated anemia and never required blood transfusion. All but one had PK activities lower than 50% of normal range but these activities did not correlate with clinical severity. We found 11 different mutations in 5 compound heterozygotes and 1 homozygote as shown in Table 1. Four mutations appeared to be novel as they were not been reported in any public databases (c.1269+3A>G; c.353A>G =p.N118S; c.865C>T =p.R289W; c.1618G>T =p.G540X). One mutation (c.941T>C=p.I314T) seemed to be recurrent since it was found in two families; one homozygous and one compounded with N118S. Beside nucleotide mutations, we found a 5006 bp deletion from intron 3 to exon 10 affecting PKLR gene. To detect these mutations in family members and further cases, we developed a long range GAP-PCR analysis to amplify the breakpoint fragment and directly sequenced to determine deletion extends and also ARMS-PCR (C.1641T>TA), PCR-RFLP (c.941T>C), mismatched PCR-RFLP for c.1403C>G, c.1463G>A and IVS9(+3)A>G). Interestingly, one index patient (PK-4) was found with only one known missense mutation (R488Q), however we could not find any mutation in KLF1 of this patient suggesting that she might have other unidentified *cis* mutation involved gene regulation of *PKLR*. Due to a limited number of patients, there was no clear genotype-phenotype correlation found in our studied population.
Summary/Conclusions: Seven confirmed cases of PK def. are reported herein. They showed a wide variation of clinical severity. Molecular basis of PKLR mutations was proven to be beneficial to provide a definitive diagnosis of PK def. and might help suggesting clinical presentation in future cases.

E1077

PRELIMINARY RESULTS OF GAU-PED STUDY: PREVALENCE OF GAUCHER DISEASE IN PAEDIATRIC PATIENTS SELECTED BY AN APPROPRIATE DIAGNOSTIC ALGORITHM

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Background: Gaucher disease (GD) is an autosomal recessive lysosomal storage disease characterized by the deficient activity of beta-glucocerebrosidase (GBA). GBA deficiency results in the accumulation of glucosylceramide in different organs, causing tissue damage. Typical GD features are splenohepatomegaly, peripheral blood cytopenias (mostly thrombocytopenia and/or anemia), growth retardation, bone involvement, gammopathies, increased risk of malignancies and, in some patients, neurological manifestations. Since symptoms are non-specific, the diagnosis can be delayed for years or missed. Enzyme replacement therapy (ERT) with recombinant β-glucocerebrosidase is safe and effective in preventing and/or reversing many clinical manifestations. However, if the diagnosis is delayed for years, major complications cannot be reversed. A useful screening method for GD is based on measuring enzyme activity on a Dried Blood Spot (DBS), while the gold standard test is still considered GBA activity in cellular homogenates. A pediatric algorithm has been proposed to promote timely diagnosis and early access to ERT (figure 1).

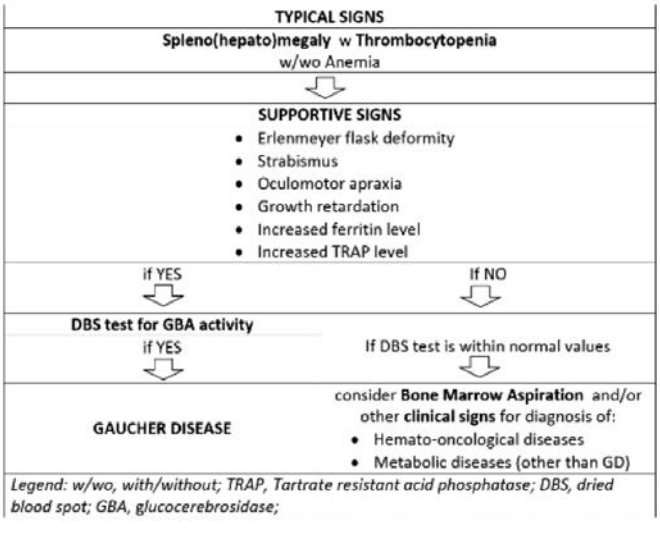

Figure 1 - Diagnostic approach for Gaucher disease in children (modified from *Di Rocco M et Al, Ped Blood & Cancer 2014;61:1905-09)*

Figure 1.

Aims: Since pediatric patients with splenomegaly and cytopenias are usually referred to pediatric hematologists, we have designed the GAU-PED study to

evaluate the prevalence of GD among children referred to the haematology paediatric units and selected according to the above mentioned diagnostic algorithm. Here, we report a preliminary analysis of GAU-PED trial.
Methods: The GAU-PED study involves 53 centers in the context of the AIEOP Study Group, the Italian clinical research consortium in paediatric hematology and oncology. Patients referring to the pediatric hematology and oncology units for splenomegaly with or without hepatomegaly and cytopenia (thrombocytopenia and/or anaemia), where other causes of splenomegaly has been excluded, are tested for GBA activity though a DBS sample. Only patients with DBS showing a GBA activity below normal values are recalled to confirm GBA enzyme deficiency using the gold standard GBA analysis in cell omogenate. For every tested patient, clinical information are also collected.
Results: After parental consent, a total of 25 DBS have been collected from 11 centers, in the first 12 months of study accrual. DBS values under 4.4 pmol/punch_1/h_1were found in 9/25 patients (36%). These patients have been recalled for the conventional enzymatic test. The diagnosis of GD has been confirmed in 5/9 patients (55%), with a prevalence of GD of 20% (95% CI, 8.8-39.1%) equal to 5/25 patients in the tested population. In all 5 patients the genetic analysis has been consistent with GD. Three patients were males and 2 females. The mean age at diagnosis was 8 years (range 2-13 years). The median time from the initial clinical presentation and diagnosis has been 12 months (range 6-50 months), while the mean time between the DBS test and the diagnosis has been 2 months. ERT has been started in all GD patients.
Summary/Conclusions: Our preliminary results support the use of DBS as screening test for GD in a selected population of children with splenomegaly and/or thrombocytopenia considered at increased risk for the disease. The use of an appropriate diagnostic algorithm is useful to increase awareness of GD among pediatric hematologists and to shorten the time to diagnosis. Taking into consideration the long life expectancy of pediatric GD patients, the early diagnosis will have a strong impact on health and quality of life.

E1078

CIRCULATING MICROPARTICLES IN CONGENITAL AND ACQUIRED HAEMOLYTIC ANAEMIA

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Background: Microparticles (MPs) are small particles budding from cells, which contain variable amounts of proteins, miRNA and cytosol from the parental cell. MPs play a role both in physiological and pathological conditions such as signal transduction, cell activation, thrombosis and cancer. Thrombotic events are a possible complication of haemolytic conditions, both congenital and acquired. Elevated levels of circulating MPs have been described in several haemolytic conditions, including sickle cell anaemia, thalassemia intermedia, haemolytic uremic syndrome, and thrombotic thrombocytopenic purpura.
Aims: To evaluate platelet MPs (PMP), tissue factor expressing MPs (TFMPs), endothelial MPs (EMPs) and microparticles expressing single antigens (CD41, CD144 or CD142) levels in in other haemolytic anaemias, such as hereditary spherocytosis (HS), elliptocytosis (HE), stomatocytosis (HSt), red cell enzymatic defects, congenital dyserithropoietic anaemia (CDA), autoimmune haemolytic anaemia (AIHA), and paroxysmal nocturnal haemoglobinuria (PNH).
Methods: To determine MPs, whole blood was collected into 0.109 M sodium citrated vacutainer tubes. Platelet Free Plasma (PFP) was prepared by double centrifugation at 2500 g for 15 min and stored frozen at -80°C until assayed. For MPs analysis 25 µl of PFP was incubated with annV-APC, CD41-FITC, CD142-PE and CD144 PerCp-Cy5.5 in Hepes buffer in the presence of 15 mM CaCl2 and 1 u/ml of r-Hirudin for 30 min. Samples were diluted with 500 µl Annexin V binding buffer and 25 µl of Flow-Count fluorospheres were added to express MP count as absolute numbers. MPs analyses were performed on a BD FACS Canto cytometer using Megamix-Plus SSC to define the MPs gate.
Results: MPs levels were evaluated in plasma of 43 patients followed-up for a median time of 9 years (range 2-34) and compared with normal controls. The median age of patients (15 male and 28 female) was 53 years (range 22-87), 9/43 (21%) had been splenectomized and 13/43 (30%) were treated at the moment of the study (steroids/immunosuppressors for AIHA, and eculizumab for PNH). Table shows Hb levels, PLT and WBC counts of the different haemolytic conditions, along with LDH and reticulocyte values. In AIHA, the number of EMP was negatively correlated with Hb levels (p<0.05), and PMPs positively with reticulocytes and LDH (p<0.05 for both); CD41+ MPs, CD142-PE+ MPs, PMPs, TF-MPs, and EMPs positively correlated with disease duration (p<0.05). In membrane defects the following positive correlation were observed: CD41+ MPs and PMPs with platelet values and EMPs with LDH (p<0.05 for all). In PNH, annV APC and CD144+ MPs were positively correlated with reticulocyte counts (p<0.05). In CDAs, the number of annV APC and PMPs negatively correlated with Hb (p<0.05). Finally, the number of annV APC was increased in PKD compared with controls (p=0.023), and positively correlated with disease duration (r=0.999, p<0.001); PMPs and TF-MPs were elevated too, although not significantly. The number of MPs here investigated were comparable between splenectomized and not splenectomized, and between naive and treated cases.

Table 1.

	AIHA (n=18)	Membrane defects (n=10)	PNH (n=7)	CDAs (n=5)	PK deficiency (n=3)	Controls (n=9)
Hb (mg/dL)	11.3 (6.7-15.4)	11.3 (9.7-16.4)	10.7 (7.6-12.7)	9.7 (7.7-10.5)	11.3 (6.7-15.4)	NR
LDH (ULN)	1 (0.7-2.8)	0.9 (0.7-1.5)	1.7 (0.8-5.3)	0.7 (0.6-0.8)	0.7 (0.7-1.2)	NR
PLT (10^9/L)	240 (97-475)	218 (229-786)	161 (79-321)	667 (293-1008)	363 (209-949)	NR
Reticulocytes (10^9/L)	89 (25-246)	116 (36-371)	126 (66-229)	37 (29-75)	131 (101-314)	NR
WBC (10^9/L)	7.4 (3.8-93)	8.2 (1.2-14.2)	3.5 (2-4.9)	6.2 (4.9-9.9)	5 (4.1-20.3)	4.8-10.8
annV APC	220 (37-787)	223 (133-631)	138 (88-972)	202 (88-612)	760 (320-1026)	223 (66-751)
CD41+ MP	251 (77-681)	246(120-461)	161 (96-239)	320 (104-622)	576 (359-1044)	190 (55-753)
CD142-PE+ MP	141 (58-505)	158 (92-372)	198 (97-408)	119 (64-374)	372 (279-447)	156 (82-261)
CD144+ MP	126 (33-417)	85 (57-286)	130 (40-243)	79 (34-171)	140 (124-201)	170 (36-360)
PMPs/µL	99 (15-399)	119 (51-181)	61 (21-124)	95 (39-536)	366 (95-948)	82 (27-659)
TF-MPs/µL	30* (7,6-86)	22* (9,2-51)	21 (15-51)	20 (8,2-35)	75 (59-399)	43 (11-145)
EMPs/µL	12,3 (1,9-43)	11,1* (3,7-19,3)	9,5* (2,7-16,1)	9,5 (2,7-24,2)	17,4 (14,8-70,7)	20 (7-48)

Values are expressed as median (range). NR = Normal Ranges (Hb: 13.6-16.7 g/dL; LDH: 240-480 U/L; PLT : 130-400 10^9/L; reticulocytes: 16-84x10^9/L; WBC: 4,8-10,8 10^9/L).
*= p< 0,05 versus controls

Summary/Conclusions: These preliminary results suggest that MPs levels are abnormal in both congenital and acquired haemolytic conditions. MPs levels correlate with the degree of anaemia and haemolysis and with the duration of disease.

E1079

THE PREVALENCE, ETIOLOGY AND PROGNOSTIC IMPACT OF ANEMIA IN OLDER POPULATION

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Background: The population of people aged ≥60 years is growing rapidly. Anemia represents a common condition among the elderly, however its prevalence and causes are not well known.
Aims: The aim of the study was to evaluate the prevalence, severity and etiology of anemia in the population aged ≥60 years. Risk factors for the development of anemia including concomitant diseases and treatment, were analysed. The association between anemia and hospitalization or all-cause mortality during follow-up was determined.
Methods: Retrospective analysis was performed on 981 Caucasian, outpatient patients aged ≥60 in Poland over 2013-2014 (median age 68, range 60-99 years, 60% females). The prevalence of anemia, defined according to WHO criteria, by gender, age groups (60-69, 70-79, and ≥80) and anemia etiology were studied. Data on the incidence of common comorbidities (coronary artery disease, heart failure, diabetes, chronic obstructive pulmonary disease, chronic kidney disease, chronic liver diseases, cancer, thyroid diseases), hospitalization, treatment used and all-cause mortality were analysed.
Results: The prevalence of anemia in the studied population was 17.2% and was higher in men than women (20.4% *vs* 15.2%, p=0.038). Anemia was present in 10.3% of patients aged 60-69, in 20.1% of those aged 70-79 and in 35.6% of patients ≥ 80 years. Incidence rates of anemia increased significantly with age (60-69 *vs* 70-79 years, p<0.001; 60-69 *vs* ≥80 years, p<0.001, 70-79 *vs* ≥80 years, p<0.001). Anemia was mild in 69.8% of patients, but a severe form was found significantly more often among men aged ≥80 years (p=0.03). Analysis of the etiology of anemia revealed three predominant types: anemia of chronic disease (33.1%), unexplained anemia (28.4%) and deficiency anemia (22.5%, including iron deficiency 13%). In comparison to patients without anemia, those with anemia were older (p<0.001), had a higher prevalence of comorbidities (p<0.001) and were more often hospitalized (p<0.001). In the multivariate logistic regression model, factors increasing the risk of anemia were: age ≥80 years (OR=2.29; 95%CI 1.19-4.42; p=0.013), the number of comorbidities (2 diseases OR=2.85; 95%CI 1.12-7.30; p=0.029, 3 diseases OR=6.28; 95%CI 2.22-17.76; p=0.001, 4 diseases OR=4.64; 95%CI 1.27-17.01; p=0.021) and the number of hospitalizations (OR=1.34; 95%CI 1.13-1.58; p=0.001). At the end of the 2-yr follow-up, the cumulative survival among patients without anemia in relation to the group with anemia was 90.76% *vs* 78.08% and the difference was statistically significant (p<0.001). In multivariate model, factors that significantly increased the risk of death in study population were anemia (HR=3.33; 95%CI 1.43-7.74; p=0.005), cancer (HR=3.31; 95%CI 1.47-7.49; p=0.004) and heart failure (HR=2.94; 95%CI 1.33-6.51; p=0.008).
Summary/Conclusions: In patients ≥60 years the incidence of anemia increases with age and male gender, number of comorbidities and frequency of hospitalization. The high rate of unexplained anemia indicates the necessity for detailed hematologic diagnosis. The occurrence of anemia among people aged ≥60 years has an adverse impact on survival.

E1080

PIEZO1 MECHANOTRANSDUCTIVE PROTEIN MUTATIONS IN RBCS: WHEN THE PHENOTYPE IS BEYOND HAEMOLYTIC ANAEMIA

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Background: Piezo proteins are integral membrane proteins with many transmembrane domains broadly expressed, including erythrocytes (RBCs). PIEZO1 proteins play an important role as an osmoreceptor, maintaining RBCs ionic homeostasis, functioning as a mechanically activated cation channels. Mutated PIEZO1 proteins have been linked to hereditary xerocytosis (HX), which is characterized by RBCs dehydration with mild to moderate compensated haemolytic anaemia and iron overload. As these clinical features are present in many different clinical conditions, the diagnosis always needs a high level of suspicion. Nowadays, besides peripheral blood smear (PBS) observation, molecular analysis, searching for mutations in PIEZO1 gene, became a tool in the diagnosis of HX.
Aims: Describe 26 patients with HX associated with PIEZO1 mutations belonging to 13 unrelated families, raising awareness of the highly variable phenotype of this patients, and the need of a highly grade of suspicion along with the morphologic evaluation of the PBS.
Methods: Collection of clinical and laboratory data on our 26 patients with HX and hyperferritinemia due to 10 different identified mutations in PIEZO1. Sanger sequencing was used to identify mutations affecting PIEZO1, encoded by *FAM38A* gene, and to confirm transmission according to the presence of disease phenotype. In all patients were excluded other known causes of hyperferritinemia (HF) and haemolytic anaemia.
Results: Of the 26 patients identified as having PIEZO1 mutations, 13 were probands and 13 were identified by family studies. Median age at diagnosis was 43 years (1-80), with female predominance (n=14; 53.9%). 4/13 probands had family history of HX (n=1) or HF (n=2). The common feature of our entire cohort of patients was the presence of xerocytes in PBS. 13/26 patients had reticulocytosis with a median reticulocyte count of $101x10^9$/L (28.1-557.3), 18/26 patients have HF with a mean value of ferritin of 556ng/mL (161-6617) and 9/26 had both. Of the 26 patients, four had splenomegaly and six gallbladder lithiasis (5/6 cholecystectomized), two of them have both. Only 5 patients presented with anaemia (Hb <12g/dL), 2 macrocytic and 3 normocytic. One patient was microcytic because he also was a β-thalassemia carrier. We detected heterozygous missense mutations in all 26 patients.
Summary/Conclusions: HX is a dominant disorder of RBCs dehydration presenting a great phenotypic variability. As shown in our cohort of patients, the anaemia may not be the main feature, in fact, the presence of xerocytes in PBS and HF were the most frequent characteristics of our patients. We would like to emphasise that in the genomics era the identification of xerocytes in the PBS keeps playing an important role for this diagnostic. Not only because, unlike other haemolytic anaemias, in HX there is a contraindication to splenectomy due to the increased risk of thrombotic events, but also because this patients present an iron overload that is not proportional to the degree of hemolysis. This iron overload may be related to a defective iron homeostasis dependent on PIEZO1 function not strictly related with Xerocytosis.

E1081

MODELLING PYRUVATE KINASE DEFICIENCY IN HUMAN PROGENITORS USING CRISPR/CAS9

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Background: Pyruvate Kinase Deficiency (PKD) is an autosomal recessive disorder caused by mutations in the PKLR gene. PKD produces chronic non-spherocytic hemolytic anemia, which can be fatal during early childhood and may result in lifelong transfusion dependence that in some instances persists despite therapeutic splenectomy. Although not considered a standard-of-care, allogeneic bone marrow transplantation has been curative in a limited number of cases. These findings, and the evidence that PKLR gene mutations are likely the sole etiology of the disorder, make PKD a candidate for gene therapy. We developed a gene therapy strategy in a PKD mouse model using a lentiviral vector (LV) carrying a codon-optimized version of the PKLR cDNA (coRPK). This vector has been recently designated as Orphan Drug for the treatment of PKD by the EMA and FDA (EMA: EU/3/14/1330; FDA: DRU-2016-5168).
Aims: To test the efficacy of the therapeutic LV, we have proposed an alternative to patient-derived PKD-hematopoietic progenitors. In particular, we have generated CRISPR/Cas9 system tools to knock-out the PKLR gene in healthy hematopoietic progenitors from healthy cord blood samples
Methods: Up to six different gRNAs were specifically generated to cleave the exons 8, 9 and 11 of the PRLR gene. All gRNAs contain at least 3 mismatches with the coRPK present in the therapeutic LV, to avoid the cleavage of the therapeutic transgene. Two gRNAs cleaved the PKLR gene both in 293T cells and primary $CD34^+$ cells. In order to identify and select edited cells, Cas9-gRNAs components were cloned into a Cas9-2A-ZsGreen1 plasmid.
Results: Cord Blood $CD34^+$ cells were electroporated, sorted and differentiated *in vitro* along the erythroid lineage. Significantly, the pyruvate kinase activity in *ex vivo* differentiated erythroid cells was impaired in gene edited cells as compared to non-edited samples.
Summary/Conclusions: Gene edit of wt CD34+ progenitors allow us to generate cells with RPK impaired. The decrease of PK activity validates this approach as a human model for PKD.

E1082

PHYSIOPATHOLOGY OF HEREDITARY XEROCYTOSIS : PIEZO1 GAIN OF FUNCTION MUTATIONS IMPACT HEMOGLOBIN OXYGEN AFFINITY

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Background: Dehydrated hereditary stomatocytosis, also called hereditary xerocytosis (HX) is a dominant non-spherocytic chronic hemolytic anemia characterized by an increased cation leak through the red cell membrane, associated with dehydration and shortened red cell survival. Clinically, most patients present a totally compensated hemolysis, with a normal hemoglobin level contrasting with a high reticulocytosis. In most cases, HX is caused by missense mutations activating Piezo1, a mechanosensitive ion channel. However, the pathophysiology of this compensated hemolysis remains largely unclear.
Aims: We studied the hemoglobin oxygen affinity parameters in HX patients and in hereditary spherocytosis (HS) subjects as controls.
Methods: Fourteen patients from 5 described and 4 unreported families with a HX diagnosis and 15 HS subjects were included. Diagnosis was based on ektacytometry and EMA assay. PIEZO1 and KCNN4 coding regions were analyzed by Sanger sequencing in all HX patients. Hemoglobin oxygen affinity was evaluated using p50 measured on venous blood on a Hemoxanalyser or a Radiometer blood gas analyzer. 2,3 diphosphoglycerate (2,3 DPG) levels were measured using a commercialized kit and expressed as a molar ratio 2,3 DPG/hemoglobin.
Results: All the 14 HX patients carried one or two missense mutations in PIEZO1, no gene variation was identified in KCNN4. Five families (9 subjects) have already been reported, with identified mutations in exon 18, 21, 42 or 51. Five subjects from 4 new families carried new mutations in exons 14, 16 and 29 and 51 for which bioinformatic softwares showed a high likelihood of pathogenicity. For all HX patients, p50 values were under the normal range (mean 21,1, range 19,7-23,4, normal range 25-29 mmHg), contrasting with HS patients for whom p50 was found to be in the normal range (mean 26,1, range 24,6-28,8 mmHg). This indicated a significant increase in the hemoglobin affinity for oxygen restricted to PIEZO1 mutated HX. Of note, p50 was not correlated with the Hb level (mean 139, range 112-180 g/L in HX patients *versus* 125, range 93-142 g/L in HS patients). Intracellular red cell 2,3 DPG level could be measured in 7 HX patients from 4 families, it was found decreased in all of them (0,43+/-0,06, normal 0,9+/-0,19), providing a pathophysiological basis for the increased hemoglobin oxygen affinity we observed.
Summary/Conclusions: The increased hemoglobin affinity for oxygen observed in HX patients reflects the decrease in the 2,3 DPG level. This may be a consequence of a high ATP requirement and an increased glycolytic activity in HX red cells at the expense of the 2,3DPG shunt in order to maintain the cell ion homeostasis. High hemoglobin affinity for oxygen may induce a relative tissue hypoxia and consequently a high reticulocytosis, providing a clue to explain the compensated hemolysis frequently observed. However, the links between PIEZO1 mutations, red cell enzymatic activity and erythropoiesis need to be clarified and a proteomic and a metabolomic approach is under investigation. Of interest, on a clinical point of view, HX diagnosis is sometimes difficult and this low p50 value, easily measured on venous blood, represents a useful new diagnosis tool for HX.

Gene therapy, cellular immunotherapy and vaccination

E1083

SAFETY AND EFFICACY OF MULTI-PATHOGEN-SPECIFIC T CELLS IN A HUMANIZED MODEL OF INVASIVE ASPERGILLOSIS: A PROOF OF CONCEPT STUDY

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Background: Viral infections, most commonly by cytomegalovirus (CMV), Epstein-Barr virus (EBV), polyoma virus type I (BK), and fungal infections, mainly by Aspergillus Fumigatus (Asp), are leading causes of transplant-associated mortality in patients undergoing allogeneic hematopoietic stem cell transplantation. Standard treatment with antiviral and antifungal pharmacological agents, is often ineffective or toxic and may lead to resistance. Due to these limitations, adoptive immunotherapy with antigen-specific T cells has emerged as an attractive alternative. Towards unleashing its full potential and treat multiple viral and fungal infections by a single T-cell product, we developed a rapid, simplified and minimally laborious protocol for the generation of multipathogen-specific T cells (mp-STs) that simultaneously target CMV, EBV, BK and Asp, from healthy donors.
Aims: Due to the lack of mouse models recapitulating the clinical condition of multiple opportunistic infections in transplanted hosts, we here aimed to test the *in vivo* safety of produced mp-STs and provide a proof of concept of their efficacy in a humanized model of invasive aspergillosis (IA).
Methods: mp-STs were generated from healthy donors by pulsing $1.5x10^7$mononuclear cells with viral (CMV: IE1, pp65; EBV: EBNA1, LMP2, BZLF1; BK: Large T, VP1) and Asp pepmixes (Crf1, Gel1, SHMT) and culturing for 10 days. The specificity of mp-STs was analyzed by IFN-γ Elispot. A total of $1.5x10^7$of immunomagnetically isolated CD3+cells (donor lymphocyte infusions-DLI) or mp-STs were infused in myelo/immuno-ablated NSG mice which had been intranasally inoculated with Asp conidia or left uninfected. Mice were evaluated by a 5-parameter sickness score and at sacrifice, tissues were assessed by histology and immunohistochemistry.
Results: We generated $23±5x10^7$ cells mp-STs (12-fold expansion). All cell lines were polyclonal expressing central and effector memory markers and specific against Asp [spot forming cells (SFC)/$2x10^5$cells: 315±82] and the targeted viruses, if derived from seropositive donors [SFC/$2x10^5$cells, CMV: 637±267; EBV: 744±158; BK: 578±118). To first address the safety issue, we asked whether mp-STs can induce acute graft-versus-host disease (aGvHD) in myelo/immuno-ablated mice. While DLI-treated mice developed clinically and histologically confirmed aGvHD and succumbed by day 20, mp-ST-mice survived free of aGvHD until the day of sacrifice (d28). To assess the *in vivo* functionality of mp-STs against IA, conditioned and Asp-inoculated mice, received mp-STs (n=5), DLI (n=4) or were left untreated (IA control, n=6). All IA- and DLI-mice succumbed to histologically evidenced IA at a median day 6, whereas 60% of mp-ST-mice survived until sacrifice, at day12. While the day-12 survivors presented high T-cell engraftment in the lung (% $CD3^+/CD45^+$: 14±7) with no histological evidence of IA, the two mp-ST-non-survivors died from IA in the absence of T-cell engraftment. Non-specific DLI failed to control IA despite T-cell presence in 3/4 DLI-mice (%$CD3^+/CD45^+$spleen: 58±12, lung: 3±1) which succumbed early, before aGvHD development.
Summary/Conclusions: Overall, engrafted mp-STs effectively controlled IA without evidence of alloreactivity. Based on the robust specificity of our mp-STs against all targeted pathogens and the clinical efficacy of virus-specific T-cells, we expect that our "four in one" T-cell product has the potential to also fight the targeted viruses and become a powerful tool for the treatment of multiple, life-threatening post-transplant infections.

E1084

DONOR LYMPHOCYTE INFUSION IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES LEADS TO DIVERSITY OF LEUKEMIA-ASSOCIATED-ANTIGENS-SPECIFIC T CELL RESPONSES AND TO REDUCTION IN REGULATORY T CELL FREQUENCY

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Background: Cytotoxic T-cell (CTL) responses against malignant cells play a major role in maintaining remission and prolonging overall survival in patients with hematologic malignancies after allogeneic stem cell transplantation (allo-SCT) and/or donor lymphocyte infusions (DLI). Graft *versus* leukemia (GvL) effects after allogeneic stem cell transplantation and/or DLI are considered to be T cell-mediated. Many groups described specific T-cell responses against several leukemiaassociated antigens (LAA) in different hematological malignancies. However, T cell responses after allo-SCT and DLI are not well characterized.
Aims: In this study, we analyzed LAA-specific T cell responses after allo-SCT and DLI. To this end, we assessed the frequency and diversity of LAA-specific CD8+ T cells using ELISpot analysis and tetramer assays in 12 patients (5 patients (pts) with acute myeloid leukemia, 2 pts with chronic myeloid leukemia, 3 pts with multiple myeloma and 2 pts with chronic lymphatic leukemia) before and after DLI. Epitopes derived from PRAME, $NPM1^{mut}$, RHAMM, WT-1 and other LAA were tested. Moreover, the frequency of regulatory T (Treg) cells was measured and the course of cytokine profiles before and after DLI was analyzed. These immunological findings were correlated to the clinical course in the respective patients.
Methods: In ELISpot and tetramer assays, an increase in frequency and diversity of LAA-specific T cells was observed in all patients. Cytokine assays using ELISA for the detection of more than 10 cytokines before and after DLI were employed.
Results: Importantly, there was a significant increase from 0 to 7 LAA-derived T cell epitopes (P=0.03) in clinical responders (R) when compared to non-responders (NR). These positive results in R *versus* NR where confirmed by tetramer-based flow cytometry assays, where an increase in frequency from 0.5 to 2.3% in the R group of LAA-specific T cell/all CD8+ T cells was observed. Interestingly, the frequency of Tregs in clinical responders decreased significantly from a median 72,9% to 54,6% (P=0.008) while the frequency of Tregs stayed stable over time in non-responding patients. T cell subset analysis did not reveal significant differences before *versus* after DLI administration. In cytokine assays using ELISA we found a significant increase of IL-4 after DLI.
Summary/Conclusions: Taken together, we detected an increase of specific CTL responses against several LAA after allogeneic stem cell transplantation and donor lymphocyte infusion. Moreover, this study suggests that broader LAA epitope-specific T cell responses as well as decreasing numbers of Tregs contribute to clinical outcome of patients treated with DLI.

E1085

GENE-MODIFIED NK-92MI CELLS EXPRESSING A CHIMERIC CD16/CD64-BB-Z RECEPTOR EXHIBIT ENHANCED CANCER-KILLING ABILITY IN COMBINATION WITH THERAPEUTIC ANTIBODY

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Background: Natural killer (NK) cells play a pivotal role in monoclonal antibody-mediated immunotherapy through an antibody-dependent cell-mediated cytotoxicity (ADCC) mechanism. NK-92MI is an interleukin-2 (IL-2)-independent cell line, which was derived from NK-92 cells with superior cytotoxicity to a wide range of tumor cells *in vitro* and *in vivo*. However, the Fc-receptor (CD16), which usually mediates ADCC, is absent in NK-92 and NK-92MI cells.

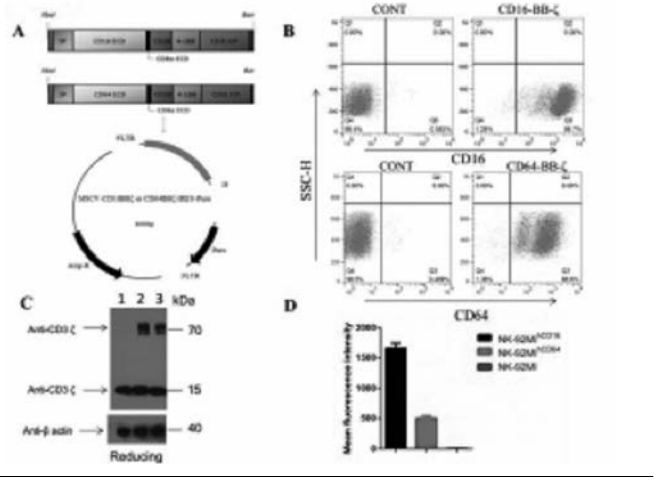

Figure 1. NK-92MIhCD16 and NK-92MIhCD64 functional validation in vitro and characterization. A. Schematic representation of the CD16-BB-ζ and the CD64-BB-ζ receptor constructs. B. Exogenous CD16 or CD64 expression on surfaces of NK-92MI cells are shown. C. Immunoblot analysis of CD3ζ fusion protein expression in NK-92MIhCD16 or NK-92MIhCD64 cells.

Aims: To apply NK-92MI cell-based immunotherapy in cancer, we designed and generated two chimeric receptors which can bind the Fc portion of human immunoglobulins in NK-92MI cells.
Methods: The construct included the low-affinity Fc receptor CD16 (158F) or the high-affinity Fc receptor CD64, with the addition of the CD8a extracellular domain, CD28 transmembrane domains, two costimulatory domains (CD28 and 4-1BB), and the signaling domain from CD3ζ. The resulting chimeric receptors, termed CD16-BB-ζ and CD64-BB-ζ, were utilized to generate chimeric receptor-modified NK-92MI cells, which were named NK-92MI^{hCD16} and NK-92MI^{hCD64} cells, respectively.
Results: We found that NK-92MI^{hCD16} and NK-92MI^{hCD64} cells significantly improved cytotoxicity against CD20-positive non-Hodgkin's lymphoma (NHL) cells in the presence of rituximab.
Summary/Conclusions: These results suggest that the chimeric receptor-modified NK-92MI cells could potentially enhance the clinical responses mediated by currently available anticancer monoclonal antibodies (mAbs).

E1086

A NOVEL *IN VITRO* METHOD TO QUANTIFY THE PHARMACOLOGY ACTIVITY OF BISPECIFIC ANTIBODIES IN HEMATOLOGICAL SAMPLES

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Background: The PharmaFlow automated flow platform has achieved 85% clinical correlation with AML samples with its novel Native Environment assay. Recently, novel Bi-specific antibodies (BsAbs) or analogous constructions acting through the formation of an immunologic synapse between T-cells (CD3) and a tumor-associated surface antigen (TAA) have been used as immunotherapy leading to T-cell activation and serial lysis of tumor cells.
Aims: The aim of the present study is develop and *in vitro* assay with multiples variables to better quantify the activity of bispecific antibodies and reflect the interpatient variability.

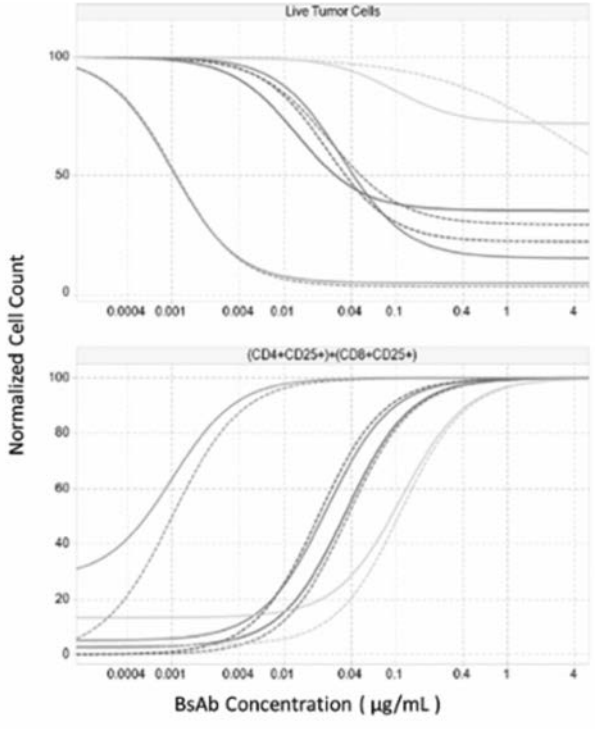

Figure 1.

Methods: For this purpose, different fresh whole Bone Marrow (BM) or Peripheral Blood (PB) were tested with their corresponding BsAbs at 8 different concentrations in different time points (24h-144h). In this sense, we tested 31 AML BM samples (5 paired BM and PB) with the CD123XCD3 (Creative Biolabs) and 7 CLL and 3 B-ALL samples with Blinatumumab (Amgen). When appropriate, basal quantification of TAA was performed by flow cytometry (FCM). The PharmaFlow platform efficiently count by FCM how many tumor cells are killed by every activated T-cells, here called effective E:T ratio. For each sample, 8-colour FCM staining was performed to simultaneously analyze the leukemic population, activated CD4 and CD8 T-cells and the residual normal cells. EC_{50} or E_{max} was calculated to evaluate potency or efficacy. Kinetics of activity was measured repeating the dose response curves in 3 different days.
Results: Most of the samples present both T-cell activation (CD25+) and an effective lysis of tumor cells after BsAbs exposure in a time and dose dependent manner (Figure 1), even starting with low basal E:T ratios (<1:100). For AML, basal quantification of CD123 by FCM density does not reflect a correlation with the *in vitro* response. By contrast, differences in T-cell cytotoxicity or leukemic immunoresistance were observed between samples in terms of EC_{50} or E_{max}, even more marked between CLL samples. The integration of effective E:T ratios, EC_{50}, E_{max}, and kinetics allow us to generate an *in vitro* response model and select those samples with higher T-cell cytotoxicity after the different BsAbs exposure. Interestingly, many of the samples for all the BsAbs leave a significant proportion of live cells, even at the higher BsAb concentrations or with a remarkable expansion of activated T-cells that suggest the use of immunecheckpoint to unblock this immnunoresistant status.
Summary/Conclusions: We have developed an automated flow cytometry assay for bispecific antibodies screening that keep intact both basal effector to target (E:T) ratios and Native environment using whole blood or bone marrow samples. In this context, the PharmaFlow platform selects different *in vitro* T-cytotoxicity effects across patients identifying best patient candidates for adoptive antitumor immunotherapy with BsAbs. The integration of Effective E:T ratios and pharmacological parameters better predict the *in vitro* response of BsAbs. Because of the high capacity of the PharmaFlow platform, additional antibodies constructions alone or in combinations with immunomodulatory agents could be tested to identify the better agents or immunotherapeutics combinations in hematological diseases.

E1087

HUMANIZED CD7 NANOBODY-BASED IMMUNOTOXINS EXHIBIT PROMISING ANTI-T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA POTENTIAL

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Background: Nanobodies, or named as VHHs, are derived from heavy-chain-only antibodies that circulate in sera of camelids. Their exceptional physicochemical properties, possibility of humanization and unique antigen recognition properties make them excellent candidates for targeting delivery of biologically active components, including immunotoxins. In our previous efforts, we have successfully generated the monovalent and bivalent CD7 nanobody-based immunotoxins, which can effectively trigger the apoptosis of CD7 positive malignant cells.
Aims: To pursue the possibility of translating those immunotoxins into clinics, we humanized the nanobody sequences (designated as dhuVHH6), as well as further truncated the *Pseudomonas* exotoxinA (PE) derived PE38 toxin to produce a more protease-resistant form which is named as PE-LR, by deleting majority of PE domain II.
Methods: Three new types of immunotoxins, dhuVHH6-PE38, dVHH6-PE-LR, and dhuVHH6-PE-LR, were successfully constructed. These recombinant immunotoxins were expressed in *E. coli* and showed that nanobody immunotoxins have the benefits of easy soluble expression in a prokaryotic expression system.
Results: Flow cytometry results revealed that all immunotoxins still maintained the ability to bind specifically to CD7-positive T lymphocyte strains without binding to CD7-negative control cells. Laser scanning confocal microscopy found that these proteins can be endocytosed into the cytoplasm after binding with CD7-positive cells, and that this phenomenon was not observed in CD7-negative cells. WST-8 experiments showed that all immunotoxins retained the highly effective and specific growth inhibition activity in CD7-positive cell lines and primary T-cell acute lymphoblastic leukemia (T-ALL) cells. Further *in vivo* animal model experiments showed that humanized dhuVHH6-PE38 immunotoxin can tolerate higher doses and extend the survival of NCG mice transplanted with CEM cells without any obvious decrease in body weight. Further studies on NCG mice model with patient-derived T-ALL cells, dhuVHH6-PE38 treatment significantly prolonged mice survival with around 40% survival improvement. However, it is also noticed that despite dhuVHH6-PE-LR showed strong anti-tumor effect *in vitro*, its *in vivo* anti-tumor efficacy is disappointed.

Summary/Conclusions: We have successfully constructed a targeted CD7 molecule modified nanobody (CD7 molecule improved nanobody) immunotoxin dhuVHH6-PE38 and showed its potential for treating CD7-positive malignant tumors, especially T-cell acute lymphoblastic leukemia.

E1088

STATINS MAY IMPROVE CAR-NK IMMUNOTHERAPY IN MM BY PREVENTING LOSS OF BCMA EXPRESSION ON MM CELLS

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Background: Chimeric Antigen Receptor (CAR) modified immune cells targeting BCMA against multiple myeloma (MM) has appeared as a feasible immunotherapy strategy to treat MM patients. However, high doses of CAR immune cells are required to achieve a response. Cord blood derived NK cells (CB-NK) is a feasible source of obtain NK cells to modify with a CAR against BCMA. We previously observed that MM cells exposed to CB-NK are able to transfer MM proteins, such as BCMA, both to CB-NK and to adjacent MM cells non-exposed to CB-NK. Furthermore, statins, which are toxic for MM cells, by altering the lipid composition of tumor cell membrane are involved in cell-cell communication. We hypothesized that statins could prevent the loss of BCMA expression which occurs in MM cells after CB-NK exposure, allowing infusing a lower CAR immune cell dose in MM patients
Aims: To evaluate the effect of statins on MM cell proliferation, on the CB-NK immune response against MM, and on BCMA expression in MM cells after CB-NK exposure.
Methods: The cytotoxicity of statins against MM cells was determined *in vitro* and *in vivo* in a murine MM model; furthermore, their impact in CB-NK cytotoxicity against MM was also determined *in vitro*. BCMA expression on MM cells after CB-NK exposure was analyzed by confocal microscopy and by flow cytometry. FACS sorting experiments were performed to analyze BCMA transfer between CB-NK exposed MM cells to neighboring non-exposed CB-NK MM cells.
Results: Atorvastatin and Fluvastatin treatment (1μM) decreased MM cell line (ARP1, RPMI, KMM1) proliferation. No effect was detected for U266 MM cells and for K562 non-MM cells. *In vivo* studies, showed that mice treated for three days I.P with Fluvastatin (1mg/kg) showed significant decreased MM disease progression. Blocking of BCMA decreased CB-NK cytotoxicity against MM cells. Furthermore, pretreatment of MM cells with Fluvastatin (3 μM) increased CB-NK cytotoxicity against all MM cell lines; no impact was observed against K562 non-MM cells. Co-culture experiments showed that, as soon as 30 minutes, CB-NK exposure led to a BCMA transfer from MM cells to CB-NK and to the extracellular milieu leading to a loss of BCMA expression on MM cells. Fluvastatin pretreatment prevented loss of BCMA expression. After two days of co-culture, alive MM cells still showed decreased BCMA surface expression, and surprisingly, increased intracellular BCMA expression. Fluvastatin pretreatment partially avoided the loss of BCMA surface expression and the increased intracellular BCMA expression. Furthermore, FACS sorting experiments showed that MM cells exposed to CB-NK, transferred BCMA to neighboring non-CB-NK exposed MM cells which was partially inhibited with Fluvastatin pretreatment.
Summary/Conclusions: Our findings show that besides the anti-MM activity of statins alone, they avoid the loss of BCMA expression on MM cells after immune cell exposure. Preventing loss of BCMA expression on MM cells might improve the efficiency of CAR immunotherapy against BCMA, suggesting the potential of statins as an adjuvant in CAR-NK immunotherapy against MM

E1089

DENDRITIC CELL VACCINATION COMBINED WITH LENALIDOMIDE AND PROGRAMMED DEATH-1 (PD-1) BLOCKADE HAS SYNERGISTICALLY INDUCED A MARKED TUMOR REGRESSION IN A MURINE MYELOMA MODEL

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Background: There is an emerging evidence that the maximal benefit of dendritic cell (DC)-based cancer immunotherapy may be achieved by combination with other therapies that act to immunomodulation and tumor microenvironment.
Aims: In this study, we tried to obtain the best efficacy of immunotherapy using DC vaccination in combination with lenalidomide and PD-1 blockade in a murine myeloma model.
Methods: After establishing myeloma-bearing mice, five treatment groups were designed to be a mimic protocol as like treatment in clinics as following: 1) PBS control, 2) DCs, 3) DCs + lenalidomide, 4) DCs + PD-1 blockade, and 5) DCs + lenalidomide + PD-1 blockade. After treatment, preclinical response and *in vitro* immunological responses were evaluated.
Results: DCs combined with lenalidomide and PD-1 blockade showed the best tumor regression among the study groups. These anti-tumor effects have meaningfully related to the decrease of immuno-regulatory populations, such as myeloid-derived suppressor cells (MDSCs), M2 macrophages, and regulatory T cells (Treg) and the increase of effector immune cell populations, including $CD4^+$ and $CD8^+$ T cells, natural killer (NK) cells, and M1 macrophages, accompanied with the activation of cytotoxic T lymphocytes (CTLs) and NK cells in the splenocytes from the treated mice. Moreover, the level of immunosuppressive cytokines, such as TGF-β and IL-10, was significantly reduced in tumor microenvironment.
Summary/Conclusions: DC vaccination in combination with lenalidomide plus PD-1 blockade has synergistically induced a strong antitumor immunity by modulating tumor microenvironment in a murine myeloma model. This protocol will become a promising translational approach to improve the efficacy of immunotherapy in the field of MM.

E1090

B- AND T-CELL IMMUNE REPERTOIRE PROFILING WITH ANCHORED MULTIPLEX PCR AND NEXT-GENERATION SEQUENCING

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Background: NGS-based analysis of the immune repertoire (IR) is a powerful tool to monitor disease, adaptive immune responses to disease, vaccination and therapeutic interventions. IR characterization by NGS usually requires large primer panels to cover its extensive combinatorial diversity, and a complex system of synthetic controls to account for differential amplification efficiency across segment combinations. Anchored Multiplex PCR (AMP™) uses molecular barcoded (MBC) adapters and gene-specific primers (GSPs), enabling NGS-based immune chain mRNA interrogation from a single side. This eliminates the need for opposing primers that bind within the highly variable V-segment, eliminating clone dropout due to somatic hypermutation.
Aims: Our goal was to develop an NGS assay based on AMP that would enable IR characterization utilizing a minimal set of unidirectional GSPs and to reduce amplification bias through the use of MBC adapters.
Methods: Upon developing our AMP-based NGS assay, we validated its quantitative reproducibility and sensitivity using mRNA isolated from PBMCs of healthy donors, B-cell chronic lymphocytic leukemia donors and formalin-fixed paraffin-embedded (FFPE) tissue.
Results: We developed the AMP-based NGS assays, Immunoverse™ IGH and TCR, for B-cell and T-cell receptor sequencing, respectively. Both assays demonstrated high reproducibility between replicates with quantitative clone tracking down to 0.01%. The ability to determine isotype, clonotype and IGHV mutational status in a single assay was demonstrated. Preliminary TCR assay data indicates that CDR3 sequence capture is possible from FFPE tissue with clonotype calling being driven by input quantity, T-cell content, and, to a lesser degree, mRNA quality.
Summary/Conclusions: AMP-based NGS with MBC quantification and error-correction is a powerful method to characterize the immune repertoire.

E1091

SYNERGISTIC ANTITUMOR IMMUNITY BY DENDRITIC CELLS IN COMBINATION WITH POMALIDOMIDE AND DEXAMETHASONE IN A MURINE MYELOMA MODEL

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Background: Pomalidomide (Pom) plus dexamethasone (Dex) could be considered one of the new treatment options in patients with relapsed and/or refractory multiple myeloma (MM). Recently, several diverse agents would be combined to improve the therapeutic efficacy of immunotherapy.
Aims: In this study, we investigated the preclinical efficacy of combined therapy with dendritic cells (DCs) and Pom-Dex in a murine myeloma model.
Methods: After establishing myeloma-bearing mice, four treatment groups were designed to be a mimic protocol as like treatment in clinics as following: 1) PBS control, 2) DCs, 3) Pom + Dex, and 4) DCs + Pom + Dex. After vaccination, preclinical and *in vitro* immunological responses were evaluated.
Results: Among four treatment groups, DC combined with POM and DEXA strongly inhibited tumor growth, compared with other groups. *in vitro* immunological analyses revealed that these enhanced anti-tumor effects were closely associated with the decrease of regulatory cell populations, such as regulatory T cells (Tregs) and type 2 macrophages (M2), and the increase of effector cell populations, including activated CD4 T cells, and type 1 macrophages (M1), accompanied with the activation of cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells in the splenocytes from vaccinated mice.
Summary/Conclusions: This study suggested thay DC combined with POM and DEXA synergistically enhance the anti-tumor immunity in a murine myeloma model, by skewing immuno-suppressive status toward immuno-supprotive status in tumor microenvironment.

E1092

ALTERATIONS IN T-CELL SUBPOPULATIONS AFTER CO-CULTURING WITH MSCS DERIVED FROM DIFFERENT DONORS

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Background: Study of interactions between lymphocytes and mesenchymal stromal cells (MSCs) *in vitro* revealed increase of HLA-DR expression on T-cells after co-cultivation with some MSCs samples. On lymphocytes derived from one donor the elevation of HLA-DR was observed after co-cultivation with half of MSCs samples (group A), on the others the HLA-DR expression level did not change (group B). MSCs were divided into two groups based on HLA-DR rise on lymphocytes. Study of T-cell subpopulations after interactions with MSCs could explain ineffectiveness of some MSCs as an immunomodulating agent in clinical applications.
Aims: The aim of the study was to discriminate variations in T-cell subpopulations, co-cultured with MSCs from two groups.
Methods: MSCs were isolated from bone marrow of 13 donors for allogeneic hematopoietic cells transplantation and cultured by a standard method. MSCs were seeded 10^5 cells per flask, and then 10^6 allogeneic lymphocytes from single donor were added to all MSCs cultures. For lymphocytes activation 5mg/ml phytohemagglutinin (PHA) was added to half of these cultures. Lymphocytes were removed from MSCs. Than MSCs were removed from the bottom of the flask by trypsin and expression of HLA-DR on their surface was measured by flow cytometry. Activation markers CD25, CD38, CD69, HLA-DR and PD-1 were studied by flow cytometry as well as distribution of naïve and effector T-cells were analyzed on 4th day of cultivation. $p<0.05$ was considered statistically significant; all data are presented as medium ± SEM.

Table 1.

Table. Subpopulations of lymphocytes that differ between group A and group B.

Subtypes of T-cells	Subpopulation	Control samples without MSCs	Group A	Group B
Non-activated lymphocytes 4 days				
CD4	HLA-DR+	8.46±1.0%	20.4±1.7%*	7.4±0.4%
	EM	29.8±1.6%	20.2±1.2%*	16.6±0.9%*
	TM	0.9±0.2%	1.2±0.2%	0.34±0.02%*
	CD127-/CD25-	17.4±5.1%	18.9±1.9%	12.4±2.0%*
Activated lymphocytes 4 days				
CD4	HLA-DR+	90.3±1.6%	72.1±3.6%	29.7±9.3%*
	CM	64.1±9.5%	53.4±2.7%	32.7±5.5%*
	TM	0.9±0.3%	1.8±0.2%	0.27±0.13%
	PD-1+	65.4±5.7%	44.5±5.6%*	17.6±3.2%*
CD8	HLA-DR+	89.0±2.5%	76.2±4.6%	35.0±13.1%*
	CM	57.7±9.3%	35.8±2.6%*	15.1±6.0%*
	EM	6.8±1.1%	16.4±2.3%*	10.1±1.5%
	TE	8.6±2.1%	6.2±0.7%	14.1±2.8%*
	PD-1+	53.3±7.8%	23.4±3.5%*	10.6±2.6%*

* indicates data in group A or B that significantly differs from control samples

Results: Expression of HLA-DR on lymphocytes after 4 days of cultivation without MSCs did not change compared to 1st day. When lymphocytes were co-cultured with some MSCs samples expression of HLA-DR was higher. Elevated percentage of HLA-DR positive cells correlates between CD4+ and CD8+ cells (R^2=0.932). Thus samples of MSCs were divided into two groups: in group A proportion of HLA-DR lymphocytes were 3 times greater than in group B. Subpopulations of lymphocytes co-cultured with MSCs from group A and B were compared. Subpopulations which significantly differed between groups A and B are presented in the table. In lymphocytes co-cultured with MSCs there were higher number of naïve cells compared to control (47.4±3.5% and 54.9±2.0% for group A and B *vs* 36.9±1.4% for lymphocytes cultured without MSCs, $p<0.001$). Group B showed lower number of EM and TM cells. Differences between groups were more pronounced when lymphocytes were activated. In group B proportion of HLA-DR CD4+ and CD8+ cells was significantly lower, compared to group A and control samples. At the same time the number of CM and PD-1+ CD4+ cells was lower in group B, but number of TE was increased. Investigation of HLA-DR expression on MSC after co-culturing with lymphocytes showed higher level of fluorescence signal (MFI) in group A then in group B (635±130 *vs* 289±18, p=0,03). These data indicated that MSCs from group A had become more immunogenic after interaction with lymphocytes and could not show immunomodulating properties in same way as MSCs from group B.
Summary/Conclusions: The immunomodulatory properties of MSCs depend on the donor. This could explain why administration of MSCs is not always successful. Preliminary study of MSCs prior to their administration may be used to predict their efficiency in the future.

The materials are supported by grant from the Russian Science Foundation, Project № 16-15-00102.

E1093

GRANULOCYTE COLONY STIMULATING FACTOR AND ERYTHROPOIETIN ENTERALLY GIVEN FOR NEONATES RECOVERING FROM GIT SURGERIES: RANDOMIZED CONTROLLED TRIAL

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Background: Feeding intolerance is a common problem among neonates recovering from surgery for congenital abnormalities of the gastrointestinal tract (GIT) such as small bowel atresia, omphalocele or gastroschisis. Feeding intolerance is a multifactorial process, but one of the important reasons is congenital maldevelopement of the small bowel villi. Disuse atrophy of the small bowel mucosa following several days of post-operative enteral fasting is one factor that can contribute to feeding intolerance. The human fetus swallows over 200 ml/kg/day of amniotic fluid and such swallowing is essential for normal small bowel development. Growth factors found in the amniotic fluid have been shown to promote proliferation of fetal intestinal cells. These growth factors include epidermal growth factor, granulocyte colony stimulating factor (G-CSF) and erythropoietin (EPO). We postulated that infants recovering from surgeries for congenital obstructive bowel abnormalities could be provided with physiologic quantities of recombinant human G-CSF and EPO by the intermittent orogastric or nasogastric administration of 20 mL/kg/day of sterile isotonic solution that contained cytokine concentrations comparable to what they would have ingested from amniotic fluid in utero.
Aims: is to test a hypothesis suggesting that feeding tolerance could be improved in neonates recovering from surgeries for congenital obstructive bowel abnormalities by enterally administering recombinant human G-CSF and EPO included within simulated amniotic fluid solution.
Methods: This double-blinded randomized controlled clinical trial was conducted on 40 neonates recovering from GIT surgeries for congenital bowel abnormalities. Hemodynamically unstable babies, and those with any contraindication to enteral feeding were excluded. Neonates were randomly divided postoperatively into 2 groups; 20 neonates received the test solution (called Simulated Amniotic Fluid-like solution given Enterally; SAFE); 20 neonates enterally received distilled water (control). Treatment was started postoperative and the test solution (or distilled water) was discontinued when enteral intake reached 100cc/kg/day. Feeding tolerance and adverse effects of treatment (if any) were assessed.
Results: All the studied neonates tolerated the received solution well without side effects that could be attributed to its intake. The study group showed better feeding tolerance as reflected by earlier achievement of 50, 100, 120 and full enteral feeds with higher enteral caloric intake 7 days after SAFE administration and higher rate of weight gain ($p<0.05$). No significant increase was found in the level of WBCs count, hemoglobin and hematocrit values either pre-initiation or 7 days after administration of SAFE ($p>0.05$).
Summary/Conclusions: This study provides further insights on the improvement of neonatal outcomes and help to decrease morbidities from post-operative malnutrition and feeding intolerance. Enteral administration of rhG-CSF and rhEPO may play a critical role in preventing villous atrophy, thereby, reducing feeding intolerance in neonates recovering from surgeries for congenital bowel abnormalities.

E1094

GENE EDITING OF HUMAN HEMATOPOIETIC PROGENITORS TO CORRECT PYRUVATE KINASE DEFICIENCY

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Background: Pyruvate Kinase Deficiency (PKD) is a rare erythroid metabolic disease caused by mutations in the *PKLR* gene which encodes the erythroid specific Pyruvate Kinase (RPK) enzyme. The defective enzyme fails to produce normal ATP levels and consequently, erythrocytes from PKD patients show an energetic imbalance and are susceptible to hemolysis. Site-specific hematopoietic stem cell gene therapy would be the safest approach to treat PKD patients. In this study, different gene editing approaches have been explored to correct PKD, either by the Knock-in of a *PKLR* cDNA sequence in the second intron of the gene, or by the site-specific correction of specific mutations.
Aims: In the Knock-in system, that previously showed to correct the PKD phenotype of PKD-iPSC lines, a recombination matrix carrying codon optimized exons 3-11 of the *PKLR* cDNA and a puromycin selection cassette was inserted in the second intron of the *PKLR* gene assisted by TALEN nucleases.
Methods: Thus, the therapeutic matrix together with specific TALENs as DNA plasmid or mRNA, for the second intron of PKLR were electroporated in purified $CD34^+$ cells from healthy cord bloods. Cells were then expanded and puromycin selected to enrich the population for gene edited ones.

Results: Although a high toxicity and low efficiency were observed with the electroporation technique used, up to 96% colony forming units showed the specific integration. Experiments directed to improve efficacy and reduce toxicity were then conducted. A high percentage of gene edited HPCs were detected by shortening the cell expansion and puromycin selection periods. Importantly, gene edited HPCs were detected after infusion in immunodeficient (NSG) mice. More recently, site-specific correction has been developed aiming at the correction of PKD patient's specific mutations.
Summary/Conclusions: Overall, we showed that gene editing in engraftable HPCs is feasible, although the efficiency of the procedure should be further improved prior to consideration of these strategies in the clinic.

E1095

BLAST KINETICS AFTER NON-ENGRAFTING HAPLOIDENTICAL MICROTRANSPLANTATION IN PATIENTS WITH REFRACTORY ACUTE MYELOID LEUKEMIA

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Background: Multiple trials have showed that granulocyte colony-stimulating factor (G-CSF) –mobilized donor peripheral-blood stem cells (GPBSCs) based on allo-graft can be effective in mediating graft-versus-leukemia (GVL) effects and promote hematologic recovery without triggering of acute GVHD.
Aims: To analyze the safety and efficacy of non-engraftment haploidentical cellular therapy for patients with refractory acute myeloid leukemia by assessment of bone marrow blast and hematopoietic cells percent kinetics.
Methods: Seven patients (4 males 57.1%, 3 females 42.9%) with refractory acute myeloid leukemia were enrolled into this Phase I/II study. They were treated with chemotherapy including fludarabine 30mg/m^2, cytarabine 1gm/m^2, etoposide 100mg/m^2, endoxan 750mg/m^2 followed by infusion of haploidentical unmanipulated GPBSCs 24 hour after last chemotherapy infusion. Morphologic assessment of bone marrow blast kinetic by bone marrow aspiration conducted before therapy, D14 and D30 after therapy. Hematopoietic cells percent kinetics (Hematologic recovery) was assessed by Complete blood count every day till Day 40.

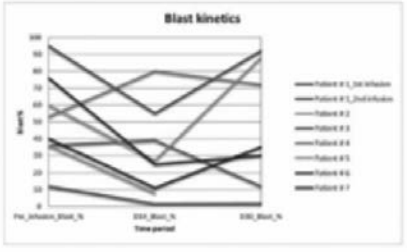

Figure 1. Blast kinetics, pre-infusion, D14 and D30 blast % for individual patients.

Figure 1.

Results: At day +30, 6 patients were evaluable for response and one patient had died. One patients out of 7 showed PR, then developed CR after a second microtransplantation and the patient who died showed PR at D14 marrow evaluation (8% blast). So collectively objective response rate was 28.6%. The patient who developed CR was consolidated with an HLA-matched sibling transplant at day +75 from the 1st microtransplantation (day +50 from 2nd microtransplantation).Three patients attain neutrophil recovery with median time 29 (range, 13-40) days, while the other four patient did not. Two patients attain platelet recovery with median time 34.5 (range, 29-40) days, while the other five patient did not. The cellular therapy did not elicit statistically significant changes in bone marrow blast% over time, F(2, 10)=1.558, p=.258, partial η2=.238., with bone marrow blast% decreasing from pre-infusion blast% (M=60%, SD=22.4%) to D14 blast% (M=39.5%, SD=24.47%) then increased to D30 blast% (M=54.8, SD=33.5%), in that order. Less than four previous chemotherapy, fludarabine-free previous chemotherapy and response naïve patients are the factors associated with good response to microtransplantation. There was a strong positive correlation between patient age (statistically significant), CR1 duration (statistically non-significant) and blast% at D30, r=.842 and .693, p=.036 and p=.307 respectively. There was a moderate negative non-statistically significant correlation between CD34+ cell dose and blast% at D30, r=-.498, p=.315.
Summary/Conclusions: The use of G-CSF primed halo-identical microtransplantation appears to be a biologically active therapy in patients with refractory AML, especially in patients received less than four previous chemotherapy, fludarabine-free previous chemotherapy, response naïve and young age patients.

E1096

ALTERATIONS IN T-CELLS SUBPOPULATIONS AFTER CO-CULTIVATION WITH MULTIPOTENT MESENCHYMAL STROMAL CELLS

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Background: Lymphocyte population depends on immunological state of organism and varies in different diseases and during treatment. Multipotent mesenchymal stromal cells (MSCs) are widely used for cell therapy due to their immunomodulatory properties. Administration of MSCs is not always effective. Immunomodulatory properties of MSCs could be induced by different cytokines, *e.g.* IFN-γ. After injection MSCs interact with activated and non-activated lymphocytes. Changes in lymphocytes subpopulations characterize the influence of MSCs on immunological state.
Aims: The aim of the study was to determine the distribution of naïve and effector cells in lymphocytes co-cultured with MSCs.
Methods: MSCs were derived from bone marrow of 13 donors (7 male and 6 female aged 22 to 62 years, median 27 years). MSCs were co-cultured with allogeneic lymphocytes in a ratio of about 1:10 for 4 days and their basic properties were analyzed over time. Lymphocytes were activated by adding to the culture medium 5mg/ml of PHA (PHA-lymphocytes). Some MSCs were treated for 4 hours with 500 U/ml IFN-g (gMSCs). Activation markers CD25, CD38, CD69, HLA-DR and PD-1 were studied by flow cytometry as well as distribution of naïve and effector cells were analyzed on 1st, 2nd, 3rd and 4th days of cultivation.
Results: By the fourth day of incubation the proportion of naive CD4+ cells reduced by 30% (from 47.5±3.0% to 32.8±3.3%) in cultured lymphocytes. It did not happen in lymphocytes co-cultured with MSCs and gMSCs (p=0.001). At the same time in cultured lymphocytes to the fourth day the number of CD4+ effector memory cells increased in 1.8 times from 19.5±1.9% to 34.6±4.2%, which did not occur when co-cultured with both MSCs and gMSCs (p=0.001). Thus, co-culturing with MSCs or gMSCs prevented naive T-lymphocytes transition into effector cells. The proportion of CD4+/PD-1+ cells increased from 8.2±1.1% to 10.9±0.7% by the 4th day of cultivation. When co-cultured with MSCs and gMSCs the proportion of these cells did not change (p=0.0125). The proportion of HLA-DR+ both on CD4+ and CD8+ cells in lymphocytes remained unchanged for 4 days. When co-cultured with MSCs and gMSCs for 4 days there was a consistent increase in the proportion of CD4+/HLA-DR+ (6.1±0.7% to 15.6±1.1%, p=0.005) and CD8+/HLA-DR+(from 9.7±0.8% to 26.0±3.7%, p=0.024). So allogeneic MSCs induced peptide presentation on lymphocytes. The proportion of CD4+ central memory cells increased in PHA-lymphocytes from 37.4±4.4 at 1st day to 68.2±6.5 at 4th day. MSCs inhibited this increase - the proportion CD4+ central memory cells increased from 24.4±2.7% to 46.6±4.5% (p=0.047). Thus the interaction of PHA-lymphocytes with MSCs inhibited their activation and preserved naïve state.
Summary/Conclusions: The composition of lymphocyte population changes during cultivation. The proportion of naive cells reduced, while the number of effector cells and the proportion of PD-1+ increased, indicating lymphocyte activation probably due to the presence of xenogeneic serum in the culture medium. Co-cultivation with MSCs maintained lymphocytes in not activated state. The interaction of activated lymphocytes with MSCs inhibits their activation and preserves naïve state. IFN-γ priming did not enhance MSCs inhibitory effect on lymphocyte activation. It is shown that MSCs both support naïve lymphocyte condition and have an inhibitory effect on their activation.
The materials are supported by grant from the Russian Science Foundation, Project № 16-15-00102

E1097

OPTIMIZATION OF TRANSDUCTION CONDITIONS WITH GMP LIKE LENTIVIRAL VECTORS FOR THE GENE THERAPY OF PYRUVATE KINASE DEFICIENCY

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Background: Pyruvate kinase deficiency (PKD) is an autosomal recessive disorder caused by mutations in the PKLR gene. PKD is the most common erythroid inherited enzymatic defect causing chronic nonspherocytic hemolytic anemia. PKD is associated with reticulocytosis, splenomegaly and hepatic iron overload, and may be life-threatening in severely affected patients. To-date, allogeneic bone marrow transplant represents the only curative treatment for severely affected patients but has been employed infrequently. Splenectomy confers reduced transfusion-dependence in many patients, but 10-15% of PKD patients remain transfusion-dependent despite splenectomy, which confers increased lifelong susceptibility to systemic infections. Preclinical gene therapy studies conducted in pyruvate kinase deficient mice have shown the safety and the efficacy of a new CPcoRPKW-17 therapeutic lentiviral vector that has been granted orphan drug designation (EMA: EU/3/14/1330; FDA: DRU-2016-5168).

Aims: In order to develop a gene therapy clinical trial for PKD we are optimizing transduction procedures compatible with a clinical application
Methods: Using a GMP-grade lentiviral vector production according to manufacturing process of the CMO VIVEbioTECH (www.vivebiotech.com) using a solid phase bioreactor iCLELLis. These viral batches have been tested for transduction efficiency in healthy cryopreserved cord blood CD34+ cells comparing different viral concentration and one or two transduction rounds.
Results: Increased doses of virus concentration revealed, as expected, increasing levels of transduction that ranged 40-90% both by scoring transduced colony forming units and by flow cytometry analysis in hematopoietic progenitors maintained for 15 days in liquid culture. Analysis of vector copy number (VCN) by qPCR ranged from 0.5 to 3 VCN/cell, demonstrating good transduction efficiency, compatible with a clinical application. Two cycles of transduction showed an increased level of transduction at limiting concentrations of the viral vector, improving the VCN up to 2-fold.
Summary/Conclusions: Transduction optimizations are being carried out in order to reduce the amount of viral vector needed to achieve optimal transduction efficiencies.

E1098

INTERACTION OF MULTIPOTENT MESENCHYMAL STROMAL CELLS WITH LYMPHOCYTES REDUCES THEIR IMMUNO PRIVILEGED PROPERTY
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Background: Multipotent mesenchymal stromal cells (MSCs) are widely used for cell therapy of autoimmune diseases and graft-versus-host disease. MSCs have long been reported to be hypoimmunogenic or 'immune privileged'. The treatment of MSCs with interferon-g (IFNg) increases their immunomodulating properties, but induce HLA-DR expression on their surface. When administered intravenously MSCs interact with activated and non-activated lymphocytes. It is impossible to follow the fate of MSCs in the recipient's organism. The only way to study the changes in the properties of MSCs after intravenous administration is *in vitro* model.
Aims: The aim of the study was to investigate the properties of MSCs after interaction with lymphocytes.
Methods: MSCs were isolated from 13 bone marrow samples used for allogeneic hematopoietic cells transplantation and cultured by a standard method. MSCs were seeded 10^5 cells per flask and a day later 500 units/mL of IFN-g were added for 4 hours to half of the cultures (gMSCs). Some cultures were seeded with 10^6 allogeneic lymphocytes, to half of these cultures 5mg/ml phytohemagglutinin (PHA) was added for lymphocytes activation (PHA-Lymphocytes). For each of the MSCs cultures the mean fluorescent signal intensity level (MFI) of CD90 PE, CD54 APC, HLA-DR APC was measured. Relative expression level (REL) of *IDO1, CFH, PTGES, IL6, CSF1, ICAM-1* was analyzed in MSCs by RT-PCR. MFI and REL were investigated on the 1st, 2nd, 3rd and 4th days of cultivation.
Results: HLA-DR expression on MSCs increased when co-cultured with lymphocytes and after IFNg treatment. PHA-lymphocytes induced HLA-DR expression significantly greater than IFNg licensing. IFNg increased the viability of MSCs when co-cultured with lymphocytes. Immunomodulating properties of MSCs were amplified both after IFNg priming and interaction with lymphocytes, so did not dependent on IFNg source (exogenous or secreted by lymphocytes). The elevated expression ICAM1 on manipulated MSCs may indicate an increase in their adhesive properties. IFNg treatment and interaction with lymphocytes induced in MSCs the increase in relative expression level (REL) of factors involved in immunomodulation (*IDO1, CFH, PTGES, IL6, CSF1*).
Summary/Conclusions: The data suggest that IFNg treatment enhances the immunomodulatory effects of MSCs in the case of intravenous injection into the body. Interaction with lymphocytes of the recipient causes the same change in the properties of MSCs as their pre-treatment of IFNg. However, this treatment stabilizes the MSCs, while maintaining their viability. Based on the results of this work it can be recommended to use: 1. autologous or obtained from the donor hematopoietic cells MSCs; 2. the short-term pre-treated with IFNg MSCs for cell-based therapy for immune modulation in order to increase MSCs survival.

The materials, supported by grant from the Russian Science Foundation, Project № 16-15-00102.

Hematopoiesis, stem cells and microenvironment

E1099

SPECIFICATION OF MURINE HEMOGENIC ENDOTHELIAL HEMATOPOIETIC PRECURSORS CEASES ABRUPTLY BY E10.25 AND CONSTITUTES A FUNCTIONALLY HETEROGENEOUS POPULATION
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Background: Hematopoietic stem cells (HSCs) arise from hemogenic endothelial (HE) precursors between embryonic day 10.5 (E10.5) and E12.5 of murine development, primarily in the aorta-gonad-mesonephros (AGM) region and the umbilical (UA) and vitelline arteries (VA). The window of specification of HE has not yet been defined in any mammalian system.
Aims: To determine the precise window of specification of HE hematopoietic precursors and interrogate at the single cell level their functional heterogeneity.
Methods: Dams pregnant with *Cdh5+/ERT2-CreROSA26Confetti/+*embryos were treated with tamoxifen (TAM) at E7.5, E8.5, E9.5, E10.5 or E11.5. Here, TAM induces the permanent and random labeling of endothelial cells and their progeny with one of the *Confetti* allele fluorescence reporters (YFP, GFP, CFP or RFP). The blood of resulting adult *Cdh5+/ERT2-CreROSA26Confetti/+*offspring was then examined for the presence of *Confetti*+ cells by flow cytometry. Clonal *ex vivo* assays: VE-Cadherin+CD45- cells, which contain HE, were isolated by FACS from E9.5, E10.5 or E11.5 embryos and co-cultured at limiting dilution *ex vivo* with either OP9 stromal cells (OP9) or AGM-derived endothelial cells engineered to express Myr-AKT (AA-ECs), which both support hematopoietic output from HE *ex vivo*. Co-cultures were scored for hematopoietic colony formation, which were then analyzed for hematopoietic re-plating activity and by flow cytometry for hematopoietic cell surface markers.
Results: To estimate the temporal window of TAM activity, CD45.2+ *ROSA26ERT2-Cre/Confetti* bone marrow (BM) cells were transplanted into CD45.2+CD45.1+ recipients treated with TAM three, two, one or zero days before transplant. Only the PB of recipients treated on the same day of transplant showed *Confetti*+cells, indicating that TAM actively labels cells for less than 24 hours. Analysis of 10 weeks old *Cdh5+/ERT2-CreROSA26+/Confetti* mice revealed that only mice exposed to TAM *in utero* at E8.5 and E9.5 had *Confetti*+ blood. Thus, specification of HE begins at E8.5 and is complete by E10.5. Next, E11.5 AGMs isolated from CD45.2+ *Cdh5+/ERT2-CreROSA26+/Confetti* embryos exposed to TAM at E10.5 were cultured as explants for three days under conditions that preserve ongoing HSC specification from HE, dissociated, and then transplanted into CD45.2+CD45.1+ mice. Remarkably, although the CD45.2+ chimerism was high (≈80%) in the blood of recipients, all CD45.2+ blood was negative for the *Confetti* label, further indicating that HE recruitment is complete by E10.5 and cannot be reactivated during explant culture. Limiting dilution co-culture of E9.5, E10.5, and E11.5 VE-Cadherin+CD45- endothelium revealed the frequency of functional HE to be 0.1, 1.1 and 0.19% at these time-points, respectively. Phenotypic analysis of primary hematopoietic colonies revealed heterogeneity in the hematopoietic output of individual HE, with only 9% of colonies producing phenotypic HSCs in AA-EC co-cultures. These data suggest the presence of HE precursors with distinct functional output or the existence of a continuum of HE at different stages of maturation.
Summary/Conclusions: We have defined the window of mammalian HE specification. The abrupt loss of ongoing HE recruitment at E10.25 suggests an active mechanism that terminates this process. We also observed large phenotypic and functional variability amongst individual HE precursors examined throughout ontogeny.

E1100

C-TYPE LECTIN-LIKE RECEPTOR 2 SPECIFIES A FUNCTIONALLY DISTINCT SUBPOPULATION OF MEGAKARYOCYTE-BIASED LONG-TERM HEMATOPOIETIC STEM CELLS
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Background: Recent studies have supported the model in which hematopoietic stem cell (HSC) compartment consists of functionally distinct subsets with discrete self-renewal and differentiation potentials. However, their immunophenotypes and the functional diversities remain poorly understood. We previously reported that the authentically identified HSC population includes a subset of cells expressing the C-type lectin-like receptor 2 (CLEC2), which efficiently give rise to megakaryocyte progenitors (MkPs) and megakaryocytes bypassing the pathway from common myeloid progenitor (CMP) to megakaryocyte/erythrocyte progenitor (MEP) (21th Congress of EHA, # P356, 2016).

Aims: In this study, we analyzed *in vivo* dynamics of CLEC2^{high} HSCs to clarify their functional roles in adult hematopoiesis.
Methods: In this experiment, we defined Lin$^-$Sca1$^+$Kit^{high} CD150$^+$CD34$^-$ cells as HSCs and Lin$^-$Sca1$^-$Kit$^+$CD150$^+$CD41$^+$ as MkP, respectively. We performed transplantation assays using HSCs isolated from EGFP transgenic (CAG-EGFP) mice to trace donor-derived HSCs and their progeny excepting enucleated erythrocytes. In reconstitutional analysis, 5x10^{2}EGFP$^+$CLEC2^{high} and CLEC2^{low} HSCs were transplanted into lethally irradiated mice, respectively. Chimerism and lineage distribution of donor-derived cells were evaluated periodically by tracing EGFP. Secondary transplantation was performed by transferring 1x10^7 BM cells from the recipient mice 16 weeks after the 1st transplantation.
Results: Bone marrow analysis revealed that both EGFP$^+$CLEC2^{high}and CLEC2^{low} donor-derived HSC populations were detected for up to 12 weeks after transplantation. Also, these subsets were capable of generating all lineages of cells in transplanted mice. Interestingly, CLEC2^{high} HSCs generated CLEC2^{low} HSCs in the recipient mice as observed in the steady-state BM, and vice versa. Consistent with these reciprocal transition, both types of HSCs could effectively reconstituted hematopoiesis in the secondary recipients. However, CLEC2^{high} HSCs showed significantly reduced repopulating activity than CLEC2^{low} cells, especially at 12 weeks after transplantation (mean of EGFP$^+$ HSC proportion in the primary recipients with CLEC2^{high} HSCs *vs* CLEC2^{low} HSCs (each n=5): 21.1% *vs* 66.1% at 4 weeks (p=0.054); 2.14% *vs* 48.3% at 12 weeks (p<0.05). In addition, the recipient mice transplanted with CLEC2^{low} HSCs kept high chimeric levels of EGFP$^+$ CMP and MEP, while these levels decreased in the recipients with CLEC2^{high} HSCs. On the other hand, CLEC2^{high} HSCs yielded 2.8-fold more MkPs than CLEC2^{low} HSCs in short term grafts (1 to 2 weeks after transplantation) (p<0.05). Consistent with this finding, CLEC2^{high} HSCs yielded more CD41$^+$ platelets than CLEC2^{low} HSCs by 6.0-fold at 1 week after transplantation (p<0.05), which peaked 10 weeks earlier than in CLEC2^{high}-recipient mice. These platelets yielded through the transient expansion of MkPs were detected at a certain level 12 weeks after transplantation. Furthermore, treatment with fostamatinib (R788), a Syk kinase inhibitor that is an indispensable component for CLEC2 signaling, blocked more potent and rapid megakaryopoiesis in the CLEC2^{high}-recipients, indicating that CLEC2 signaling is essential for rapid and enhanced megakaryopoiesis from CLEC2^{high} HSCs.
Summary/Conclusions: Here, we showed that CLEC2 expression on HSCs demonstrates their oscillation for serving as a potent source of megakaryopoiesis, and found that CLEC2/Syk signaling would be involved in differential regulation between CLEC2^{high} and CLEC2^{low} HSC subtypes.

E1101

PRE-TRANSPLANT DEFECTS OF BONE MARROW ENDOTHELIAL CELLS MAY CAUSE THE OCCURRENCE OF POOR GRAFT FUNCTION AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Poor graft function (PGF) is a serious complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT). PGF is defined as complete donor hematological chimerism with no residual or recurrent leukemia, but a hypo- or aplastic bone marrow (BM) with 2 or 3 of the following: (1) neutrophils ≤0.5×10^9/L; (2) platelets ≤20×10^9/L; and/or (3) hemoglobin concentration ≤70 g/L for at least 3 consecutive days after day +28 post-HSCT. The exact pathogenesis of PGF remains unclear. Mouse studies suggest that endothelial progenitor cells (EPCs), one of the major components in BM vascular microenvironment, modulate the proliferation, self-renewal and differentiation of hematopoietic stem cells(HSCs). In this regard, we previously reported that PGF patients had impaired BM EPCs post-HSCT (*BBMT* 2013; *BMT* 2016). Moreover, we recently reported that the impaired BM EPCs post-HSCT, which could be quantitatively and functionally improved by atorvastatin in vitro, may induce the occurrence of PGF (*Blood, 2016*). However, whether the BM EPCs in subjects with PGF are impaired pre-HSCT and the reconstitution kinetics of BM EPCs post-HSCT remains to be elucidated.
Aims: To investigate whether the BM EPCs in subjects with PGF are impaired pre-HSCT. To compare the reconstitution kinetics of BM EPCs, HSCs and their ROS levels in subjects with PGF and good graft function (GGF) post-HSCT.
Methods: A total of 115 patients who will receive allo-HSCT were prospectively recruited and randomly selected as training group (n=32) and validation group (n=83). The percentage of BM CD45-CD34+VEGFR2+ EPCs, CD34+ HSCs, and reactive oxygen species (ROS) levels in EPCs and HSCs were evaluated in all of the enrolled patients pre-HSCT by flow cytometry. Furthermore, 59 patients were monitored for the frequency and ROS levels of BM EPCs and HSCs at +1, +2 month post-HSCT by flow cytometry. In order to identify risk factors for PGF, pre-HSCT risk factors with a *P*<0.10 on univariate logistic analysis were included in the multivariate logistic regression analysis, and factors with a *P* <0.05 were considered independently associated with PGF.
Results: A total of 18 patients including 5 patients in training group (15.63%) and 13 subjects in validation group (15.67%) developed PGF post-HSCT. Both in training group and validation group pre-HSCT, significantly reduced percentage of BM EPCs were observed in PGF patients than those in GGF patients, whereas no significant differences were found in the percentage of BM HSCs between PGF and GGF patients. Meanwhile, similar ROS levels were demonstrated in BM EPCs and HSCs between PGF and GGF patients. Although there was no difference in transplanted CD34+ cell dose between the PGF and GGF groups, significantly lower percentages of BM EPCs and HSCs, whereas remarkably higher ROS levels were observed in BM EPCs and HSCs in PGF patients than those in GGF patients at +1 month and +2 month after allo-HSCT. Moreover, inverse correlations were observed between BM EPCs frequency and their ROS levels post-HSCT, as well as BM HSCs frequency and their ROS levels post-HSCT. Multivariate analyses revealed that the reduced BM EPCs and the disease status pre-HSCT were independent risk factors for the occurrence of PGF following allo-HSCT.
Summary/Conclusions: We identified that patients with impaired BM EPCs pre-transplant were at a high risk for the occurrence of PGF post-allotransplant. Moreover, persistent low percentage and high levels of ROS in post-transplant BM EPCs may hamper the hematopoietic reconstitution of engrafted donor HSCs in patients with PGF. Therefore, strategies to repair the impaired BM EPCs appear to be a promising therapeutic approach in patients with PGF after allo-HSCT.

E1102

EFFICIENT LYMPHOID DIFFERENTIATION OF HEMATOPOIETIC STEM CELLS REQUIRES CXCR4 DESENSITIZATION

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Background: The Warts, Hypogammaglobulinemia, Infections and Myelokathexis Syndrome (WS) is a rare immunohaematological disorder characterized notably by a chronic lymphopenia. It is mostly caused by inherited heterozygous autosomal gain-of-function mutations in *CXCR4*, which engender a distal truncation in the C-tail domain and lead to a desensitization-resistant receptor. Given that CXCR4 is widely expressed on non-hematopoietic cells and virtually on all leukocytes at multiple stages of development, one possibility could be that the WS-associated peripheral blood lymphopenia is a consequence of skewed production, differentiation or distribution of lymphocytes related to altered CXCR4-mediated signaling. Recently, we have been able to replicate the hematologic phenotype of WS using a knock-in mouse strain that harbors the WS-linked heterozygous *CXCR4^{S338X}* mutation causing a distal truncation of the last 15 residues of the C-tail domain (Balabanian et al., Blood, 2012). Mutant mice displayed lymphocytes with enhanced migration to Cxcl12, phenocopied severe lymphopenia and failed to maintain antibody titers after immunization (Biajoux et al., Cell Reports, 2016). First-line analyses of +/1013 mice suggested developmental defects at the pro/pre-B cell stage in the bone marrow (BM) and during the early double-negative stages of thymocyte maturation. However, whether impaired lymphopoiesis stems from an upstream cell-intrinsic hematopoietic defect remains to be established.
Aims: We took advantage of our relevant knock-in model and the access to blood samples from WS patients to investigate the impact of CXCR4 desensitization on BM and extra-medullary splenic hematopoiesis and recirculation of hematopoietic stem and progenitor cells (HSPCs).
Methods: The global hematopoietic development, including quiescence, cycling and survival properties of HSPCs, was examined in non-manipulated and BM-chimeric mice using flow-cytometric- and clonogenic-based assays. Cxcr4 expression and function were assessed using internalization, chemotaxis, *in vivo* homing and AMD3100-promoted mobilization experiments. Both multipotency and self-renewal abilities of HSPCs have been assessed using serial BM transplantation experiments. Immunophenotypic and clonogenic analyses of HSPCs were performed from blood samples of five WS patients and age-, sex-matched healthy donor volunteers.
Results: We showed that Cxcr4 desensitization is required for quiescence/cycling balance of murine short-term HSCs and their differentiation into multipotent (MPPs) and downstream lymphoid-biased progenitors (*i.e.* LMPPs and CLPs). Alteration in this negative feedback mechanism resulted in dramatic decrease of circulating HSPCs in five patients with WS. This was also evidenced in WS mice and mirrored by accumulation of HSPCs in the spleen, where enhanced extramedullary hematopoiesis occured.
Summary/Conclusions: Efficient Cxcr4 desensitization is critical for the lymphoid differentiation of HSPCs and its impairment is a key mechanism underpinning the lymphopenia observed in mice and likely in WS patients.

E1103

A SUBSET OF ADULT HSC DERIVES FROM GATA4-EXPRESSING PROGENITORS LOCATED IN THE PLACENTA AND LATERAL MESODERM OF MICE

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Background: GATA4 is a transcription factor expressed in mesoderm and endoderm during development. Members of the family such as GATA1-3, but not GATA4, are critically involved in hematopoiesis. An enhancer (G2) of the mouse *Gata4* gene directs its expression throughout the lateral mesoderm and the allantois, beginning at E7.5, becoming restricted to the septum transversum by E10.5, and disappearing by midgestation (Rojas et al., *Development*, 2005, 132:3405). Our previous work has shown that inactivation of *Gata4* using this $G2^{Cre}$ driver is lethal by midgestation (Delgado *et al.*, Hepatology, 2014, 59:2358). The anemia observed in the $G2^{Cre;}Gata4^{flox/flox}$ embryos was attributed to a failure in the expansion of the hematopoietic progenitors in the fetal liver. Interestingly, a small population of hepatic YFP^+ cells from $G2^{Cre;}R26R^{EYFP}$ embryos was positive for leukocyte and megakaryocyte markers, suggesting that a lineage of hematopoietic cells could derive from GATA4-expressing progenitors.
Aims: To study in our murine models the origin and properties of the hematopoietic lineage derived from progenitors expressing GATA4 under control of the G2 enhancer.
Methods: We analyzed hematopoietic organs of G2-$Gata4^{Cre;}R26R^{YFP}$ mice, adults and embryos, by flow cytometry, RT-PCR and confocal microscopy. Cells obtained from different tissues were cultured and transplanted to analyze *in vitro* and vivo potential.
Results: YFP+ cells represented about 20% of the hematopoietic system of adult mice and contributed in the same proportion to the lymphoid, myeloid and erythroid lineages. Adult YFP+ hematopoietic stem cells (Figure 1) constituted a long-term repopulating, transplantable population. Fetal YFP+ hematopoietic progenitors were much more abundant in the placenta than in the aorta-gonad-mesonephros area. These placental YFP+ progenitors were clonogenic in the MethoCult assay and fully reconstituted hematopoiesis in myeloablated mice (Cañete et al., *Haematologica*. doi: 10.3324/haematol.2016.155812. [Epub ahead of print]).

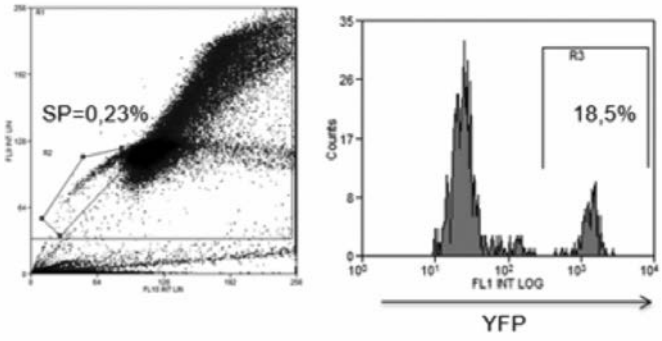

FIGURE 1: The side population of the bone marrow from $G2^{Cre;}$ $R26R^{EYFP}$ mice identified by Hoescht 22242 staining, which contains adult HSC, includes a fraction of YFP+ cells. (from Cañete et al., 2017)

Figure 1.

Summary/Conclusions: A lineage of adult hematopoietic stem cells in mice is characterized by the expression of GATA4 in their embryonic progenitors and probably by its extraembryonic (placental) origin. Both lineages basically showed similar physiological behavior in normal mice, but this finding raises a number of questions, for example: Does this hematopoietic stem cell subpopulation show a different response in physiopathological conditions? Does this subpopulation show a differential profile of gene expression? Does a similar heterogeneity exist in human HSCs? We are currently investigating the transcriptome of the G2-GATA4 linage HSC in order to answer these questions.

E1104

EXPLORING THE MECHANISM OF FOG1-DEPENDENT TRANSCRIPTIONAL REGULATION IN ERYTHROID CELLS

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Background: GATA-1 is a hematopoietic zinc-finger transcription factor, which controls the development of erythrocytes and megakaryocytes. Numerous proteins have been reported to be associated with GATA-1 to alter its activity. Among them, Friend of GATA-1 (FOG1), a nine-zinc finger protein, is expressed in a tissue-specific pattern that overlaps markedly with that of GATA-1. FOG1 is an essential coregulator of GATA-1 during hematopoiesis, which mediates transcriptional activation and repression of GATA-1 target genes; yet, the mechanisms by which FOG1 exerts its activating and repressing functions remain unknown.
Aims: We explored a novel role of FOG1 by forcibly expressing FOG1 in the human K562 erythroleukemia cells.
Methods: *FOG1* mRNA was cloned into Flexi HaloTag vector (Promega) and pBABE-puro retrovirus vector (Addgene), and FOG1 was overexpressed in K562 cells. Quantitative ChIP analysis was performed using antibodies against GATA-1, GATA-2, TAL1, FOG1, histone H3 acetylated-K4 (H3K4ac), H3K9ac, H3 trimethylated-K4 (H3K4me3), and H3K27me3. *PU.1* regulatory element was cloned into luciferase plasmid (pGL4.10, Promega) and mutation within the *cis*-element was introduced using a site-directed mutagenesis kit (Agilent). TAL1 loss-of-function analysis was conducted with specific siRNA. For transcription profiling, Human Oligo chip 25K (Toray) was used.
Results: Forced FOG1 expression in K562 cells induced the expression of erythroid genes (*HBA*, *HBB*, and *SLC4A1*), whereas repressed that of *GATA-2*, which have been reported to be FOG1-dependent GATA-1-target genes (Lee *et al*, Mol Cell 2009). On the other hand, FOG1 overexpression did not affect the expression of master regulators of erythropoiesis, such as *GATA-1* and *TAL1*. Next, we conducted microarray analysis to comprehensively characterize FOG1-regulated gene ensemble. The analysis demonstrated that 942 and 180 genes were upregulated and downregulated (> 2-fold), respectively, in the FOG1-overexpressed cells. Noticeably, we found that the expression of *PU.1*, known as a myelo-lymphoid-promoting transcription factor, was strongly downregulated by FOG1 overexpression, indicating that *PU.1* is another FOG1-dependent GATA-1 target. Because GATA-1 ChIP sequencing analysis identified a GATA-1 peak in the *PU.1* promoter (Fujiwara *et al*, Mol Cell 2009), which contained evolutionally conserved consensus GATA-binding motif (AGATAG), we performed a transient luciferase promoter analysis to test whether FOG1-mediated transcriptional repression of *PU.1* could be regulated at the GATA site located at its promoter. Co-transfection of the FOG1 expression vector significantly reduced the promoter activity of *PU.1*, and this effect was clearly diminished by disruption of the GATA motif, suggesting that this motif has an important role in FOG1-mediated transcriptional repression of *PU.1*. Quantitative ChIP analysis demonstrated increased GATA-1 chromatin occupancy at both FOG1-activated (globins, *SLC4A1*) as well as FOG1-repressed (*GATA-2*, *PU.1*) gene loci. However, while TAL1 chromatin occupancy was significantly increased at FOG1-activated gene loci, it was significantly decreased at FOG1-repressed gene loci. When FOG1 was overexpressed in TAL1-knocked down K562 cells, FOG1-mediated activation of *HBA*, *HBG*, and *SLC4A1* was significantly compromised by TAL1 knockdown, suggesting that FOG1 may require TAL1 to activate GATA-1 target genes. To estimate the molecular mechanisms by which FOG1 confers transcriptional repression, we evaluated the epigenetic landscape at FOG1-repressed gene loci. Quantitative ChIP analysis demonstrated that activating marks (H3K4ac, H3K9ac, and H3K4me3) were significantly decreased, whereas repressive H3K27me3 was not affected, by FOG1 overexpression.
Summary/Conclusions: Our results provide important mechanistic insight into the role of FOG1 in the regulation of GATA-1-regulated genes and suggest that FOG1 has an important role in inducing cells to differentiate toward the erythroid lineage rather than the myelo-lymphoid one by repressing the expression of *PU.1*.

E1105

THE STEM CELL ZINC FINGER 1 (SZF1) / ZNF589 PROTEIN INHIBITS TUMOR DEVELOPMENT IN A K562 XENOGRAFT MOUSE MODEL, BLOCKING CELL CYCLING AND INDUCING PREMATURE CELLULAR SENESCENCE

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Background: The stem cell zinc finger 1 (SZF1) / ZNF589 protein, a member of the family of Krüppel associated box domain-zinc finger (KRAB-ZNF) transcription factors, has an isoform exclusively expressed in CD34+ hematopoietic stem/progenitor cells (HSPCs), suggesting its role as epigenetic regulator of specific genes involved in hematopoiesis. The SZF1/ZNF589 gene exhibits a human-specific evolutionary nucleotide DNA-change leading to a complex molecular organization and to a protein structure peculiar for humans, as compared to all other primates, potentially conferring human-specific functional properties. SZF1/ZNF589 has recently been shown to control cell viability in the hematopoietic system. It is regulated by the HIF-1α hypoxia-induced transcription factor and is differentially expressed in a cytokine-dependent manner during hypoxia in CD34+ HSPCs (Venturini et al., Exp Hematol 2016; 44: 257-268). Thus, SZF1/ZNF589 may play a role in the maintenance of hematopoietic stem cell quiescence and survival, known to be influenced by the hypoxic state in the bone marrow niche.
Aims: We studied the effects of SZF1/ZNF589 overexpression *in vitro* and evaluated its tumor suppressor potential *in vivo*.

Methods: K562 (BCR-ABL positive chronic myeloid leukemia in blast crisis)-Luciferase-control or K562-Luciferase-SZF1/ZNF589 cells were directly injected into the femurs of NSG mice and tumor development was monitored by bioluminescence. Furthermore, K562 cells with or without SZF1/ZNF589 overexpression were studied by proliferation assay, cytomorphology, flow cytometry, cell cycle analysis, cyclin B1 expression and beta-galactosidase assay.
Results: K562-dependent tumor growth was efficiently inhibited in NSG mice transplanted with K562-Luc-SZF1/ZNF589-cells as compared to mice transplanted with K562-Luc-control-cells, leading to significantly prolonged survival, demonstrating a strong tumor suppressive potential of SZF1/ZNF589 *in vivo*. In vitro, overexpression of SZF1/ZNF589 dramatically inhibited proliferation of K562 cells which, instead of dying, became giant and dysplastic, without other significant morphological changes and in absence of polyploidy. Cell cycle analysis revealed a blockade in G2/M phase, with cyclin B1 accumulation characteristic for mitotic arrest. As suggested by morphology and beta-galactosidase assay, these cells were entering premature senescence.
Summary/Conclusions: SZF1/ZNF589 controls survival of hematopoietic cells mediated by mitotic arrest and premature senescence, exhibiting tumor suppressing functions *in vivo*.

E1106

THE FUNCTIONAL RELEVANCE OF DNMT3A SPLICE VARIANTS IN HEMATOPOIETIC DIFFERENTIATION

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Background: DNA methyltransferase 3A (DNMT3A) plays a pivotal role for *de novo* DNA methylation (DNAm) during development. It seems to be of particular relevance in hematopoietic differentiation because it is frequently mutated in acute myeloid leukemia or clonal hematopoiesis. So far, it is unclear how DNMT3A governs the multitude of lineage-specific DNAm patterns – it is conceivable that this can at least partly be attributed to alternative splicing of *DNMT3A*.
Aims: In this study, we followed the hypothesis that specific splice variants of *DNMT3A* impact on hematopoietic differentiation or DNAm patterns. Therefore we addressed the role of specific splice variants of *DNMT3A* in hematopoietic stem and progenitor cells (HSPCs).
Methods: Expression of *DNMT3A* splice variants was modulated in HSPCs: transcript 1+3 (Tr.1+3), transcript 2 (Tr.2), or transcript 4 (Tr.4) of *DNMT3A* were either knocked down by short hairpin RNA or constitutively overexpressed by lentiviral infection. Expression changes were validated by qRT-PCR. Subsequently, we evaluated the impact on colony formation potential (CFU assay), proliferation (CFSE assay), and the immunophenotype (CD34+ and CD133+). Global DNAm profiles were generated with the Infinium HumanMethylation450 BeadChip platform and gene expression profiles with the Human Affymetrix Gene ST1.0 platform.
Results: Downregulation of either Tr.2 or Tr.4 reduced the proliferation rate of HSPCs significantly (n=3, p<0.05). HSPCs maintained CD34 expression for a higher number of cell divisions upon knockdown of Tr.2 (n=3; p<0.05). In colony forming unit (CFU) assays downregulation of Tr.4 resulted in a clear bias towards erythroid colonies (n=3, p<0.05). Overall, CFU frequency was reduced by knockdown of *DNMT3A* transcripts, whereas it was increased by overexpression. Subsequently, we analyzed the impact of specific *DNMT3A* variants on the DNAm patterns: several CpG sites revealed significant differences in DNAm levels upon knockdown of Tr.2 and Tr.1+3 (8,905 and 352 CpGs, respectively; n=3, adjusted p-value <0.05). Notably, these patterns were regulated in the opposite direction upon overexpression of the same transcripts. Knockdown of Tr.4, which does not have the DNA-methyltransferase domain, did not evoke significant changes in DNAm. Furthermore, modulation of *DNMT3A* splice variants resulted in transcript-specific gene expression changes, which may at least partly be attributed to the DNAm changes.
Summary/Conclusions: Our results demonstrate that the various splice variants of *DNMT3A* have different functional sequel on HSPCs. Knockdown and overexpression resulted in opposite and transcript-specific DNAm changes. Thus, alternative splicing of *DNMT3A* is relevant for site-specific epigenetic modifications in hematopoietic development.

E1107

ERYTHROPOIETIN STIMULATES TRANSDIFFERENTIATION OF BONE MARROW PRO-B CELLS INTO BONE-RESORBING OSTEOCLASTS

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Background: Erythropoietin (EPO) is a crucial kidney-derived hormone responsible for erythropoiesis; however, its extra-erythroid effects are substantial and correlate with EPO receptor (EPO-R) expression in both hematopoietic and non-hematopoietic tissues. Bone turnover is regulated by the coupled actions of osteoblasts, the bone-forming cells, and monocyte-derived osteoclasts, which mediate bone resorption. In this regard, we have recently reported that EPO directly stimulates bone resorption *via* activation of EPO-R signaling in the monocytic lineage (Hiram-Bab et al., 2015). Monocyte differentiation into osteoclasts relies on monocyte-macrophage colony-stimulating factor (M-CSF) and the receptor activator of nuclear factor kappa B ligand (RANKL). B cells are also known to regulate bone metabolism, chiefly *via* paracrine signals. Osteoclasts and B cells arise from distinct myeloid and lymphoid progenitors, respectively, which are downstream of a common multipotent progenitor cell.
In the bone marrow (BM), Pro-B cells sequentially differentiate into Pre-B and immature B cells. Whether BM B cells can transdifferentiate into osteoclasts remains controversial, since osteoclast differentiation from residual monocytic precursors in the cultures was not excluded in earlier studies.
Aims: We set to determine whether B cells can transdifferentiate to osteoclasts and to assess the effect of EPO on this process.
Methods: Experiments were conducted on C57BL/6J or CD19-Cre;R26R-EYFP, 8-12-week-old female mice in accordance and with the approval of the Institutional Animal Care and Use Committee of Tel-Aviv University (M-14-043). BM cells were flushed from femurs, tibias, and pelvic bone and red blood cells were lysed. Cells were stained with labelled anti-mouse antibodies: PE-B220, FITC-CD19, PerCP-IgM, PeCy7-CD43, and APC-M-CSF receptor/CD115; and sorted by flow cytometry. Cells were then cultured in α-MEM containing 10% fetal bovine serum, M-CSF and RANKL. Multinucleated osteoclasts were stained for tartrate-resistant acid phosphatase (TRAP) and pit resorption was assessed.

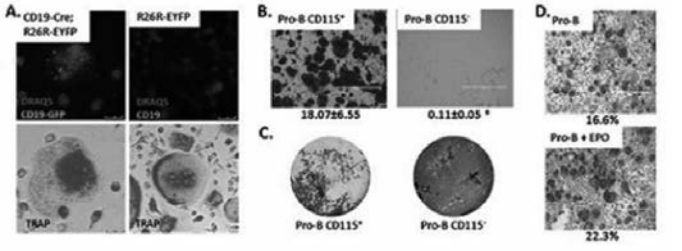

Figure 1.

Results: B cells isolated from BM of CD19-Cre:R26R-EYFP mice cultured with M-CSF and RANKL differentiated into TRAP$^+$ multinucleated osteoclasts that were also positive for EYFP, thus tracing back their B cell origin (Figure 1A). Next, we dissected which B cell precursor subtype possesses this osteoclastogenic capacity and found that only Pro-B (B220$^+$CD19$^+$CD43^{High}IGM$^-$), but not Pre-B (B220$^+$CD19$^+$CD43^{Low}IGM$^-$) nor immature B cells (B220$^+$CD19$^+$CD43$^-$IGM$^+$) could transdifferentiate into osteoclasts (16%±3.7 *vs.* 0.79%±0.28 and 0.48%±0.13 osteoclasts' area, respectively). Moreover, among the Pro-B cells, only those expressing M-CSF receptor (CD115) could transdifferentiate into functional osteoclasts (18%±6.55 *vs.* 0.11%±0.05 osteoclasts' area, respectively, Figure 1B and C). Using an anti-EPO-R specific antibody we detected EPO-R on the surface of B cells and noted that EPO enhanced the differentiation of the Pro B cells into osteoclasts by as much as 70% (p=0.04) (Figure 1D). **Figure 1: Osteoclastogenesis** *in vitro* **from sorted B cells. (A)** Transdifferentiation of 180,000 cells/well CD19-Cre;R26R-EYFP into osteoclasts. DRAQ5 (blue) and anti-GFP (green) for CD19-Cre;R26R-EYFP and CD19-Cre cells. Bottom - TRAP staining. Confocal images (x20 magnification) **(B)** TRAP staining of osteoclasts derived from the indicated sorted cells from BM (10,000 cells/well) and cultured with M-CSF and RANKL. Left – Pro-B cells expressing CD115 (B220$^+$CD19$^+$CD43^{High}IgM$^-$CD115$^+$). Right – Pro-B cells not expressing CD115 (B220$^+$CD19$^+$CD43^{High}IgM$^-$CD115$^-$). Data are mean±SEM of osteoclast area, n=5 mice in each group; *p<0.05. **(C)** Pit resorption area from the indicated sorted cells cultured on calcium phosphate-coated plates. **(D)** TRAP staining of osteoclasts derived from sorted Pro-B cells (B220$^+$CD19$^+$CD43^{High}IgM$^-$ cells; 180,000cells/well)±5U/ml EPO, n=7 mice in each group. Data are% osteoclasts of a representative image; EPO *versus* Control was 70% increase, p<0.05.
Summary/Conclusions: Taken together, our data suggest a new physio-pathological role for BM B-cell precursors in bone metabolism via their capacity to differentiate into functional osteoclasts, and a possible role for EPO in this process.

E1108

Abstract withdrawn.

E1109

Abstract withdrawn.

E1110

BONE MARROW MYELOPOIESIS INDEPENDENTLY OF CANONICAL NOTCH SIGNALING

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Background: Notch signaling is a highly conserved pathway important in multiple developmental processes. Canonical signaling through all Notch receptors converges on the Csl transcription factor recombination signal binding protein for immunoglobulin kappa J region (Rbpj). In haematopoiesis, Notch is critical for the emergence of definitive hematopoietic stem cells (HSCs) in the embryo and in thymic T cell development. Contrastingly, canonical Notch signaling has been shown to be dispensable for HSC homeostasis in the adult bone marrow (aBM). Recent studies have however suggested a role of Notch in promoting megakaryocyte (Mk) and erythroid (E) progenitor cell development as well as in suppressing granulocyte-macrophage (GM) progenitor expansion and acting as a tumor-suppressor in myeloid malignancies. However, these findings were largely made through genetic approaches potentially also affecting regulatory pathways distinct from canonical Notch signaling.
Aims: To unambiguously investigate the role of canonical Notch signaling in aBM myelopoiesis, in steady-state and following transplantation.
Methods: B6-SJLCD45.1, *Rbpj$^{fl/fl}$, Mx1-Cre*, *Vav-Cre* and *Vwf-eGFP* BAC mice were used. Flow cytometry (FACS) was applied for phenotypic analyses. Gene expression levels were measured by real-time reverse transcription-PCR. Mk/E and GM *in vitro* colony forming potentials were applied in mouse colony assays. For transplantation study, lethally irradiated recipients were competitively transplanted (1:1) and reconstitution assessed 7-9 weeks after transplantation.
Results: FACS staging of GM, Mk and E progenitors in aBM of *loxP*-flanked *Rbpj* mice crossed to both *Mx1-Cre* and the pan-hematopoietic *Vav-Cre* strains was applied. As expected, HSCs were unaffected. Not previously investigated, FACS analysis of distinct stages of GM, Mk and E progenitors revealed no defects, at any progenitor stage, in *Rbpj*-deficient mice. To demonstrate that this lack of a phenotype was not due to BM cells escaping *Rbpj* deletion, we FACS purified HSCs and all GM, Mk and E progenitor stages from *Rbpj*-deficient mice and verified a virtually complete deletion of *Rbpj* in all populations. In further agreement with canonical Notch signaling not being required for steady-state generation, maintenance or stepwise differentiation of adult GM, Mk and E progenitors, the number of GM, E and Mk colonies generated from unfractionated aBM cells as well as circulating platelet counts were also unaffected in *Rbpj*-deficient mice. We next sought to address whether we could uncover a role of the Notch pathway in regulation of GM, Mk and E progenitors by establishing BM chimeras in which *Rbpj*-deficient progenitors compete with wild type (WT) progenitors for replenishment and differentiation in lethally irradiated recipients. No deficiencies were observed in the replenishment of HSCs and any stages of GM, Mk and E progenitors in mice competitively transplanted with *Rbpj*-deficient as compared to control WT BM cells. Moreover, transplanted *Rbpj*-deficient and control progenitors contributed equally well to platelet reconstitution. We next investigated whether loss of canonical Notch signaling might nevertheless impact on expression of genes for key regulators the Mk and E lineages at distinct progenitor stages for these lineages, as previously implicated. Notably, transcript levels of genes encoding key Mk/E regulators were unaffected in *Rbpj*-deficient Mk/E progenitors. In previous studies, expression of Notch target genes in Mk and E progenitors in aBM has been implicated as reflecting activation through Notch signaling. However, since the expression of Notch targets might also be regulated by other pathways besides Notch pathway, we investigated whether the expression of key Notch target genes (*Hes1, Hes5, Nrarp* and *Gata3*) in Mk/E progenitors in aBM was dependent on canonical Notch signaling. Neither in HSCs or any Mk/E progenitor was the expression of these Notch genes negatively affected by *Rbpj*-deficiency, demonstrating that their low expression levels in aBM HSCs and Mk/E is independent of canonical Notch signaling.
Summary/Conclusions: Studies implicating canonical Notch signaling as a critical regulator of aBM Mk, E and GM progenitors potentially failed to target only canonical Notch signaling. Herein, we demonstrate that canonical Notch signaling is dispensable for generation and replenishment of Mk, E and GM progenitors in aBM in steady-state as well as following BM transplantation.

E1111

IDENTIFICATION OF NOVEL HUMAN HEMATOPOIETIC STEM CELL SUBPOPULATIONS VIA COMPREHENSIVE SURFACE MARKER ANALYSIS

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Background: All hematopoietic cells are derived from hematopoietic stem cells (HSCs), which exhibit capacities for multilineage differentiation and long-term self-renewal. Human HSCs can be isolated by Fluorescence-activated cell sorting (FACS) with the combination of several surface markers, such as CD34, CD38, CD45RA and CD90. The HSC fraction reportedly consists of functionally heterogeneous subpopulations, including multi-potent and/or lineage-biased progenitors {Notta:2016hj} and HSC-like populations with reduced self-renewal capacity {Notta:2011bg}; however, prospective isolation of bona fide human HSCs is still challenging due, at least in part, to the lack of specific markers.
Aims: The goal of this study is to identify a novel HSC-specific surface marker(s) that enables prospective isolation of functionally-distinct HSC subpopulations.
Methods: We examined expression levels of 342 cell surface markers in the HSC fraction (Lin-CD34+CD38-CD45RA-CD90+) by FACS using commercially-available antibodies. Single-cell gene expression profilings of isolated subfractions were performed using Fluidigm C1 system in combination with Biomark. Differentiation potential of each HSC fraction was assessed by single-cell colony assays in methylcellulose. *in vitro* lineage tracing in liquid culture were performed to determine hierarchical relationships among subfractions.
Results: Among 342 cell surface proteins examined, only CD35, CD115 and CD215 were detected in the HSC fraction. We focused on CD35, which is also known as complement receptor type 1 (CR1), as its expression was most distinct among the three markers. CD35-positive population accounted for 15~50% of the human HSCs, defined as Lin-CD34+CD38-CD45RA-CD90+ cells, in adult bone marrow and cord blood. HSCs exhibited multi-lineage reconstitution capacity without lineage-biased differentiation in a single-cell colony assay regardless of the CD35 levels. CD35+HSCs gave rise to CD35-HSCs in lineage tracing experiments, suggesting that CD35+HSCs reside upstream of CD35-HSCs in the hierarchy of hematopoietic differentiation. Single-cell gene expression profiling of CD35-positive or -negative HSCs indicated that CD35+HSCs, but not CD35-HSCs, are phenotypically homogenous, expressing cell cycle-related genes and lineage-specific markers at low levels.
Summary/Conclusions: Our data suggest that HSCs can be further subdivided into subfractions based on CD35 levels. CD35 might be a useful marker to prospectively isolate the most primitive human HSC fraction. *in vivo* functional assays using xenotransplantaion models are currently underway, and the results will be discussed at the meeting.

E1112

DEVELOPMENT OF A 3-DIMENSIONAL CULTURE TO MIMICK THE BONE MARROW MICROENVIRONMENT AND RECAPITULATE DRUG RESISTANCE FOR IN VITRO STUDY

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Background: Chronic myeloid leukemia (CML) is a haematological malignancy caused by acquisition of the *BCR-ABL1* oncogene. Demonstration of the central role of BCR-ABL1 kinase activity in CML pathogenesis led to the development of imatinib, an ABL1-specific tyrosine kinase inhibitor. Most patients on imatinib attain good clinical and molecular responses, despite the persistent presence of a low level of therapy-refractory leukaemia stem cells (LSCs), which reside in the bone marrow niche. However, in a significant minority of patients these cells eventually provide a reservoir for disease relapse and subsequent malignant progression. A greater understanding of the biology of imatinib-resistant LSCs could therefore be of significant clinical benefit. One of the proposed mechanisms of drug resistance in CML LSCs is close contact with the surrounding microenvironment, however an in-vitro model of the bone marrow matrix is currently lacking.
Aims: Development of a 3-Dimnsional culture using fibre scaffolds to mimick bone marrow microenvironment in order to study the mechanism of resistance to anti-leukaemia agents
Methods: Scaffold production: PMMA solution was prepared by dissolving PMMA in chloroform and adding appropriate amount of hydroxyapatite to polymer solution followed by pressurized gyration and collection of the PMMA fibres using a rod - collector. **2 Dimensional (2D) Cell culture:** K562 and HL60 cell lines were cultured and treated with or without imatinib or doxorubicin respectively.**3D Cell culture:** The same experiment was performed in the presence of scaffolds. **Co-culture of HS-5 with HL60 or K562 cells:** GFP$^+$ stromal cells HS-5 cells were added to PMMA-HA scaffold. K562 and HL60 cells were treated in the presence or absence of imatinib and doxorubicin in HS-5 cells plus scaffold environment respectively and were investigated for proliferation and viability 72h later.
Results: We produced a PMMA-based 3D scaffold and compared the growth of CML and AML cell lines grown in this scaffold in the presence and absence of cytotoxic or targeted therapy to that of cells grown in 2D culture. PMMA-HA scaffold was not toxic to the leukaemia cells as primary AML cells and also K562 cells grew in the presence of scaffold and also concentrated around the

fibres. Treatment of K562 or HL60 cells with imatinib or doxorubicin respectively resulted in a lower level of apoptosis in cells grown on the 3D scaffold compared to those grown in 2D culture. Further development of this 3D culture by adding stromal cells HS-5 to the scaffold reduced even further the sensitivity of K562 or HL60 to imatinib or doxorubicin, respectively.

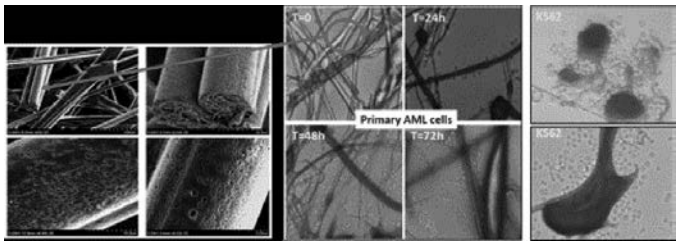

Figure 1.

Summary/Conclusions: The relative resistance to either imatinib or doxorubicin that we observed in cells grown in 3D culture supports a role for the bone marrow matrix in the protection of leukaemic cells against chemotherapeutic agents. A combination of the PMMA-HA with HS-5 cells made this system more similar to the bone marrow microenvironment as this is a model in which all the basic components of the bone marrow microenvironment such as scaffold, stromal cells and cytokines (secreted by HS-5) are present. The results of this study show adding extra complexity to the microenvironment changes the sensitivity of the cells to therapeutic agents, better recapitulating the situation observed in-vitro. Three dimensional cultures using the PMMA-HA/HS-5 model may prove useful in the investigation of therapy resistance in leukaemia and for the discovery of new agents capable of eradicating quiescent leukaemic stem cells.

E1113

WHOLE EXOME SEQUENCING REVEALED SEQUENTIAL GAIN OF MUTATIONS IN TWO CASES OF DONOR CELL HAEMATOLOGICAL MALIGNANCY AFTER HEMATOPOIETIC TRANSPLANTATION

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Background: The leukemic transformation of otherwise healthy donor stem cells provides a useful *in vivo* model to study the mechanisms involved in leukemogenesis.
Aims: We report two cases of donor cell derived haematological malignancy in which whole-exome sequencing (WES) was performed in bone marrow (BM) samples from recipient at different times after allogeneic hematopoietic stem cell transplantation (allo-HSCT) in order to study the dynamics of emergence of mutations that precede the development of donor cell leukemia (DCL) and donor cell myelodysplastic syndrome (DC-MDS).

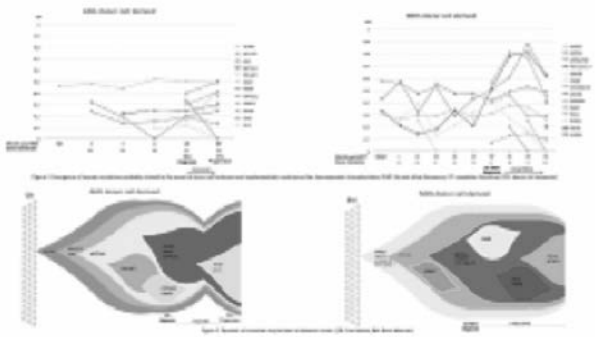

Figure 1.

Methods: Case 1: A 43-year-old female diagnosed with lymphoblastic leukemia-B t(1;19), who developed acute myeloid leukemia (AML) with normal karyotype, *NPM1*+of donor origin 16 months after unrelated cord blood transplantation (UCBT). Case 2: A 65-year-old male diagnosed with mantle cell lymphoma, who developed MDS 45,XX,-7,del(12)(p12) of donor origin, 57 months after allogeneic BM transplantation from his HLA-identical brother. WES (SureSelect-XT Human-exon 50Mb) was performed by next generation sequencing (Hiseq) on donor stem cells (SCs) infused as well as on BM samples from recipient after allo-HSCT. The exome of donor SCs and 5 BM samples, from case 1, were aligned to the human reference genome (GRCh 37/hg19) and donor SCs and 9 BM samples were aligned to GRCh 38/hg38 in the second case.
Results: WES analysis revealed progressive emergence of multiple somatic mutations probably related to the development of leukemia in bone marrow samples post allo-HSCT (Figure 1). Both SCs showed alterations that may be involved in leukemogenesis. (Case 1: *SH2B3* and case 2: *KMT2C, KMT2A, ARHGAP26* and monosomy 7). Somatic mutations, acquired over time, fall into genes that play well-established roles in signalling pathways. Mutations in leukemic subclones that disappear after chemotherapy were indentified, as well as the acquisition of new mutations in resistant subclones. We propose a possible model of leukemogenesis in these cases (Figure 2).
Summary/Conclusions: The present study reveals a process of sequential clonal expansions, promoted by the acquisition of additional somatic mutations in donor hematopoietic cells. Detection of heritable or acquired gene mutations in donor associated with predisposition to haematological malignancies could have clinical implications for the patients undergoing to allo-HSCT. Although the cause of donor cell derived haematological malignancy onset seems to be multifactorial, the infusion of a SCU with pre-leukemic potential in a context of residual toxicity in recipient as a result of pre-transplant chemotherapy, a post-transplant environment characterized by a decreased immune surveillance may well have played role in these cases. The study of a greater number of DCL cases by next generation sequencing could help to understand this process and to detect new mutations involved in the emergence of AML.

E1114

LEUKEMIC STEM CELL-RELATED MRNA EXPRESSION ANALYSIS USING A NOVEL FLOW CYTOMETRY-BASED ASSAY

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Background: Gene expression analysis of protein-coding (mRNA) and non-coding RNA in paediatric and adult acute myeloid leukaemia (AML) has become of paramount importance for therapeutic decision-making, revealing prognostic information and for the identification of novel therapeutic targets. AML is a clinically, phenotypically and molecularly heterogeneous haematological malignancy, with different leukemic cell populations organized in a hierarchical fashion, and leukemic stem cells (LSCs) residing at the apex herein. Unfortunately, gene expression profiling is commonly performed on unfractionated bulk samples, leading to 'expression averaging' of these heterogeneous leukemic cell populations. Multicolor flow cytometry (FCM) is capable of distinguishing heterogeneous cell populations based on the phenotypic characterization at a single-cell level. However, fluorochrome-conjugated antibodies are not available for intracellular RNA targets.
Aims: To evaluate the applicability of a novel flow cytometry-based technique, PrimeFlow™ RNA assay, to measure cell-of-interest RNA expressions in heterogeneous AML samples.
Methods: Technical assessment was performed using six neuroblastoma cell lines with varying levels of *MYCN* gene amplification. Correlation to expression data obtained by the gold standard RT-qPCR, performance in rare (0.1%) cell populations, effects of cryopreservation and off-target effects were evaluated. Next, diagnostic material of *de novo* AML patients was used to measure target gene (*Wilms' tumor 1* (*WT1*)) and reference gene (*RPL13a, GAPD*) expression. Expression analysis was performed in unfractionated bulk leukemic cells as well as blasts and rare subsets of leukemic cells, *e.g.* LSCs. FCM analyses were performed on a FACSCanto II (BD Biosciences) with set-up according to EuroFlow guidelines. Infinicyt™ (Cytognos®) was used for data analysis and mean fluorescence intensities (MFI) values (with/without normalisation) were interpreted. P-values < 0.05 were considered significant.
Results: mRNA expression quantified by PrimeFlow™ significantly correlated with data obtained by RT-qPCR and remained detectable in rare (0.1%) cell populations. *WT1* expression was shown to be statistically significantly higher in bulk leukemic cells of those patients characterized by *WT1* overexpression, as defined by RT-qPCR, showing a mean 52% MFI upregulation by PrimeFlow™ if WT1 overexpression was present, as shown by the gold standard RT-qPCR. Moreover, *WT1* overexpression could be detected within heterogeneous cell populations, *e.g.* the CD34+CD38+ cell population and the LSC (defined as CD34+CD38-), showing a 63% and 45% MFI upregulation, respectively, compared to patients with normal *WT1* expression levels, although only statistically significant in the former population. *GAPD* and *RPL13a* expression differed intra- and interpatient in the bulk (defined as CD34+) and both of the aforementioned populations. Interestingly, reference gene expression was consistently higher in CD34+/CD38+ cells compared to LSCs.

Summary/Conclusions: Key mRNA target expressions in AML, *e.g. WT1* gene expression, could be evaluated using PrimeFlow™ RNA assay, including rare and heterogeneous cell populations herein, *e.g.* LSCs. This study demonstrates that PrimeFlow™ is a technique of interest for the discovery of novel LSC-specific targets.

E1115

POTENTIAL PREDISPOSING GERMLINE MUTATIONS IN PATIENTS WITH CONCOMITANT MYELOID AND LYMPHOID MALIGNANCIES

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Background: Recent findings have suggested that mutations predisposing the development of either acute myeloid leukemia (AML) or chronic lymphocytic leukemia (CLL) may arise in pre-leukemic hematological stem cells. In addition, genes involved in epigenetic regulation, such as *TET2*, and RNA processing, such as *SFB31*, are mutated in both myeloid and lymphoid malignancies. This could indicate a possible genetic link between myeloid and lymphoid malignancies. Therapy related AML (t-AML) is a known complication to treatment with cytotoxic drugs such as alkylating agents and topoisomerase inhibitors. The susceptibility of developing t-AML has been associated with variation in DNA-repair pathways, drug metabolism and transport.
Aims: In this study, we aimed to investigate a possible common genetic origin of hematological cancers in patients with concomitant CLL and *de novo* AML or myelodysplastic syndrome (MDS) and in patients with concomitant therapy-related AML (t-AML) and CLL.
Methods: The presence of concomitant lymphoid and myeloid malignancies in patients is rare, however we managed to include 3 patients with *de novo* AML and CLL, one patient with MDS and CLL, one patient with chronic myelomonocytic leukemia(CMML) and CLL, and two patients with t-AML and CLL. The patients' diagnoses were based on the evaluation of the morhophology, immunohistochemsitry, cytogenetics, and flowcytometry analysis in accordance to the WHO classification. For each patient mononuclear cells (MNCs) from blood or bone marrow were isolated using Ficoll gradient centrifugation and used for fluorescence activated cell sorting (FACS) of the malignant clones and the T-cells. Paired end exome sequencing (2x150) aiming for an average coverage of 50-100x was performed using either the HiSeq2500 or NextSeq500 platforms from Illumina. Raw sequencing data was processed using CASAVA-1.8.2. Mapping to the human genome (hg19/GRCh37 UCSC) was performed using CLC Biomedical Genomics Workbench (Qiagen) mapping cell software. Variants with a frequency of 5% or above were called.
Results: We identified possible pre-disposing germline mutations in all 7 patients by comparing variants between the myeloid malignant clone, CLL cells, and T cells, as well as using saliva to aid in characterizing the mutations as either germline or only present in the hematological compartment. In all the patients except one with *de novo* AML and CLL, we identified a potential damaging germline variant in a DNA-repair related gene, such as *ATM (*387dupA, D130fs*4), *SMARCAL1 (*2114C>T, T705I), *HELQ (*393_397delAGGTG, G132fs*16), *SWI5* (652C>T, R218*), *LIG1*(2168A>G, Q761R) and *PRKDC*(902G>A, C301Y). In the remaining patient with concomitant *de novo* AML and CLL, we identified a potential damaging germline variant in an epigenetic regulator believed to play a role in normal and malignant hematopoiesis, *KDM2B(*44delC, P15fs*92). Furthermore, we identified the somatic mutational landscapes of the malignant clones using T-cells as germline tissue for the patients with concomitant *de novo* AML/MDS/CMML and CLL and for the two patients with t-AML and CLL. The somatic mutational landscapes of the malignant clones in the *de novo* concomitant cases and the cases with CLL and t-AML were quite similar to what has previously been reported in isolated cases of disease. The myeloid and lymphoid malignant clones did not share any of the mutations, indicating development of two independent malignancies.
Summary/Conclusions: Our results suggest a possible role of germline variations in the susceptibility to development of concomitant *de novo* hematological cancers as well as t-AML. However, further studies including more patients are needed to confirm this hypothesis.

E1116

THE MUTATIONAL LANDSCAPE OF DNMT3A MUTATIONS IN CLONAL HAEMATOPOIESIS OF INDETERMINATE POTENTIAL. CHIPPING AWAY AT THE PROBLEM

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Background: Dysfunction of epigenetic modifiers contributes significantly to the pathogenesis of acute myeloid leukaemia (AML). One frequently mutated gene involved in epigenetic modification is DNMT3A (DNA methyltransferase-3-alpha). Approximately 22% of de-novo AML and 36% of cytogenetically normal AML are found to have DNMT3A mutations and around 60% of these mutations affect the R882 codon. In particular, the R882H mutation has been associated with a poor prognosis and survival outcomes for patients. A large number of DNMT3A mutations are present in clonal cells in healthy individuals with no characteristics of haematological malignancy and is termed as clonal haematopoiesis of indeterminate potential (CHIP).

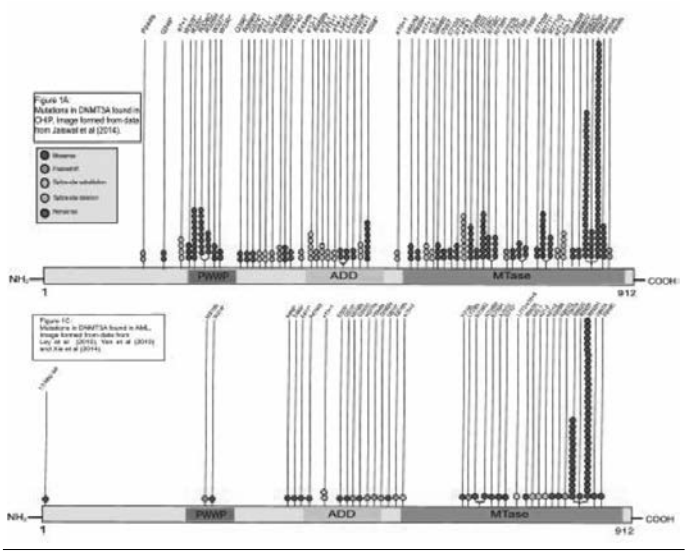

Figure 1.

Aims: We aimed to compare here the locations and types of mutations identified in AML and in CHIP in the DNMT3A gene by several different studies.
Methods: To review the mutations found in CHIP and AML, we carried out an extensive literature search of CHIP studies and AML studies that had mapped a large number of mutations in this gene. Mutations were collated to form several diagrams illustrating and comparing these findings.
Results: When DNMT3A mutations in CHIP were compared to mutations in AML the R882 residue was still found to be the most frequently mutated residue in both CHIP and AML. Figure 1 clearly illustrates the mutations in comparison to AML. However, only 13% of all reported mutations were found at the R882 residue in CHIP, while in AML 60% DNMT3A mutations are found at the R882H mutations.
Summary/Conclusions: Analysis of the mutational landscape of CHIP has clearly highlighted the role of DNMT3A mutations in clonal haematopoiesis in older healthy individuals, the significance of such preleukaemic clones is yet to be determined.

E1117

NEXT-GENERATION REFERENCE INTERVALS FOR PEDIATRIC HEMATOLOGY

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Background: Interpretation of hematology analytes in children is challenging due to extensive changes in hematopoiesis with age leading to pronounced sex- and age-specific dynamics. To facilitate clinical decision making based on quantitative hematology test results, reference intervals are used to classify samples according to upper and lower limits, and age-related change is represented using reference intervals partitioned into separate age groups. However, this approach can only approximate the continuous physiological dynamics of hematological analytes in childhood and does not enable appropriate quantification of test results in relation to the reference distribution. Conversely, percentile charts as used in anthropometric quantities (e.g. pediatric weight-for-age charts) would allow adequate appreciation of pediatric hematology test results. However, the ethical and practical challenges unique to pediatric reference intervals have restricted the creation of such percentile charts, and limitations in current approaches to laboratory test result display prevent their integration into clinical decision making.
Aims: To create percentile charts for hematology analytes from birth to adulthood using a data-mining approach and to demonstrate their integration into clinical care with benefits in clinical decision making.

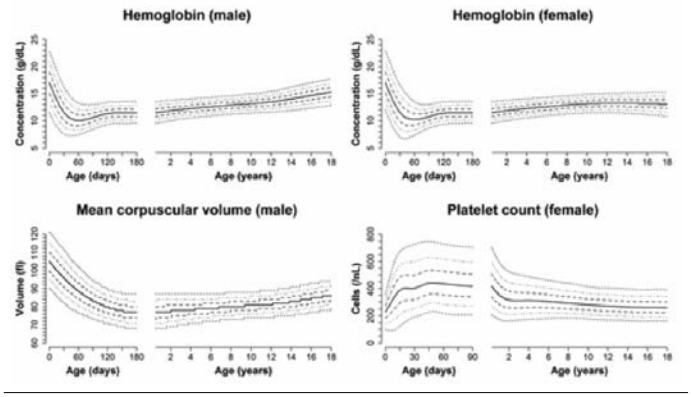

Figure 1.

Methods: We applied a data-mining algorithm to generate percentile charts for hematology analytes using laboratory data collected during the clinical care of patients. A total of 9,517,245 samples from 343,463 patients (72,614–337,011 samples per analyte) from 8 German tertiary care centers and 2 German laboratory service providers were examined. Percentile charts were calculated using an established statistical approach which extracts the proportion of samples from healthy individuals from the unfiltered input dataset containing both non-pathologic and pathologic samples. To evaluate the clinical benefit of hematology test result interpretation using percentile charts, accuracy and speed of pediatricians assessing eight different predefined clinical situations were measured in comparison to conventional test result representations.
Results: We created percentile charts for hematology analytes in girls and boys from birth to 18 years which can be used as common reference intervals. Results are provided for hemoglobin, hematocrit, red cell indices, red cell count, red cell distribution width, white cell count, and platelet count, example charts for hemoglobin, mean corpuscular volume, and platelet count are shown in the accompanying figure. A web application at www.pedref.org/hematology demonstrates hematology test result interpretation using percentile charts and z-scores with special consideration of pediatric dynamics. Comparison of pediatricians' decision times when assessing different clinical scenarios using percentile charts and conventional representations shows more correct decisions (75.9% *vs* 68.4%, p<0.01) which are made in shorter time (2.7 s *vs* 3.8 s, p<0.01) when using percentile charts.
Summary/Conclusions: The created percentile charts enable the appropriate differential diagnosis of changes in hematology analytes due to disease and changes due to physiological development. Integration of suitable forms of result reporting using the provided percentile charts into clinical decision making improves assessment of the unique dynamics in pediatric hematology.

E1118

GROWTH FACTOR INDEPENDENCE 1 (GFI1) REGULATES THE AML SUPPORTING FUNCTION OF MESENCHYMAL STROMAL CELLS

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Background: Mesenchymal stromal cells (MSCs) harbor and support the function of normal hematopoietic stem cells. Less is known about their interaction with leukemic cells, *e.g.* in acute myeloid leukemia (AML). The prognosis of AML, a clonal malignant disease of the bone marrow (BM), is still poor with only 25% of patients living longer than 5 years.
Aims: In the current study, we investigated the interaction between MSCs and AML cells. and we also investigated the underlying molecular mechanism.
Methods: We used cell cultures using primary cells from human and mice and cell lines of MSCs and AML cells. Different Mouse models of human AML were used in our study to confirm the results obtained from human sample. MSCs were characterized by differentiation assay, flow cytometry and RT-PCR. Matrigel test was also applied in this study.
Results: MSCs from AML patients called AML-associated MSCs (AMSCs) or from murine models of human leukemia enhance significantly *in vitro* the growth of leukemic cells compared to AML cells growing without MSCs or in presence of MSCs from non-leukemic patients or mice. Among other, AMSCs increased entry of leukemic cells into the cell cycle, and at the same time protected the leukemia cells against exogenous toxic events such as chemotherapy or irradiation. The interaction between AMSCs and leukemia cells is dependent on cell-to-cell contact. *In vivo*, absolute and relative numbers of AMSCs and other stromal cells, *i.e.* endothelial cells and osteoblast lineage cells were highly expanded in the BM of mice modeling of human AML. AMSCs showed a higher efficiency of capillary tube formation in the matrigel assay than normal MSCs which gives an additional indication that AMSCs were polarized by leukemia cells towards a tumor-supporting state. On a molecular level, the polarization of MSCs towards an AML-supporting state depends on upregulated expression of the transcription factor Growth factor independence 1 (Gfi1). Loss of Gfi1 diminished the tumor-supporting state of AML-associated MSCs.
Summary/Conclusions: We conclude that leukemia cells polarize AMSCs towards a leukemia-supporting state in a Gfi1-dependent manner, which could open the way to new therapeutic approaches.

Hodgkin lymphoma - Clinical

E1119

BASELINE LEUKOCYTE AND EOSINOPHIL COUNTS PREDICT OUTCOME IN RELAPSED OR REFRACTORY CLASSICAL HODGKIN LYMPHOMA PATIENTS TREATED WITH PD1 INHIBITION

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Background: Despite encouraging efficacy of anti-PD1 antibodies in relapsed or refractory (r/r) classical Hodgkin lymphoma (cHL), not all patients achieve a lasting response, with few complete remissions (CR) observed. Thus, identification of predictive biomarkers is important. Recently, two models using readily available differential blood count parameters have been suggested to predict outcome in melanoma patients treated with immune checkpoint inhibition.
Aims: In this study, we aimed to identify baseline differential blood count parameters associated with response and progression free survival (PFS) in r/r cHL patients treated with anti-PD1 antibody nivolumab.
Methods: We retrospectively investigated baseline differential blood count parameters and their association with response and progression free survival (PFS) in 30 r/r cHL patients treated with the anti-PD1 antibody nivolumab. All 30 patients had previously received multiple lines of treatment, including treatment with high dose chemotherapy followed by autologous stem cell transplant (ASCT) for r/r disease; the median number of prior treatment lines was 5 (2-11) and 21 patients received prior brentuximab vedotin. To investigate the association of baseline blood count parameters (white blood cell count (WBC), relative monocyte count (RMC), relative neutrophil count (RNC), relative lymphocyte count (RLC) and relative eosinophil count (REC)) with outcome after PD1 inhibition, we used the last differential blood count performed immediately prior to the first received dose of nivolumab.
Results: RMC, RNC and RLC did not have a prognostic impact on PFS, whereas higher WBC ≥ 7.78x10³/µl and lower REC<1.7% were associated with worse PFS in both univariate and multivariate analysis. We constructed a simple score to prognosticate PFS. By adding 1 point each for WBC ≥ 7.78x10³/µl and REC<1.7% to the score, we could clearly differentiate a low (score=0), intermediate (score=1) and high risk (score=2) group for disease progression (p<0.001). Only one PFS event occurred in the best prognostic group (n=10, median PFS (days): NA]) whereas 5 out of 11 patients in intermediate (median PFS (days): 365 [129-NA]) and 7 out of 9 patients in high risk group progressed (median PFS (days): 197 [50-NA]). Evaluation of best response achieved according to the initial risk score showed a trend towards higher CR-rates in low risk group, but was not significant.

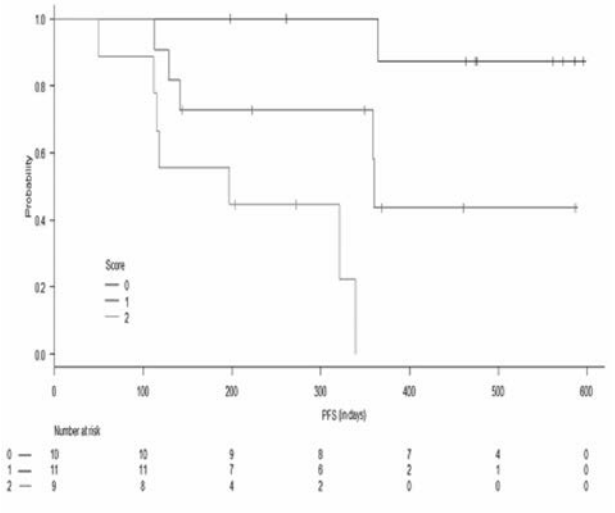

Figure 1.

Summary/Conclusions: Our simple prognostic model, mainly characterized by a normal to high REC, robustly discriminates three risk groups for PFS. Almost all patients in the low risk group achieved a durable remission without disease progression throughout the study period, despite often achieving just a partial response. In contrast, high-risk patients often progressed quickly despite initially achieving a partial or complete response. Further validation of this score which is easily available from routine clinical parameters in a larger cohort of patients and further investigation of its potential predictive impact is needed. Moreover, efforts to clearly understand a possible mechanistic role of eosinophils in cHL patients treated with PD1-inhibiton are warranted.

E1120

THE PROGNOSTIC SIGNIFICANCE OF BETA-2 MICROGLOBULIN (B2M) LEVELS IN PATIENTS WITH HODGKIN LYMPHOMA (HL) TREATED WITH ABVD OR EQUIVALENT (ABVDEQ) CHEMOTHERAPY OR COMBINED MODALITY THERAPY (CT/CMT)

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Background: The prognosis of HL primarily depends on clinical stage (CS) as well as limited-stage risk classification schemes and the International Prognostic Score (IPS), both of which delineate further prognostic subgroups within stages. B_2m is a well-established prognostic factor for several hematologic malignancies, but its role in HL is yet controversial. Between 1993 and 2016, several reports from other groups have yielded heterogenous results in small-to-medium-sized patient series of no more than 220 patients, frequently under variable treatment.
Aims: Our aim was to investigate the prognostic significance of serum b2m levels in HL.
Methods: We analyzed 864 patients with HL treated with ABVDeq CT/CMT (1990-2016) and selected solely based on the availability of pretreatment b_2m levels. B_2m [P1] levels (upper normal limit 2.4mg/L) were analyzed according to other baseline features and prognostic factors as well as according to the outcome: Freedom From Progression (FFP) was defined as time between treatment initiation and treatment failure (primary refractoriness, PR with switch to alternative CT or relapse); deaths of unrelated causes were censored. Overall Survival (OS) was measured from treatment initiation to death of any cause. ROC curves and sequential cut-offs (1.8-3.5 by 0.1 increments) were used to explore the potential impact of b_2m on FFP and OS.
Results: The median follow-up for currently living patients was 88 months. *Univariate Analysis:* FFP was significantly inferior in patients with higher b2m at all tested cut-off points. At 2.4mg/L (normal *versus* elevated) the 10-year FFP was 81% *vs* 71% (p=0.003). However, the best cut-off was the observed median value of this series, calculated at 2.1mg/L, with 10-year FFP rates of 84% *vs* 71% (p<0.0001). In early stages (IA/IIA) significant results were obtained at cut-offs between 1.8 and 2.1mg/L. The best cut-off was 1.9mg/L, a close approximation of the median b_2m level of early stage patients, with 10-year FFP of 89% *vs* 78% (p=0.003). In advanced stages, none of the cut-offs yielded statistically significant results (borderline at 2.0mg/L; 10-year FFP 77% *vs* 67%, p=0.057). *Multivariate Analysis:* B_2m levels remained significant for FFP after adjustment for IPS factors, ESR and B-symptoms at both 2.1mg/L and 2.4mg/L cut-offs [hazard ratio (HR) 1.78, p=0.001 and 1.41, p=0.04 respectively) in the whole series of 864 patients. In early stages, b2m was a significant predictor of FFP at the cut-offs of 1.9mg/L and 2.1mg/L (HR 2.00, p=0.01 and 1.83, p=0.02 respectively), but only borderline at the cut-off of 2.4mg/L (HR 1.65, p=0.07). In advanced stages, b2m emerged as an independent prognostic factor for FFP at the cut-off of 2.2mg/L (HR 1.59, p=0.046 despite the lack of significance in univariate analysis), but was not significant at the 2.4mg/L cut-off. The 10-year OS was lower in patients with high b_2m levels (10-year rates 91% *vs* 76%, p<0.0001).
Summary/Conclusions: Higher serum b_2m emerged as a significant independent predictor of FFP at the cutoff of 2.0mg/L for the whole series and 1.9mg/L for early-stage patients. The prognostic significance in advanced stages was weaker (best cut-off 2.2mg/L). Serum b_2m was also highly predictive of OS. This is by far the largest report on the prognostic significance of b_2m in HL, highlighting the significance of the cut-off used to define "high" levels. Its significance is more pronounced in early stage disease. The optimal cut-off for the evaluation of serum b_2m in HL may be stage-dependent and appear to lie between 1.9 and 2.2mg/L, thus performing better than a "normal *versus* high" evaluation (cut-off 2.4mg/L).

E1121

THE PREDICTIVE VALUE OF INTERIM PET-CT IN ELDERLY PATIENTS WITH HODGKIN LYMPHOMA

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Background: Hodgkin lymphoma (HL), a disease of mostly young patients, also peaks in the elderly. Despite the profound improvement in the clinical outcome of young patients, in the elderly, 5 year overall survival (OS) is estimated at only 40-55%. Interim PET-CT (iPET), known to be highly predictive for progression free survival (PFS) in young patients with HL, has not been sufficiently validated in elderly patients, nor have many other outcome predictors in HL of the elderly.
Aims: The objective of the present study was to evaluate the significance of iPET in elderly patients with HL.
Methods: All consecutive patients (age ≥60) diagnosed with HL between 1998-2016 were retrospectively reviewed in this multi-center study. Baseline characteristics as well as PET-CT results at diagnosis, interim analysis and end of treatment (EOT) were recorded and analyzed. PET-CT results were classified as no evidence of disease (NED), partial response (PR), stable disease (SD) and progressive disease (PD).
Results: Ninety five patients from 5 centers were identified. Median age was 71 (range 60-89) years. Subtype was nodular sclerosis in 48% and mixed cellularity in 23%. Sixty three (69%) patients had advanced disease and mean international prognostic score (IPS) was 3.5±1.4. Fifty nine (63%) patients received first line treatment with ABVD, in 13 (14%) chemotherapy was followed by involved field radiotherapy. At EOT, sixty seven (82%) patients achieved CR, 6 (7%) achieved PR, 10 (11%) were primary refractory and 2 (2%) died during treatment. Fifteen (16%) patients experienced relapse. Five year PFS and OS were 56% and 78%, respectively. ABVD treated patients had 5 year PFS and OS of 59% and 82% as opposed to 48% and 68% for all other regimens, but these differences were not statistically significant. Seventy two (76%) patients had undergone both iPET and EOT-PET. 50 patients had NED on iPET, 20 had PR, 1 SD and 1 PD. NED EOT-PET was achieved in 47/50 (94%) patients who had NED iPET, 12/20 (60%) patients who had PR iPET and none of the patients with SD/PD iPET (p<0.01). In patients with either NED or PR on iPET, relapse occurred in 11 (15%) patients and 5 year PFS and OS were 62% and 85%, respectively. The 5 year PFS of these patients differed according to the depth of response on iPET - 69% *vs* 45%, (p=0.02, fig.1) in patients achieving NED *vs* PR, while 5 year OS did not reach statistical significance, 90% *vs* 71% (p=0.08). Restricted analysis, evaluating only 59 patients who were treated with ABVD, showed similar results with 94% of NED iPET *vs* 45% of PR iPET achieving NED on EOT-PET (p<0.01). Outcome differed according to the depth of response in iPET with 5 year PFS rates of 74% *vs* 34%, in patients achieving NED *vs* PR, respectively (p<0.01). 5 year OS rates were 92% *vs* 76%, in patients achieving NED *vs* PR (p=0.1).

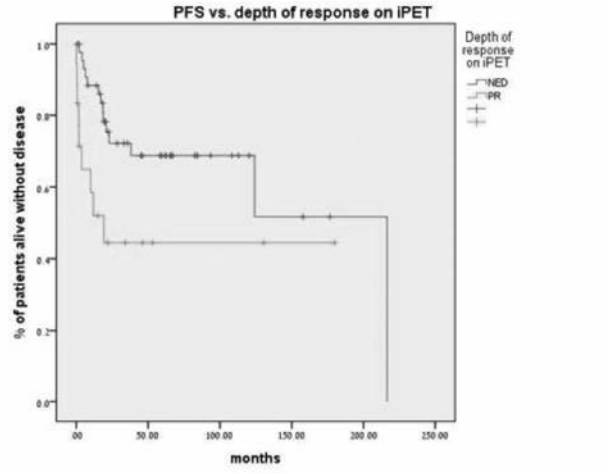

Figure 1.

Summary/Conclusions: We present a cohort of elderly patients with HL, most were treated with ABVD. Outcome was comparable or even superior to previously published cohorts. Traditional outcome measures for HL have not been extensively validated in the elderly. iPET and EOT-PET, known to be highly predictive for PFS in young HL patients, appeared to be highly predictive in elderly individuals. The improved prognosis, suggested by our results, may be related to the high rate of iPET which was used in this cohort. The importance of this tool in HL in the elderly is emphasized by the diminished prediction power of the traditional outcome measures in elderly HL patients.

E1122

HIGH-DOSE BENDAMUSTINE PLUS BRENTUXIMAB COMBINATION IS EFFECTIVE AND HAS A FAVOURABLE TOXICITY PROFILE IN THE TREATMENT OF REFRACTORY AND RELAPSED HODGKIN LYMPHOMA

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Background: The management of patients with refractory or relapsed Hodgkin lymphoma (HL), especially after autologous stem cell transplantation (ASCT), remains controversial. Bendamustine has demonstrated efficacy in several lymphoproliferative disorders but limited data are available regarding the schedule in patients with HL, in particular its dosage and the possible combinations for a synergistic effect. Brentuximab Vedotin is a CD30-directed antibody-drug conjugate, currently approved for the treatment of relapsed or refractory HL.
Aims: The objective of this retrospective observational trial was to evaluate efficacy and safety of salvage cytotoxic regimens in patients with refractory and/or relapsed HL. Three different regimens were evaluated.
Methods: From May 2011 to December 2016, 32 consecutive patients (19 M/13 F) with a median age of 31.7 years (range, 16-73) received a salvage regimen after failure of ASCT. Patients were by chance assigned to one of these three arms: standard dose bendamustine (90mg/sqm) days 1 and 2 plus standard DHAP schedule (every 4 weeks) x 3 cycles (Arm A, n= 10 cases), brentuximab single agent 1.8mg/kg (every 3 weeks) x 4-8 cycles (Arm B, n= 11 cases), high dose bendamustine (120mg/sqm) days 1 and 2 plus brentuximab 1.8mg/kg (day 3) x 4-6 cycles (Arm C, n= 11 cases). Each cycle in arm C was repeated every 28 days and growth factor support was systematically administered, in association with antimicrobial prophylaxis. The treatment efficacy in each arm was evaluated according to Revised Response Criteria for Malignant Lymphoma by Cheson *et al.* Any adverse event occurred was recorded and classified for type and grade using NCI-CTCAE criteria (v 4.0).
Results: In arm A, the overall response rate (ORR) was 40% (4/10 patients), with 4 (40%) complete remission (CR) and 6 (60%) progressive disease (PD). Hematological toxicity was grade 3 thrombocytopenia in 4 patients (40%) and bone marrow aplasia in 1 patient (10%); extra-hematological toxicity was gastrointestinal toxicity of grade 2 in 6 patients (60%) and grade 1 in 3 patients (30%), In arm B, ORR was 63.6% (7/11 patients), with 5 (45%) CR, 2 (18%) partial response (PR) and 4 (36%) PD. Hematological toxicity was grade 2 neutropenia in 4 patients (36%), extra-hematological toxicity was grade 3 neuropathy in 2 patients (18%), In arm C, ORR was 100% (11/11 patients), with 11 CR followed by SCT (second autologous transplant, 6 cases; and haploidentical transplant, 5 cases) with persistence of complete remission in all patients at a median follow-up of 33.4 months (range, 12-60). Hematological toxicity was grade 3 thrombocytopenia in 4 patients (36.3%); extra-hematological toxicities were increase of transaminase (grade 2) in 3 patients (27%) and cytomegalovirus (CMV) reactivation in 2 patients (18%), treated successfully with valganciclovir. Three patients had fever during infusion at first cycle, together with a skin rash, managed with corticosteroid injections, and a successful antihistamine plus corticosteroid prophylaxis in the next cycles of treatment.

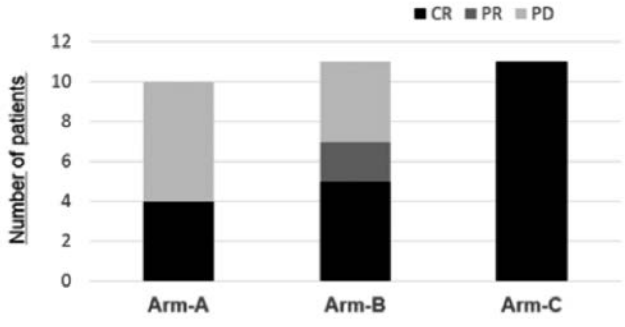

Figure 1.

Summary/Conclusions: High-dose bendamustine plus brentuximab has shown relevant efficacy and a relatively good safety profile in a setting of heavily pretreated patients with HL. Adequate monitoring of CMV reactivation is recommended. This combination could be considered as a bridge to second autologous or allogenic SCT. However, these results should be validated by controlled and prospective studies involving larger number of patients.

E1123

NEED OF HORMONAL THERAPY TO PRESERVE FEMALE FERTILITY IN HODGKIN E NON-HODGKIN LYMPHOMA PATIENTS FOLLOWING CHEMOTHERAPY: A TWO-CENTER SURVEY

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Background: In the last decades, Hodgkin and Non-Hodgkin Lymphoma (HL-NHL) therapies have resulted in high cure rates and increased survival. However, younger patients (< 50 years), experienced severe late toxicities, such as, gonadal toxicity that can result in permanent sterility.
Aims: to evaluate different aspects of fertility (menstrual status, pregnancy, and menopause) in women with HL and NHL in reproductive age before and after chemotherapy.
Methods: By a phone interview we administered a questionnaire to the patients. The interview was composed of questions concerning reproduction (pregnancies, menses and abortion) and also menopausal status. The analyses were made using data collected in a cohort 109 women patients from two Italian hematologic centers. Statistical analysis was carried out in Graphpad® system, data were compared by the chi-square (P value <0.05 was consider to be statistically significant)
Results: the median age (in years) at the time of the treatment was 31 (range 16-49), 69/109 (63%) had HL and 40/109 (37%) NHL, 74/109 [ES1] (64%) of the patients had a stage I-II. All HL patients were treated with ABVD, whereas the remaining NHL patients were treated with R-CHOP (20%) or similar regimens (16%), respectively. Radiotherapy was delivered to the 62/109 (57%) of the sample. Complete Remission (CR) was obtained by the 101/109 (93%) and only 16/101 (16%) relapsed. Considering the gynecologic history of the patients there were no statistically significance difference between the regularity of menses and the event of an abortion pre and post treatment. As for pregnancies, 35% of patients had children before therapy and 17% after. Among these 109 patients, 68/109 (62%) received gonadotropin-releasing hormone (GnRH) analogues and/or oral contraception, while 41 (38%) were not treated with hormonal therapy. Among the 68 patients who received hormonal therapy regular menses recovered in 61/68 (90%) while in those of the control group a recover of menses was observed in 20/41 (48%). This difference was statistically significant (P<0.05). The same was observed as for early menopause. In this case excluding patients who had a natural menopause, a lower cases of early menopause was observed in those who received hormone therapy (8/65, 12% *vs* 23/38, 61%; P<0.05). Considering only the 81/109 (74%) patients who had regular menses after chemotherapy, 61/81 (75%) received hormonal therapy and 20/81 (25%) were not treated with hormonal therapy. Before treatment for lymphoma, 16% of patients belonging to the hormonal group had pregnancies *versus* 45% of the control group (P<0.05). Following therapy, pregnancies were observed in 23% of those receiving hormonal therapy *vs* 5% of the control group (P<0.05).
Summary/Conclusions: The use of hormonal therapy is fundamental not only to favor of pregnancies and motherhood but in particular to avoid the consequences of an irregular cycle or an early menopause with its symptoms and clinical implications.

E1124

25(OH)VITAMIN D SERUM LEVELS IN HODGKIN LYMPHOMA

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Background: Vitamin D has pleiotropic effects on cellular differentiation, proliferation, apoptosis and angiogenesis in addition to maintaining serum calcium and skeletal homeostasis. Several studies suggest that low serum 25(OH)D levels may be associated with inferior outcome in solid tumors as colorectal and breast cancer, and in Non-Hodgkin lymphomas [Drake *et al*, J Clin Oncol 2010; 28:4191] as diffuse large B cell lymphoma [Bittenbring *et al*, J Clin Oncol 2014; 32:3243], and follicular lymphoma [Kelly *et al*, J Clin Oncol 2015; 33:1482]. 25(OH)Vitamin D levels have not been reported for Hodgkin Lymphoma (HL).
Aims: To evaluate vitamin 25(OH)D levels in patients with HL and analyze for associations with clinical characteristics and clinical outcome.
Methods: We studied 76 patients with cHL (40 females, 36 males, median age 33 years), diagnosed at our Institution between 2014 and 2016. Treatment consisted in ABVD (66 patients), BEACOPP d.e. (7 patients), and COPP (2 patients). One patient received only radiotherapy. Serum samples for vitamin D quantification were collected before the first day of chemotherapy. 25(OH)D was measured in patients' sera using a standardized clinical assay, the DiaSorin LIAISON 25-OH Vitamin D TOTAL. 25(OH)D levels were defined according to three conditions: deficient (<10 ng/ml), insufficient (10-30 ng/ml), and sufficient (>30 ng/ml).
Results: The median 25(OH)D level at diagnosis was 20.6 ng/ml (range: 5.5 to 42.3 ng/ml). 25(OH)D levels were considered normal in 8 (10.5%) patients, insufficient in 59 (77.5%) patients, and deficient in 9 (12%) patients. Looking at patient characteristics, 25(OH)D levels were lower in patients with age over 60 years (p=0.002), reduced performance status (ECOG>1) (p=0.01), stage IV disease (p=0.01), and IPS (Hasenclever) score >2 (p=0.002). Furthermore levels were lower in patients with hemoglobin below 10,5 g/dl (p=0.06). No association was found with gender, albumin level, B symptoms. In addition, there was a significant seasonal variation, with 25(OH)D levels to be lowest in the first quarter and highest in the third quarter (p=0.03). FDG-PET evaluation after 2 cycles of chemotherapy according to the 5-point Deauville scale was available in 66 patients. Vitamin D levels were not associated with interim PET response. With a median of 12 months follow-up of patients is still short. Patients with deficient levels (n=9) had a significantly worse PFS than patients with higher levels (n=67) (p=0.002). The probability of progression-free survival at 12 months was 87% (95% C.I., 75-94%) in patients with 25(OH)D levels>10 ng/ml, while patients with levels<10 ng/ml had a 12 months PFS of 47% (95% C.I., 12-76%). We included 25(OH)D levels, IPS (that includes age, stage and hemoglobin level), ECOG and season in a multivariate Cox analysis. Deficient 25(OH)D level had a borderline significance (HR 5.65, 95% C.I.,0.98-32.55; p=0.05).
Summary/Conclusions: 25(OH)Vitamin D serum levels are frequently low in patients with Hodgkin Lymphoma and are associated with patient-related and disease-related characteristics. Our preliminary analysis suggests that low 25(OH)D levels might be associated with worse prognosis.

E1125

NODULAR LYMPHOCYTE PREDOMINANT HODGKIN LYMPHOMA: A NEW RISK ADAPTED TREATMENT STRATEGY BASED ON RITUXIMAB

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Background: Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) is a rare variant of Hodgkin's lymphoma (HL), that only accounts for 5% of all HL. Due to its rarity, consolidated and widely accepted guidelines of treatment still lack for this type of HL. Due to NLPHL cells expression of CD20, targeted therapy with Rituximab (R), a chimeric anti-CD20 monoclonal antibody, has been explored as a treatment option.
Aims: This study analyzed two different risk-adapted therapeutic strategies to cure patients newly diagnosed with NLPHL. The aim was to compare the efficacy and safety of the conventional chemotherapy plus irradiation *versus* the R-including treatment of patients with NLPHL.
Methods: Within a retrospective study, we collected the medical records of 24 consecutive adult patients with NLPHL, taken from the total of 484 patients with HL, who referred to our institution from 1 October 2001 to 31 July 2014. According to our institutional guidelines, the 12 patients diagnosed from October 2001 to November 2007 received a treatment based on ABVD with/without involved-field radiotherapy (IFRT). Treatment was modulated according to the stage. The 9 patients with stages I and II received 4 courses of ABVD plus IFRT, while 3 patients in stages III or IV received 6 cycles of ABVD. The subsequent 12 patients (diagnosis from December 2007 to July 2014) received R (375mg/m^2) alone or combined with ABVD. The stage-adapted strategy of therapy was applied for these patients, as well. The 5 patients with early favourable disease, according to the stage and baseline EORTC risk factors, received R as single agent (once per week for four consecutive weeks) followed by R maintenance (MR) (once every three months for 2 years); the 2 patients with early unfavorable stage were treated with R (once per month on day 1) plus 4 cycles of ABVD, while the remaining 5 advanced stage patients received R (on day 1 and 15) plus ABVD for 6 cycles. The primary end-point was DFS rate, and secondary end-points were ORR and treatment-related toxicity evaluation.

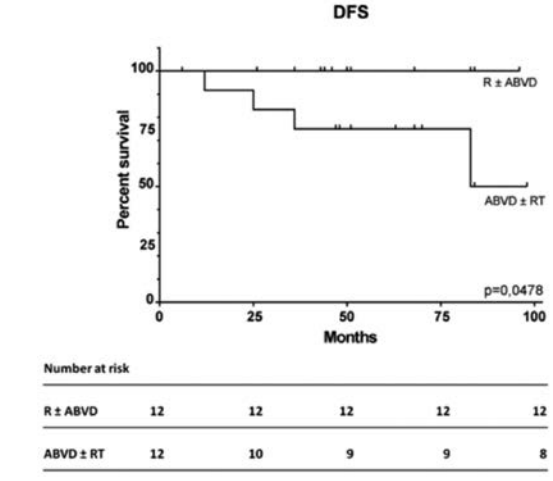

Figure 1.

Results: At final restaging, 4 weeks after the cycle of treatment or completion of IFRT, 23/24 patients (95.8%) were in CR while one patient showed refractory disease and was addressed to rescue therapy with autologous hematopoietic

stem cell transplantation (ASCT). Patients treated with R alone or R+ABVD had better DFS (p=0.04) than those treated with ABVD with/without IFRT. Specifically, the year Kaplan-Meier estimates for DFS were 100% for the R treated group *versus* 50% for those treated with ABVD with/without IFRT. Four patients in the latter group, showed insufficient response to the therapy: 1 refractory disease in the early stage group and 3 recurrent diseases in the advanced stage group were recorded. The median follow-up time of the entire cohort of patients was 4.3 years (range, 0.5-8.2 years). Over the study period, one patient died for infectious pneumonitis due to severe neutropenia following the last cycle of R-ABVD. Of the 9 patients treated with addition of IFRT, adverse events regarded mainly thyroid (4), bone (2), lung (1) and salivar glands (1). Nobody developed a secondary malignancy.

Summary/Conclusions: Our results confirm the value of R in NLPHL and show that R induction and maintenance combined with chemotherapy only in the presence of risk factors or in more advanced stages give excellent treatment results respect the conventional radio-chemotherapy either in term of ORR and of DFS while sparing long term toxicity usually seen in patients affected by classical HL who receive chemo and irradiation.

E1126

CASE-BASED LEARNING IN CONTINUING EDUCATION: IMPROVING HEMATOLOGIST/ONCOLOGIST EVIDENCE-BASED DECISIONS FOR PREVENTING HOGDKIN LYMPHOMA POST-TRANSPLANT RELAPSE

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Background: Several prognostic factors have been identified as associated with a higher rate of relapse after autologous stem cell transplant (ASCT) for patients with Hodgkin lymphoma (HL). Due to the rarity of this disease, many hematologists/oncologists (hem/oncs), especially those in the community setting, lack experience in correctly identifying patients who may be at risk of post-transplant relapse. Proper risk assessment and understanding of treatment options in the pre- and post-transplant setting are critical to ensure optimal longer progression-free survival for qualified patients.

Aims: Underlying clinical practice gaps and educational needs were identified and a study was conducted to determine whether an online, case-based educational intervention could improve knowledge, competence, and confidence of hem/oncs in managing patients with HL.

Methods: The educational format presented patient case scenarios (2) followed by a series of 4-5 questions that "tested" learner knowledge and competence before delivering the education focused on the optimal approach to the case using evidence-based medicine. Case questions assessed degree of patient risk for disease relapse or progression prior to ASCT and consolidation strategies, taking into consideration patients' prior received therapies. To assess educational effectiveness, participants served as their own controls by responding to a sample of same questions again after (post-assessment) exposure to the content. For all questions combined, the McNemar's chi-square test assessed differences from pre- to post-assessment. P values are shown as a measure of significance; P values <.05 are statistically significant. Cramer's V calculation determined the change in proportion of 184 participants who answered questions correctly from pre- to post- assessment and who qualified for the study.

Results: At post-assessment, there was a large effect to the education (V=0.442), indicating a sizable improvement in evidence-based choices and significant improvement in knowledge, competence, and confidence related to managing patients with HL, including: 138% relative improvement regarding the implications of type and number of prognostic factors on risk of HL relapse and benefit of consolidation brentuximab vedotin after ASCT (*P*<.001); 101% relative improvement in knowledge that a higher rate of relapse after ASCT is associated with a CR duration of less than 1 year, extranodal disease at relapse, and the presence of symptoms at relapse (*P*<.001); 5% relative improvement in knowledge regarding the efficacy of brentuximab vedotin in relapsed/refractory HL after ASCT (*P*<.001); Responses to a self-efficacy question indicated that 42% of hematologists became more confident in managing a patient on consolidation therapy for HL after participating in the education.

Summary/Conclusions: This study demonstrated the success of an online, case-based format using a predisposing pre/post assessment was effective in improving the evidence-based practice patterns of hem/oncs in the management of patients with HL. Despite the marked improvement in knowledge, competence, and confidence, hematologist education needs specific to accurate risk assessment, treatment selection, and adverse effect monitoring remain. The education gaps uncovered during this intervention and the evolving treatment landscape outside of the United States lay a foundation for future global education initiatives to bridge education gaps in HL.

E1127

QUANTITATIVE PET PARAMETERS PREDICTS OUTCOME IN PATIENTS WITH HODGKIN'S LYMPHOMA

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Background: Positron emission tomography [18F] fluorodeoxyglucose (FDG-PET) has emerged as the standard response assessment after 1st line therapy for classical Hodgkin's lymphoma (HL). Quantitative PET parameters are not well established as a predictive factor for disease progression in HL.

Aims: Thus, the aim of this study was to test the hypothesis that tumor burden characterized by mean standardized uptake value (SUVmean), maximum SUV (SUVmax), metabolic tumor volume (MTV) and total lesion glycolysis (TLG) could be independent prognostic factors.

Methods: We analyzed the relation of absolute value PET parameters, negative predictive value (negative PET scan and no treatment failure, NPV) and positive predictive value (positive PET scan and treatment failure, PPV) with event-free survival (EFS) or overall survival (OS). Quantitative PET parameters of the baseline (PET-1), interim (PET-2) and end of treatment (PET-3) PET-CT scans were investigated in the retrospective study. MTV was computed by using the 41% maximum SUV thresholding method, and the optimal cut-off for survival prediction was determined.

Results: Thirty one patients with HL with a stage I-II–51.6%, III-IV–48.4% consecutively admitted from April 2009 to December 2016, by 5 Ukrainian hematological centers were included in the analysis. Patients were staged at baseline, after 2-4 cycles of chemotherapy with PET/CT and at the end of chemotherapy. All patients were treated with ABVD, BEACOPP-14/esc. All 31 patients achieved CR or PR and 67,7% had a negative PET-2, while 16,3% had a positive PET-2. Patients with negative PET-2 and positive PET-2 had CR rates of 64,5% and 12,1%, respectively, which yielded a PPV of 26% and NPV of 74%. ROC analysis revealed that PPV and NPV are an important markers associated with EFS in patients with HL (Se=100%; Sp=100%; AUC=1.0). 3-year EFS was 100% for NPV patients and 12% for PPV patients, which was statistically significant (p<0.01). 3-year OS was 100% and 75% for NPV and PPV patients, respectively (p<0.01). Quantitative parameters at PET-1 and PET-2 were not statistically significant in predicting clinical outcome in this study. This may be due to the small sample size in our study. PET-3 was negative in 67,7% cases. ROC analysis showed that ∑MTV at PET-3 is an important marker associated with reduced EFS in patients with HL (Se=75%; Sp=100%; AUC=0.97, p<0.0001). 3-year EFS was 89% and 25% in patients with ∑MTV <4.75 and ∑MTV >4.75, respectively (p=0.005). Also, ROC analysis revealed that TLG at PET-3 was associated with decrease EFS in patients with HL (Se=75%; Sp=100%; AUC=0.97, p<0.0001). Multivariate analysis confirmed ∑MTV and TLG at PET-3 were the only significant variables for EFS with HRs of 1.07 [95% confidence interval(CI) 1.0–1.15, p=0.003] and 2.9 [95% (CI)0.9-1.03, p=0.05), respectively. The PET-3 SUVmax and SUVmean were not statistically significant in predicting EFS.

Summary/Conclusions: Quantitative PET parameters may play a predictive role for identifying patients at high risk of treatment failure. These results should be evaluated prospectively in larger cohorts with longer follow-up.

Indolent Non-Hodgkin lymphoma – Clinical

E1128

Abstract withdrawn.

E1129

BIOMARKER ANALYSIS OF PATIENTS WITH FOLLICULAR LYMPHOMA TREATED WITH IBRUTINIB IN THE PHASE 2 DAWN STUDY

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Background: Ibrutinib, a first-in-class, oral, covalent inhibitor of Bruton's tyrosine kinase, has demonstrated robust clinical activity and is approved in various B-cell non-Hodgkin's lymphomas. To assess the efficacy and safety of ibrutinib in patients (pts) with follicular lymphoma (FL), the DAWN study (FLR2002, NCT01779791) investigated single-agent ibrutinib in chemoimmunotherapy (CIT)-refractory FL pts. Ibrutinib may exert immune-modulatory effects on T-cell activity via inhibition of ITK, a key regulator of T-cell activity, possibly through inhibition of T-helper 2 (Th2)-polarized CD4 T-cells and activation of Th1 cells (Dubovsky, *et al. Blood* 2013). Here, we describe the effect of ibrutinib treatment on T-cells and cytokines in pts in the DAWN study.
Aims: To determine the effect of ibrutinib on circulating T-cells, chemokines, and cytokines in ibrutinib-treated CIT-refractory FL pts.

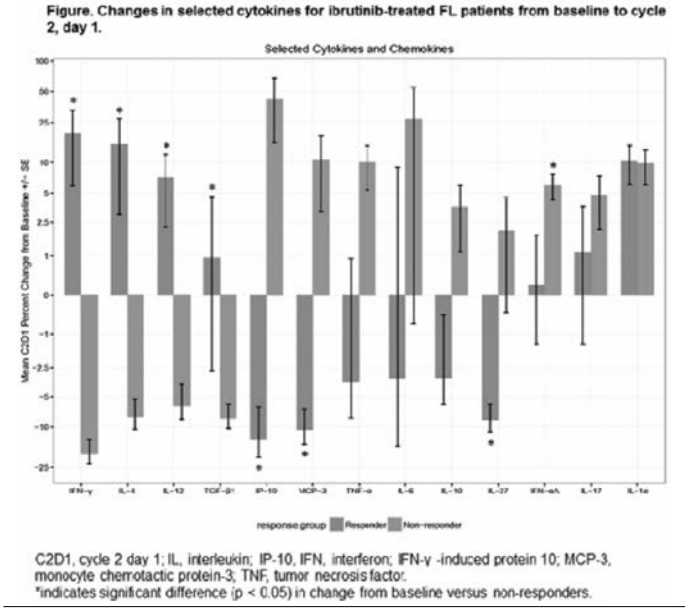

Figure 1.

Methods: The DAWN study was an open-label, multicenter, single-arm, phase 2 study of ibrutinib in pts with CIT-refractory (*i.e.*, ≥2 prior lines of therapy and progressive disease [PD] ≤12 months after last dose of a CIT regimen). All pts received ibrutinib (560mg QD) on a 21-day cycle until PD or unacceptable toxicity. The primary end point was Independent Review Committee (IRC)-assessed overall response rate (ORR) (complete response [CR] + partial response [PR]). Flow cytometry assessed T-cell subsets in peripheral blood at baseline (C1D1) and at cycle 3 (C3D1) for 57 pts (14 responders and 43 nonresponders); cytokine and chemokine analyses were performed at C1D1 and at cycle 2 (C2D1) for 50 pts (21 responders and 29 nonresponders).
Results: Results from the DAWN study have been presented previously (Gopal A, *et al.* ASH 2016). Briefly, 110 pts with a median age of 61.5 years and a median of 3 prior therapies were enrolled. Ibrutinib achieved an ORR of 20.9% (CR rate, 10.9%) and a median duration of response of 19.4 months. Flow cytometry analysis revealed significant downregulation of CD4+CD25+CD127-T_{regs} at C3D1 in 14 responders (CR + PR, mean decrease 17 to 12.9% CD4, p=0.02) but not in 43 nonresponders (SD + PD, 11.5 to 10.4% CD4, p=0.17). From a large panel of inflammation-related cytokines and chemokines, some of the most significant changes at C2D1 were the Th1 cytokines interferon (IFN)-γ and interleukin (IL)-12, both of which were increased in responders but decreased in nonresponders (p=0.0025 and p=0.035, respectively; **Figure 1**). Conversely, the chemokines IFN-γ-induced protein 10 (IP-10) and monocyte-chemotactic protein 3 (MCP-3) were decreased in responders but increased in nonresponders (p=0.022 and 0.016, respectively).
Summary/Conclusions: Here we show immunomodulatory effects of ibrutinib in pts with CIT-refractory FL, which may be related to response to therapy. In responding pts at early time points, downregulation of T_{regs} was observed, along with increases in Th1-associated cytokines IFN-γ and IL-12. This shift in T-cell population may be linked to the antitumor response; in nonresponders, these cytokines were decreased but T_{regs} were not. Chemokine changes observed also indicate variation in chemoattraction of T-cells and monocytes/macrophages. These data suggest that immunomodulatory effects of ibrutinib could play a role in its antitumor activity in FL, so combinations with other immune-oncology therapies may prove beneficial.

E1130

DYNAMO: THE CLINICAL ACTIVITY OF DUVELISIB IN PATIENTS WITH DOUBLE-REFRACTORY SMALL LYMPHOCYTIC LYMPHOMA IN A PHASE 2 STUDY

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Background: Duvelisib is an oral, dual inhibitor of PI3K-δ,γ in development for the treatment of hematologic malignancies. DYNAMO is a Phase 2 study to evaluate the safety and efficacy of duvelisib in a double refractory iNHL population, which included 28 patients (pts) with small lymphocytic lymphoma (SLL).
Aims: The primary objective was to evaluate the antitumor activity of duvelisib monotherapy in pts whose disease is refractory to rituximab and to either chemotherapy or RIT, with an additional objective to further characterize the safety duvelisib.
Methods: DYNAMO is an open-label, single-arm, safety, and efficacy study in patients (pts) with FL, small lymphocytic lymphoma (SLL), or marginal zone lymphoma (MZL), whose disease is double refractory to rituximab (monotherapy or in combination) and to chemotherapy or radioimmunotherapy. Pts received duvelisib 25mg BID in 28-day treatment cycles until disease progression or unacceptable toxicity. The primary endpoint is overall response rate (ORR) as assessed by an independent review committee (IRC) per revised IWG criteria. Secondary endpoints include duration of response (DoR), progression-free survival (PFS), overall survival (OS), time to response (TTR), adverse events (AEs), and changes in safety laboratory values. *Pneumocystis jiroveci* pneumonia (PJP) prophylaxis was mandated for all pts.
Results: 129 pts with iNHL were treated on study. Of these, 28 pts with SLL received duvelisib with a median duration of exposure of 9 mo. (range 6.5-12). Median age was 65 years; 68% were male. Most SLL pts had an ECOG performance status score at baseline of 0 (43%), followed by 1 (54%) and 2 (4%). Most SLL pts had either Stage 3 (25%) or Stage 4 (61%) disease at baseline. Median time from last anticancer therapy to first dose of duvelisib was 3 months. SLL pts received a median of 3 prior anticancer regimens (range: 1-18); 43% of pts received ≥4 prior anticancer regimens, 29% ≥6 regimens. The ORR for SLL pts was 68% (95% CI: 48, 84) per IRC assessment. All responses (19) were PRs. Four (14%) pts had a best response of SD and 3 (11%) pts had a best response of PD. 2 pts were unevaluable for response. Per Investigator assessment, the ORR was 79% (including 1 CR). Median time to IRC response was 1.9 months (range 1.4-5.5). 93% of pts had a reduction in nodal target

lesions. Among the 19 SLL pts with a response per IRC, the median DOR was 9.9 months. The median PFS among all SLL pts was 11.3 months, while the median OS was not reached. The estimated probability of survival at 12 months was 83.9%. Among all pts treated (n=129), AEs were mostly Gr 1-2. Most common ≥ Gr 3 AEs were transient cytopenias (neutropenia [23%], anemia [12%], and thrombocytopenia [10%]), and diarrhoea (15%). 4 SLL pts had SAEs that led to discontinuation of duvelisib: NSCLC, neuroendocrine carcinoma of the skin, pseudomembranous colitis, and pneumonia. Two SLL pts has a fatal AE, 1 pneumonia and 1 viral infection.
Summary/Conclusions: In DYNAMO, duvelisib showed clinical activity in a double-refractory SLL population (68% ORR, median DOR 9.9 mo., 93% with a reduction in target lesions). Duvelisib was generally well tolerated, with a manageable safety profile with appropriate risk mitigation. Duvelisib monotherapy appears to have a favorable benefit-risk profile in double refractory SLL. Updated clinical data will be available at the time of presentation.

E1131

Abstract withdrawn.

E1132

WALDENSTROM MACROGLOBULINEMIA: UK REAL WORLD EXPERIENCE
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Background: There are few randomised controlled trials in Waldenström macroglobulinaemia (WM) due to its rarity and indolent nature. As a result, there is no standard treatment approach and management is variable.
Aims: The aim of this retrospective study was to review "real world" management of WM in the UK and correlate this with survival outcomes.
Methods: All patients with a diagnosis of WM seen at ULCH between 01/07/2002 and 31/12/2016 were included. Patient characteristics, presenting features, lines of treatment, responses and overall outcome were recorded. IPSSWM where available, was calculated at time of first treatment. Survival was estimated using Kaplan-Meier analysis from time of first treatment and *P* values calculated using the log-rank test.

Figure 1.

Results: A total of 211 patients were identified (116 M/ 95 F), median age 60 yrs (range 34-89). Presenting symptoms included anaemia, n=33; neuropathy, n=19; fatigue, n=18; hyperviscosity symptoms, n=13; lymphadenopathy; n=5; progression frommgUS, n=5; B symptoms, n=5; other, n=28; unknown, n=55. Mutated MYD88 was seen in 59 of 72 cases analysed (82%). Of these 59 cases, 13 were CXCR4 mutated. IPSSWM was known in 150 cases of whom 64 were in low, 63 intermediate and 23 high risk groups. Median follow-up from first appointment was 64 months (range 0-394). The median number of lines of therapy was 2 (range 0-9). Dexamethasone, rituximab and cyclophosphamide (DRC) was given to 62 patients upfront, 52 had other cyclophosphamide containing regimens *e.g.* CHOP +/- rituximab, 29 had Chlorambucil-based regimen, 14 R-bendamustine, 15 fludarabine-based with a minority getting R-cladribine (5) or R-bortezomib (4), 9 patients had no treatment at data cut-off. Notably, DRC was given to 1 patient before 2009, 28% of patients between 2009 and 2013, and 41% from 2013. In the 149 cases with known responses to first line treatment, 11% achieved a CR (7 patients with R-CHOP, 4 DRC, 2 fludarabine containing regimen, and 3 patients other treatment), 63% PR/ VGPR, 21% no response or PD and 5% stopped due to toxicity. For the 52 patients who had DRC chemotherapy, median PFS was 61 months. Of those patients who had at least 3 lines of chemotherapy (n=62), median time between 1st and 2nd line treatment was 10 months and 3 months between 2nd and 3rd line. Transplants were performed on 28 patients after a median of 2 lines of chemotherapy. Median overall survival (OS) has not been reached in the 195 patients with available data. Stratifying by IPSSWM shows median OS for the low risk group has not been reached, 11 years for the intermediate risk and 9 years for the high risk group, *P*=0.29 (Figure). Patients had a significantly reduced OS if they developed Bing Neel syndrome or high grade transformation compared to other known complications of WM. Despite differences in chemotherapy strategies over the past two decades, there was no difference in outcome in patients treated before 2005, between 2005-2009, 2009-2013 and 2013 onwards. Of the 34 deceased patients, the cause of death was unknown in 3 cases, due to PD in 16 and other causes in 15 cases.
Summary/Conclusions: The management of patients with WM in this large case series reflects the variability of treatment given over time and also geographically. UCLH treats both a local and tertiary referral patient population, thus it is not completely typical. Survival data confirms the IPSSWM is likely to still differentiate patients into prognostic groups but the overall prognosis is better than when first published. With the advent of targeted therapies, it is imperative to perform randomised controlled trials and to collect data prospectively in order to elucidate the optimal management. To this end, a WM Biobank and Registry has been set up at our centre.

E1133

CLINICAL CHARACTERISTICS AND LONG-TERM RESULTS OF TREATMENT OF INDOLENT NON-HODGKIN'S LYMPHOMA ASSOCIATED WITH HEPATITIS C (IL + C)
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Background: According to the WHO classification (2008) hepatitis C virus is one of the causesof non-Hodgkin lymphoma. The incidence of chronic hepatitis C (HCV) in patients with indolent B-cell non-Hodgkin's lymphoma (IL + C) is 15%. Diagnosis of hepatitis C related lymphoma (IL + C) is established in cases where the tumor tissue of patients express proteins of hepatitis C virus. These proteins could idefined by immunohistochemistry (IHC).
Aims: The aim of this work was evaluation of the results of treatment of IL associated with hepatitis C in comparison with a control group of patients with IL without viral hepatitis markers.
Methods: The study included 107 patients with indolent lymphoma who were identified in the blood markers of hepatitis C.
Results: Histological types were follicular lymphoma - 74%, marginal zone lymphoma - 32%. The age of patients ranged from 28 to 82 years (median 50). Men / women ratio was 1: 1. Stage I + II were in 3%, III stage was in 24% of patients, IY stage was at 73% of patients. Primary extranodal lymphoma was diagnosed in 33% of patients. Extranodal lesions: splenic lesion -in 53% of patients, liver injury - 21% of the patients, the bone marrow - 62% of patients. LDH ≥ 450 IU / l was at 76% cases, ALT ≥40 IU / l was at 82% of cases, albumin <35 g / l was at 31% of patients. 57 patients were treated with interferon and Ribavirinum as a first-line treatment. Treatment lasted for 2 years after reaching the antitumor effect. 50 patients were treated with immunochemotherapy (R-CHOP, R-CVP) as a first-line treatment. Antiviral therapy was effective in 88% patients, immunochemotherapy was effective in 64% of patients. Median progression-free survival in patients with IL + C treated with antiviral treatment was 42 months, in patients with IL + C treated with immunochemotherapy - 19 months (p=0.00001). Five-year overall survival was 67% and 32%, respectively (p=0.0003). It was diagnosed disease relapses after immunochemotherapy in 39 patients. All the patients in the second-line was received antiviral treatment. Response to the ongoing antiviral therapy was achieved in 77% of cases. Median progression-free survival in relapsed lymphoma was 31 months.
Summary/Conclusions: Antiviral therapy in first-line and relapse of disease surpasses all the indicators of efficiency of treatment IL + HCV. In this category of patients preferred option is to conduct anti-viral treatment.

E1134

90Y-IBRITUMOMAB-TIUXETAN AS FIRST-LINE CONSOLIDATION IN COMPLETE RESPONSE FOLLICULAR LYMPHOMA PATIENTS. SINGLE CENTER ANALYSIS AFTER SIX YEARS MEDIAN FOLLOW-UP
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Background: Follicular lymphoma (FL) accounts for around 22% of all non-Hodgkin lymphomas. His natural history is characterized by multiple relapses and progressively shorter response durations after every new line of therapy for this is desirable to offer the best first-line approach to each patient. In the current guidelines several first line options are included: immunotherapy (Rituximab (R) x4 or Lenalidomide+/- R), immunochemotherapy (CHOP, RCVP, Bendamustine + R), radioimmunotherapy for elderly patients. Moving forward, the consolidation with radioimmunotherapy or extended dose immunotherapy

(R every 8 weeks for 4 or 12 doses) still appears as an optional part of the therapy (NCCN V3.2016). Radioimmunotherapy with 90Yttrium-ibritumomab tiuxetan (90Y-IT) is available in our institution since 2006 and more than 100 patients have been treated with RIT since then. Here an institutional analysis focus in their use as consolidation is presented

Aims: To analyze the experience with 90Y-IT as a consolidation therapy in patients in CR after first-line therapy.

Methods: A retrospective analysis was performed including all the patients that have received RIT with 90Y-IT. Inclusion criteria were: patients 18 years or older with a grade 1-2a follicular lymphoma, RIT was received as a consolidation therapy in complete response (CR) after a first-line therapy. Demographic and follow-up data were included. International working group (IWG) criteria of response was used. Progression free survival (PFS) was calculated from the date of RIT to the date of a confirmed relapse according IWG criteria, overall survival (OS) was calculated from the FL diagnosis to the last contact.

Results: A total of 31 FL patients have received 90Y-IT been in CR after a first-line of therapy and were included for the study. Mean age at diagnosis was 61.2 (29-86) years with a female predominance (19, 61.3% *vs* 12, 38.7%). 80.6% (26) with ECOG 0-1 and 19.4 ECOG 2. A third of them (10, 32.3%) were diagnosed with low tumor burden (stage I-II), 2 (9.7) of them presented extra nodal infiltration (subcutaneous and gut) and 12 (38.7%) showed bone marrow infiltration demonstrated by flow cytometer or biopsy. There were no patients with bulky disease. Stages: I: 7 (22.6%), II: 3 (9.7%), III: 9 (29.1%), IV: 12 (38.7%). As first-line therapy the patients received: Rx4: 11 (35.5%) cases, R-Cyclophosphamide vincristine prednisone (COPx4): 3 (9.7%) cases and 17 (54.8) R-cyclophosphamide doxorubicin, vincristine and prednisone (R-CHOPx4-6). The median follow-up was 58.0 (10-107) months. During this time only 5 (16.1%) of patients have relapsed and need another therapy. None of the patients that have received R-CHOP+90Y-IT have relapsed; the relapsed patients received Rx4 (4) and R-COP (1). The median PFS after 90Y-IT has not reached, the mean was 83.3 (71.7-94.98) months, see Fig 1. Four (12.9%) patients have died, none of them were relapsed and the mortality was due other causes. The median OS was not reached, the mean was 95.8 (85.6-106.1) months. As long-term events one 82 years old patient developed a colon cancer after 67 months of RIT, one 72 years old female a breast cancer after 17 months of RIT and one 71 years patient amgUS after 24 months of RIT, none of them related with mortality events.

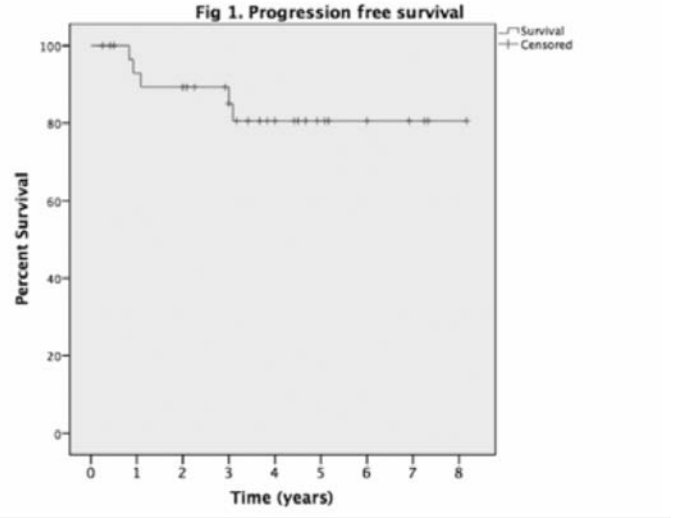

Figure 1.

Summary/Conclusions: The use of immunotherapy with rituximab or combined schedules with immunochemotherapy (R-COP and R-CHOP) followed by consolidation with 90Y-IT remains as a valid option for follicular lymphoma patients. After ~6 years of follow-up: 63.6% (Rx4+RIT), 66.7% (R-COP+RIT) and 100% (R-CHOP+RIT) of patients continue with complete response and off of therapy.

E1135

ASSESSING RISK OVER TIME IN PATIENTS WITH SYMPTOMATIC WALDENSTRÖM MACROGLOBULINEMIA (WM). A STUDY ON 114 PATIENTS (PTS)

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Background: By contrast, with follicular lymphoma (J Clin Oncol 2015;33:2516) or other chronic hematological malignancies (Blood 2009;114:1299; Blood 2016;128:902), few reports attempted to decipher the evolution of pts with WM, a disorder associated with delayed response to therapy in some pts.

Aims: To assess the prognostic role during the clinical course of initial international prognostic index (IPSSWM), response and progression (according to 6th International Workshop criteria).

Methods: We took advantage of our continuously updated clinical database for reviewing a series of 114 symptomatic WM pts treated in our 2 institutions between 1993 and 2016 (median age 70, male/female ratio=1.91, high, low/intermediate and unavailable IPSSWM in 57, 36 and 21 pts respectively). Response rate after 1st line therapy was 70%. Sixty-two, 37 and 19 pts received a 2nd a 3rd and 4 to 6 lines of therapy respectively according to the 2nd International Workshop guidelines. Monitoring of serum monoclonal immunoglobulin concentration (SMIC) throughout the evolution of the disease was available in 106 pts. Informed consent was obtained according to the protocol submitted to the Institution Review Board

Results: Median survival after 1st line was estimated 79 months. It was estimated 69 and 65 months after 2nd line and 3rd line respectively. High IPSSWM (hIPSSWM *vs* low/intermediate) retained prognostic value for survival after 1st treatment initiation (SAFTI, p=0.0005). However, plot of hazard function showed a decrease of hazard ratio over time with a departure from the proportional hazard hypothesis (Grambsch and Therneau test: p=0.053). Consequently, Dxy concordance index obtained in multiple landmarks analyses decreased from 0.27 to 0.12, during the first 6 years of follow-up. In Cox model of SAFTI with time dependent covariate, onset of response (whatever cut-off in SMIC) had no prognostic value. By contrast, onset of progression and initiation of 2nd line therapy, retained prognostic values for SAFTI (p=0.0038 and p=0.004 respectively). Only 2 thresholds in SMIC defined a response status (observed between onset of response and progression) of prognostic value for SAFTI: namely >25% reduction in SMIC (*i.e.* minor response or better: p=0.041) and 50% (*i.e.* partial response or better: p=0.056). In similar Cox models with hIPSSWM, onset of progression (p=0.0034) and 2nd treatment initiation (p=0.0031) retained independent prognostic value beside hIPSSWM (p<=0.0026). Times elapsed from the initiation of 1st line therapy to 1st progression and to the initiation of 2nd line therapy had no prognostic value for subsequent survival. In similar Cox model of survival after 2nd line therapy with time dependent covariate no threshold in SMIC were found to be associated with a significant value of onset of response or response status. Neither onset of progression nor next treatment initiation had significant prognostic value. Similar results were observed after the 3rd line of therapy.

Summary/Conclusions: The prognostic value of initial IPSSWM decreased in part during the first 6 years of evolution. Onset of progression and 2nd treatment initiation provided additional prognostic information for predicting SAFTI. Therefore progression-free survival or time to next treatment may be satisfactory surrogate endpoint of SAFTI in WM. Further international collaborative studies are mandatory for this purpose. Assessing response in more advanced phase of the disease may require specific tools.

E1136

TIME TO NEXT TREATMENT ANALYSIS FOR EARLY AND ADVANCED STAGES OF MYCOSIS FUNGOIDES /SEZARY SYNDROME TREATED WITH BEXAROTENE AND PUVA IN COMBINATION

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Background: Bexarotene is a syntetic retinoid effective in early and advanced stages of Mycosis Fungoides (MF)/Sezary Syndrome (SS) both in monotherapy and combination schemes. Time to next treatment (TTNT) seems to be a clinically meaningful endpoint that incorporates both symptom control and disease progression. It has been investigated in few retrospective studies focusing on retinoids in monotherapy both in limited-stage and advanced stage MF, but up to now no data are available concerning the use of retinoids in combination.

Aims: We aimed to evaluate TTNT together with the usual time-to-event measures (OS and EFS) in our series of 21 refractory and/or relapsed patients with MF and SS treated with Bexarotene and PUVA in combination, that we recently published (Rupoli et al, *EJD 2016*). The follow-up of these protocols was prolonged up to February 2017.

Methods: We recruited patients with stages I-IV MF who had failed PUVA (early disease) or several systemic regimens (early and advanced disease). We designed "mini" and "standard" protocols in which Bexarotene dose and PUVA administration were individually titrated, and tailored during induction and maintenance according to previous therapy, disease stage and toxicity. Survival curves for each efficacy endpoint were calculated according to Kaplan-Meier.

Results: We enrolled 21 patients, 12 males and 9 females, with median age of 67 years (range, 30-77), of which 15 affected by early MF (13 with stage IB, 2 with stage IIA) and 7 by advanced disease (2 with stage IIB, 2 with stage IIIA, 1 with stage IIIB and 1 with stage IVA). Six patients had previously received PUVA therapy only, while fifteen patients had received other therapies.The protocol proved to be effective, well tolerated and able to induce an overall response of 85.6% at the end of induction phase (93.4% of early stage patients and 66.6% of advanced stage patients) and of 76.2% at the end of maintenance phase (86.7% of early stage patients and 14.2% of advanced stage patients). Median follow up for all patients was 85 months (6-118) with respectively 98 months (21-118) for early stages and 46 months (6-102) for advanced stages. For the entire cohort, median OS, PFS and TTNT was not reached; mean values of OS, PFS and TTNT were respectively, 90, 92 and 72 months and median EFS was 33 months. For the early stage MF cohort, the median OS, PFS and TTNT were not reached; mean values of OS, PFS and TTNT were respectively, 105, 103 and 79 months, and median EFS was 58 months. For advanced stage patients, median OS, PFS, EFS and TTNT were 32, 29, 18 and 39 months respectively.

Summary/Conclusions: Our combination treatment seems to have superior TTNT compared to data published in the literature for PUVA and bexarotene used in monotherapy. When considering early and advanced MF, 66% of our patients are estimated to be free from further treatment at 2 years, a higher percentage compared to the results of Hughes *et al.* (*Blood, 2015*) for patients treated with PUVA (54.2%) or bexarotene (36.8%) as single agents. Moreover, TTNT seems to be longer in our study than in the study by Hanel et al (*AJH* 2016) on patients treated by retinoids in monotherapy, respectively 79 *vs* 60 months (mean TTNT values) in the early stages and 39 *vs* 9 months (median TTNT values) in the advanced stages. We believe that our results strongly suggest a synergistic or additive effect between PUVA and bexarotene compared to either agent alone in the treatment of both limited-stage and advanced stage MF.

E1137

PERIPHERAL BLOOD INVOLVEMENT IN PATIENTS WITH ADVANCED STAGE FOLLICULAR LYMPHOMA: CLINICAL-BIOLOGICAL CHARACTERISTICS AND PROGNOSTIC IMPACT

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Background: Follicular lymphoma (FL) is a clinically heterogeneous indolent lymphoma. The majority of patients have a non-aggressive clinical course, but a small percentage shows a rapidly progressive disease, including histological transformation in some cases. Although disseminated disease and bone marrow infiltration are common, only a small percentage of FL patients have peripheral blood (PB) involvement. The clinical significance of the PB involvement is unclear.

Aims: To describe the clinico-biological characteristics and to determine the prognostic impact of blood involvement in patients with advanced FL.

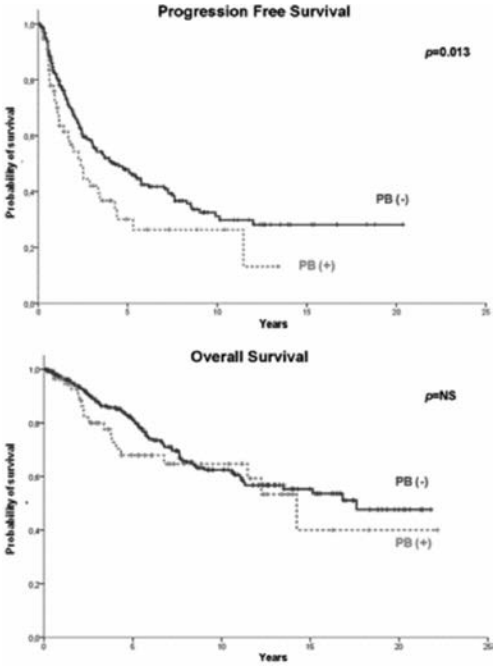

Figure 1.

Methods: We selected 304 patients in stage IV out of 654 patients diagnosed with FL between 1991 and 2014 in a single institution. Patients with a diffuse large B-cell lymphoma component, histological grade 3b and primary cutaneous FL were not included. Fifty-six (18%) had PB expression (PB+) defined by the presence of circulating FL cells by morphology, further confirmed by immunophenotype. The main clinical and biological characteristics, response to treatment and outcome were analyzed.

Results: Patients with PB+ more frequently had splenic involvement, anemia, elevated β2-microglobulin and LDH and high FLIPI score than those without PB involvement (PB-) and differences were statistically significant. There were no differences concerning the proportion of patients undergoing a watchful waiting approach (7% *vs* 9%), type of treatment, or overall response rate (83% *vs* 88%) and complete response rate. Overall, 149 patients had refractory disease or relapsed, including 34/52 (65%) PB+ and 115/225 (51%) PB-. The median follow-up was 7 years (range 0.7 - 22.2 years). The 5-year progression-free survival (PFS) of treated patients in PB+ group was 28% (95% CI: 14-42%) compared with 48% in the PB- (95% CI: 41-55%) (p=0.013). However, when the analysis was restricted to patients receiving rituximab combination regimen, 5-year PFS was 45% (95% CI: 24-66%) *vs* 64% (95% CI: 54-74%) (p=NS). Ninety-six patients died during the follow-up (19 PB+ and 77 PB-), with a 5-year overall survival (OS) of 68% (95% OR: 54-82%) in the PB+ group and of 81% (95% CI: 76-86%) in the PB- group (p= NS) (Figure). Finally, there was no difference in the risk of histological transformation or second malignancies.

Summary/Conclusions: Peripheral blood involvement in FL is associated with particular clinical features, higher tumor burden load and shorter PFS, although in the short-term it appears that has not impact on overall survival.

E1138

TREATMENT PATTERNS OF PATIENTS WITH FOLLICULAR LYMPHOMA IN A LARGE US-INSURED DATABASE FROM 2010 TO 2014

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Background: Follicular lymphoma (FL) is the second most common type of non-Hodgkin's lymphoma. While there are therapeutic options for patients with FL, it remains an incurable disease with conventional therapies. Furthermore, real-world treatment patterns for patients with FL are not well characterized in the literature.

Aims: To characterize the real-world treatment patterns by line of therapy (LOT) for patients with FL in a large US-insured database.

Methods: Using the Optum integrated database, patients with FL were identified and included if 1) they were diagnosed with the International Classification of Diseases, Ninth Revision (ICD-9) codes 202.0 or 202.00 to 202.08 between January 2010 and December 2014; 2) their age was ≥ 18 years at the index date (defined as date of FL diagnosis); 3) they did not have any other primary cancers during the period from 3 years prior to index date up to 1 month post-index date; and 4) they had continuous insurance coverage for 365 days prior to index date. All reporting was done using descriptive statistics.

Table 1.

Table 1. Share of regimens used in 1180 patients treated with ≥ 1 NCCN-recommended therapy.

Therapy	LOT1 (n = 1180)	LOT2 (n = 711)	LOT3 (n = 479)	LOT4+ (n = 897)	All LOTs* (n = 3267)
Rituximab monotherapy	21.3%	27.1%	30.1%	29.9%	26.2%
Bendamustine-rituximab	14.0%	10.8%	10.4%	11.4%	12.1%
Steroids	10.9%	14.2%	15.9%	9.9%	12.1%
R-CHOP	11.4%	9.7%	9.8%	10.3%	10.5%
R-CHOP-containing	4.6%	6.6%	9.4%	15.9%	8.8%
R-CVP/R-CVP-containing	3.6%	1.0%	1.0%	0.4%	1.8%
Obinutuzumab-containing	0.0%	0.1%	0.2%	0.2%	0.1%
Other rituximab-containing	12.4%	11.1%	8.6%	7.1%	10.1%
Other chemotherapies	21.8%	19.3%	14.6%	14.8%	18.3%

*Patients may receive multiple LOTs.

Results: A total of 2569 patients with FL met the inclusion criteria and were included in the analysis. In this cohort, the mean age was 60 years; 51% were male; 72% were Caucasian, 5% African American, 2% Asian, and 20% other. The median duration of follow-up was 610 days. Across all LOTs, 1180 patients (46%) had at least one National Comprehensive Cancer Network (NCCN) guideline-recommended treatment for FL, and 153 patients (6%) had steroids only in their follow-up. Across all LOTs, rituximab monotherapy (RTX) was the most frequently used regimen (26%; average duration of therapy [DOT]: 96 days), followed by rituximab-cyclophosphamide-doxorubicin-vincristine-prednisolone (R-CHOP) or R-CHOP-containing regimens (19%; average DOT: 75 days) and bendamustine-rituximab (BR) (12%; average DOT: 128 days). These regimens represented 21%, 16%, and 14% of the first LOT, and 27%, 16%, and 11% of the second LOT, respectively. Across all LOTs, the use of other FL treatments was very low, including rituximab-cyclophosphamide-vincristine-

prednisolone (R-CVP) (2%) and obinutuzumab (< 1%). Utilization trends of R-CHOP-containing regimens and RTX increased in successive LOTs, while all the other regimens remained unchanged or declined, particularly in later LOTs. RTX-containing regimens were used in 69.5% of regimens across all LOTs. The share of regimens used in different LOTs and overall are described in Table 1.
Summary/Conclusions: The use of NCCN guideline-recommended treatments in less than half of this cohort of patients with FL suggests that many patients with this form of indolent lymphoma are not adequately treated. As expected, rituximab was found to be the mainstay of treatment. Further research is needed to examine the relationship between LOT and long-term outcomes in patients with FL.

E1139

A PHASE 1 STUDY EVALUATING THE SAFETY AND PHARMACOKINETICS (PK) OF VENETOCLAX (VEN) IN JAPANESE PATIENTS (PTS) WITH NON-HODGKIN LYMPHOMA (NHL) AND MULTIPLE MYELOMA (MM)
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Background: The antiapoptotic protein BCL-2 is commonly overexpressed in hematologic malignancies. VEN is a potent, selective, orally bioavailable BCL-2 inhibitor that has demonstrated acceptable safety and antitumor activity in NHL and MM pts.
Aims: To evaluate the safety, PK profile, and preliminary antitumor activity of single-agent VEN in Japanese pts with NHL or MM.
Methods: Phase 1 open-label, dose-escalation study of VEN in Japanese pts with relapsed or refractory (R/R) NHL or MM (NCT02265731). Dose escalation followed a 3+3 design. After a 2-week ramp-up period with weekly dose escalation, VEN was administered daily at final doses of 300, 600, 900, or 1200mg on 21-day cycles until progression. All pts received tumor lysis syndrome (TLS) prophylaxis (allopurinol, hydration, hospitalization and monitoring) starting at least 72 hours before the first VEN dose and before each dose escalation. Adverse events (AEs) were assessed by NCI CTCAE v4.0. Dose-limiting toxicities (DLTs) were determined during the ramp-up period and during cycle 1. Responses were assessed by 2007 IWG (NHL) or 2006 IMWG (MM) criteria. Informed consent was obtained from all pts.
Results: As of January 19, 2017, 20 pts (50% male; median age 65 years [39–81]) have been enrolled: 3 pts in the 300-mg, 7 pts in the 600-mg, 7 pts in the 900-mg, and 3 pts in the 1200-mg VEN dose cohorts. Eighteen (90%) pts had NHL (stage III/IV, n=14), including 11 with follicular lymphoma (FL), 6 with diffuse large B-cell lymphoma (DLBCL), and 1 with concurrent FL+DLBCL; 2 (10%) pts had MM at diagnosis. Treatment-emergent AEs (all grades) in >20% pts were lymphopenia (80%), neutropenia (60%), leukopenia (50%), and anemia (25%), and non-hematologic toxicities including nausea (55%), vomiting, diarrhea, and nasopharyngitis (30% each). Grade ≥3 treatment-related AEs were lymphopenia (45%), neutropenia (40%), and leukopenia (30%). One pt in the 600-mg dose cohort experienced grade 3 pemphigus as DLT after receiving 2 doses of 100-mg VEN on day 2 of the dose ramp-up period. One DLBCL pt died while on study due to disease progression. No TLS events were reported. Steady-state VEN exposures were nearly dose proportional across 300-mg to 900-mg doses. At the 1200-mg dose, exposures to VEN increased less than dose proportionally, which is consistent with non-Japanese subjects. VEN exposures were comparable between Japanese and non-Japanese pts at the 300-mg dose. At higher doses, individual exposures were generally within the range observed in non-Japanese pts but mean exposures were 30–100% higher. OR was seen in 8/19 (42%) pts evaluable for efficacy. Among pts with NHL, 8/17 (47%) had an OR (CR+PR), including 2 (12%) CR (both pts with FL) and 6 (35%) PR; 6/17 (35%) pts had SD and 3/17 (18%) pts progressed while on treatment. The 2 (100%) pts with MM (1 with t[11;14]) had PD. Time on study for all pts ranged from 2 to 795 days; at data cutoff, 7/20 (35%) pts were still on study, with treatment duration ranging from 98 to 795 days.
Summary/Conclusions: VEN monotherapy has a tolerable safety profile in Japanese pts with R/R NHL or MM, with most common toxicities being hematologic. Individual exposures were generally within the range observed in non-Japanese pts but mean exposures were 30–100% higher. Overall, the OR rate was high, with nearly half the pts with NHL achieving an OR. Further evaluation of VEN in Japanese pts with hematologic malignancies is ongoing.

E1140

A SIMPLIFIED APPROACH IN THE ASSESSMENT OF T-CELL CLONALITY BY FLOW CYTOMETRY
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Background: T-cell lymphoprolferative disorders are amongst the most challenging diagnoses in haematology. Flow cytometric T-cell receptor (TCR)-V(β) repertoire analysis (TCR-V(β)-R) is a sensitive method for detection of T-cell clonality; however, the assay is cumbersome owing to the required eight-part analyses that limit its clinical utility.
Aims: Here we describe a simplified flow cytometric method utilising a monoclonal antibody Jovi-1 that targets the T-cell receptor β constant domain 1 (TRBC1). The α/β TCR is a pan T-cell antigen, expressed on >90% of T-cell lymphomas and all normal T-cells. A feature of the TCR is that the β-constant region comprises 2 functionally identical genes: TRBC1 and TRBC2. Each T-cell expresses only one of these. Consequently, normal T-cells will be a mixture of individual cells expressing either TRBC1 or 2, while a clonal T-cell disorders will exclusively express TRBC1 or 2.
Methods: Using multiparameter flow cytometry we assessed the expression of Jovi-1 in normal donors (n=19), T-cell leukaemia cell line (n=1), T-LGL (n=9), T-NHL (n=3), Sezary syndrome (n=3) and patients with reactive lymphocytosis (n=20). A comparison of Jovi-1 and TCR-V(β)-R was also performed to compare the two approaches.
Results: Jovi-1expression within the CD4 and CD8 compartments of T-cells in normal donors was a median of 42.6% (range 33.7%> 49%) and 36.4% (range 22.3%> 48.5%) respectively. The T-cell line, Jurkat was exclusively positive for Jovi-1. Of the 9 patients with T-LGL, 7 patients shared a common T-cell phenotype CD3+/CD8+/CD4-, one patient was predominantly CD4+ and the other patient was dual negative for CD4 and CD8. Jovi-1 expression within the abnormal T-cell population of this group of patients was >90% restricted to one compartment; these findings were confirmed by TCR-V(β)-R analysis. Similar results were also obtained in each case of T-NHL and Sezary syndrome, more than 90% of T-cells from the population with an abnormal phenotype (CD3dim/CD4+/CD8-, CD3+/CD4+/CD8+, CD3+/CD4+/CD8- and CD3+/CD4+/CD7- respectively) were either positive or negative for Jovi-1. Patients with persistent lymphocytosis were also assessed for Jovi-1 expression. Within this group all patients had Jovi-1 positive and negative compartments within CD4 and CD8 T-cells.
Summary/Conclusions: In summary we have demonstrated a novel approach in the assessment of T cell clonality by targeting T-cell receptor β constant domain 1 (TRBC1). The addition of Jovi-1 in routine practice could improve the clinical evaluation of abnormal T-cell populations by flow cytometry.

E1141

A HIGHER AMOUNT OF LILOTOMAB PRE-DOSING INCREASES THE ACTIVITY-ADJUSTED AUC AND HAS A PROTECTIVE EFFECT AGAINST MYELOSUPPRESSION OF LUTETIUM (177LU)-LILOTOMAB SATETRAXETAN IN INDOLENT NHL PATIENTS
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Background: lutetium (^{177}Lu)-lilotomab satetraxetan (Betalutin®) is a novel CD37-binding murine IgG_1 antibody radionuclide conjugate (ARC), in a ready-to-use formulation currently in Phase 1/2 clinical development for the treatment of indolent non-Hodgkin lymphoma (iNHL). Previously, pharmacokinetic (PK) data have been reported from 2 treatment arms of the ongoing LYMRIT-37-01 study. In this abstract PK data from 4 treatment arms are presented for the first time.
Aims: This PK sub-study in iNHL patients (pts) was designed to determine the PK profile of ^{177}Lu-lilotomab when administered after four different pre-dosing schedules.
Methods: Patients with relapsed incurable indolent NHL, with platelet counts ≥150 x10^9/L and <25% bone marrow involvement were eligible for inclusion in the study. All pts received either one or two doses of rituximab to deplete normal B cells. In addition, prior to ^{177}Lu-lilotomab administration pts also received:
- 40mg lilotomab prior to 10, 15 or 20 MBq/kg ^{177}Lu-lilotomab;
- no pre-dosing prior to 10 or 15 MBq/kg ^{177}Lu-lilotomab;
- 250 or 375mg/m^2 rituximab prior to 15 MBq/kg ^{177}Lu-lilotomab;
- 100mg/m^2 lilotomab prior to 15 or 20 MBq/kg ^{177}Lu-lilotomab.

PK samples were collected at 0 and 5 minutes, then after 1, 2, and 20 hours and at 2, 3, 4, 7, 14 and 21 days post-^{177}Lu-lilotomab administration. The patients were followed with weekly blood counts for 12 weeks post-treatment.
Results: A total of 22 pts were enrolled into this PK sub-study, 19 with follicular lymphoma, 2 with mantle cell and 1 with marginal zone histologies. The number

of prior therapies ranged from 1 to 7, median body weight was 79 kg (range: 58-118kg).The administered activity across all treatment groups ranged from 746 to 1982 MBq. The table below shows a summary of the median PK and haematology safety results for ^{177}Lu-lilotomab by treatment group. The activity-adjusted $AUC_{0-\infty}$ of ^{177}Lu-lilotomab increased with 100mg/m^2 of lilotomab compared to the other pre-dosing regimens ($p<0.001$ compared to 40mg lilotomab). The median volume of distribution and clearance were both reduced with 100mg/m^2 of lilotomab compared with the other pre-dosing regimens. However, activity adjusted Cmax was similar. Smaller percentage post-treatment reductions in platelet and neutrophil counts were observed in patients receiving 100mg/m^2 lilotomab. Most common grade 3/4 AEs were hematological and were transient and reversible.

Table 1.

Median/Pre-dose regimen	40 mg lilotomab N=9	No-predosing N=3	Rituximab N=4	100 mg/m^2 lilotomab N=6
Activity adjusted $AUC_{0-\infty}$(h*kBq/mL)/MBq	8.91	4.34	6.08	13.07
Activity adjusted Cmax (kBq/mL)/MBq	0.21	0.18	0.24	0.22
Vz L	10.5	17.8	12.5	5.3
Cl mL/h	126	230	167	77
% platelet count reduction #	82	94	88	39
% neutrophil count reduction#	64	94	86	58

#15 MBq/kg only. N=3 for each arm except with no pre-dosing n=2

Summary/Conclusions: A higher pre-dose of lilotomab increases the activity-adjusted AUC and decreases the volume of distribution and clearance rate of ^{177}Lu-lilotomab in iNHL pts. Despite the increase in AUC the percentage reductions in neutrophil and platelet counts were smaller, indicating that a higher dose of lilotomab may have a protective effect against the myelosuppression associated with ^{177}Lu-lilotomab. Further characterisation of 20 MBq/kg dose of ^{177}Lu-lilotomab with 100mg/m^2 of lilotomab pre-dosing is ongoing and will be presented.

E1142

PHARMACOKINETICS AND TOLERABILITY OF OFATUMUMAB AND BENDAMUSTINE IN PATIENTS WITH INDOLENT B-CELL NON-HODGKIN'S LYMPHOMA

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Background: Anti-CD20 antibody rituximab (R)-based immunochemotherapy is the standard treatment for untreated or relapsed indolent B-cell non-Hodgkin lymphoma (iNHL). Due to the inevitable relapse of patients with iNHL, an unmet need remains for active and well-tolerated novel therapies. Bendamustine (BEN) is approved for the treatment of refractory iNHL, and the combination therapy BEN-R showed efficacy in the treatment of relapsed iNHL. Ofatumumab (OFA) is an anti-CD20 human monoclonal antibody (mAb) with high binding affinity and slower dissociation from a distinct membrane-proximal epitope on both small and large loops of CD20. OFA is indicated for the treatment of chronic lymphocytic leukemia (CLL) and is being investigated for the treatment of iNHL. The combination of OFA and BEN may provide additional clinical benefit in patients with iNHL and therefore the potential for drug-drug interaction was investigated.
Aims: The study aimed to evaluate the pharmacokinetics (PK) of OFA and BEN alone and in combination, along with the safety and tolerability assessments in patients with previously untreated or relapsed iNHL.
Methods: In this Phase I open-label, multicentre study, patients (aged ≥18 years) with previously untreated or relapsed iNHL were randomized 1:1 to Arm A (OFA + BEN) or Arm B (OFA alone) to receive at least four cycles and up to eight cycles of treatment (cycle length 28 days). All patients provided informed consent. Arm A patients received single-sequence treatment of BEN, then OFA + BEN, BEN (90mg/m^2) on days 1 and 2 every 28 days for up to 8 cycles, and OFA (1000mg) on day 1 of weeks 2, 3, and 4 of cycle 1 and on day 1 of cycles 2-8. Patients in Arm B received OFA alone at same dosing schedule. Blood samples including all end-of-infusion (EOI) PK samples were collected for plasma concentration over time. The primary PK parameters C_{max}, AUC_{last}, AUC_{inf} were derived using non-compartmental analysis. All adverse events (AEs) and severe AEs (SAEs) were recorded for safety assessments.
Results: Thirty two patients were randomized (15 in Arm A and 17 in Arm B), 3 patients in Arm A discontinued study treatment due to consent withdrawal (2 patients) and infusion related AE (1 patient). All 32 patients were included for safety and PK concentration analysis while 30 patients (15 in each arm) were included for PK parameters. Patient and disease characteristics were similar between treatment arms; the majority of patients from both arms did not receive prior NHL therapy. PK concentration profiles and PK parameters of OFA were comparable when administered alone or co-administered with BEN (Table 1). As compared to OFA alone, there was a decrease of 14% in C_{max} and 15% in AUC_{last} when OFA was co-administered with BEN, which was not considered relevant (Table 1). BEN PK concentration profiles and PK parameters were comparable with or without OFA co-administration (Table 1).
All patients reported AEs. The most frequent treatment-related AEs were infusion related reaction in 53% and 47%, nausea in 33% and 35%, fatigue in 33% and 18% patients in Arm A and Arm B, respectively. The percentages of patients with grade 3/4 AEs were higher in Arm A (53%) compared to Arm B (24%). Cytopenias were present in 40% of patients in Arm A and 6% in Arm B. Four SAEs were related to study treatment in Arm A while none in Arm B.

Table 1.

Table 1. Primary PK parameters and Statistical Analysis of the primary PK parameters for OFA and BEN

	Geometric mean (95% CI)		%CV$_b$		Median (Min-Max)		Geometric Least Square Mean		
OFA – PK parameter population	OFA + BEN	OFA alone	OFA + BEN	OFA alone	OFA + BEN	OFA alone	OFA + BEN	OFA alone	Ratio (90% CI)
*AUC_{inf} (hr*µg/mL)	56100 (.)	65400 (8490-504000)	-	23.0	56100 (56100-56100)	66300 (55700-76800)	56100	65400	0.857 (0.148-4.97)
AUC_{last} (hr*µg/mL)	91700 (77400-109000)	108000 (84500-138000)	28.7	46.4	94500 (47500-131000)	126000 (50300-204000)	91700	108000	0.850 (0.666-1.08)
C_{max} (µg/mL)	383 (341-429)	443 (380-515)	19.2	28.0	363 (276-517)	481 (275-606)	383	443	0.864 (0.741-1.01)
BEN – PK parameter population	OFA + BEN	BEN alone	OFA + BEN	BEN alone	OFA + BEN	BEN alone	OFA + BEN	BEN alone	Ratio
AUC_{inf} (hr*µg/L)	6740 (5540-8210)	6880 (5390-8770)	35.2	44.0	6610 (3580-13900)	6320 (4120-14100)	6720	6780	0.991 (0.842-1.17)
AUC_{last} (hr*µg/L)	6740 (5540-8210)	6880 (5390-8770)	35.2	44.0	6610 (3580-13900)	6320 (4120-14100)	6720	6780	0.991 (0.842-1.17)
C_{max} (µg/L)	5880 (4640-7440)	6060 (4910-7500)	42.6	38.0	5440 (3250-11600)	58600 (3430-11700)	5880	6020	0.977 (0.797-1.20)

* No conclusion could be drawn with respect to AUC_{inf} due to very limited sample size (n=2 in OFA alone arm and n=1 in OFA + BEN arm); %CV$_b$, Between-subject coefficient of variation CV$_b$ (%)

Summary/Conclusions: No relevant drug-drug interaction between OFA and BEN was observed in this study. OFA alone or in combination with BEN exhibited manageable safety profile in patients with iNHL.

Infectious diseases, supportive care

E1143

ASSESSMENT OF INTERNATIONAL CONSENSUS GROUP FOR HEMATOLOGY (ICGH) SMEAR REVIEW RULES FOR AUTOMATED PLATFORMS IN THE DETECTION OF MALARIA

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Background: Peripheral blood smear review (SR) is a useful adjunct to the full blood count (FBC) and differential white cell count (DWCC), but is labor intensive and time consuming. For this reason, the international consensus group for hematology (ICGH) published guidelines to reduce SR rates in clinical laboratories using rules based on a combination of blood parameters and instrument suspect flags. These rules have reduced SR rates in many laboratories, but adjustment is often required to accommodate for local pathology/clinician preferences. As malaria is common in Johannesburg (JHB) (although not endemic), this study was undertaken to retrospectively evaluate the performance of modified ICGH SR rules for detection of malaria at the Chris Hani Baragwanath Academic Hospital Laboratory (CHBAH) (part of the National Health Laboratory Service (NHLS) network) in JHB, South Africa.
Aims: To assess the performance of the CHBAH NHLS SR rules in the detection of malaria.
Methods: Malaria test results (*P. falciparum* antigen & thick/thin SR) were extracted from the laboratory information system and corresponding FBCs assessed in those with parasitemia. All ICGH rules were applied to patients with both a FBC and DWCC requested, while only the parameter related rules were applied to patients with only a FBC performed. Samples were processed using a Sysmex XE-5000 analyser (Sysmex, Kobe, Japan), and the rules violated for each FBC recorded. As a retrospective laboratory based study, informed consent was not obtained. However, the study was approved by the Human Resarch Ethics Committee of the University of the Witwatersrand.
Results: Of the 153 samples included, all had *P. falciparum* parasitemia and 37 were collected from patients with severe malaria. A FBC with a DWCC was performed in 72/153(47.1%) patients, and a FBC alone in 81/153(52.9%). SR rules were triggered in 132(86.3%) patients (68(84.0%) in those with only a FBC performed and 64(88.9%) in those with a FBC and DWCC). The thrombocytopenia (platelets (Plt) <100x10^9/l) and anemia (Hb <7g/dl) rules were the most common, triggering in 105(79.5%) and 24(15.7%) patients respectively. Common analyzer morphology flags included those querying the presence of atypical lymphocytes, immature granulocytes and blasts, but 1/more of these triggered in the absence of parameter flags in only 5(7.9%) patients with a DWCC requested. There were 21(13.7%) unflagged (false negative (FN)) samples, of which 20(95.2%) had a Hb level ≥10g/dl, 14(70.0%) had both a Hb ≥10g/dl and a plt count >120x10^9/l and 8(38.1%) had a Hb ≥10g/dl and a plt count >150x10^9/l. On SR, malaria parasites were missed in a further 13.0% of cases, predominantly when the parasitemia was low (median 0.35% in those missed *vs* 3.1% in those with parasites identified). Reassuringly, SR rules were triggered in all the patients with severe malaria, and the parasites identified in 90.5% of these.
Summary/Conclusions: ICGH SR rules are FN in 13.7% of patients with malaria, largely in those with near-normal blood counts. Furthermore, SR failed to identify the parasites in a further 13.0% of cases (particularly those with very low parasitemia). Elimination of a proportion of FN samples is thus not likely to be possible, and clinical vigilance for this condition is required. Reassuringly, SR rules were flagged in all patients with severe malaria, thus increasing the likelihood of this entity being diagnosed if not tested for specifically.

E1144

A PROSPECTIVE MULTICENTER STUDY OF CANDIDEMIA IN NEUTROPENIC PATIENTS WITH HEMATOLOGICAL DISEASES: INCIDENCE, RISK FACTOR AND OUTCOMES

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Background: Candidemia is one of the most common nosocomial bloodstream infections and is associated with high morbidity and mortality, especially amongst the immunocompromised population. Several articles focus on the epidemiology of candidemia, but most of them were from cancer patients, patients with hematological malignancies, patients receiving solid organ transplant, or patients receiving hematopoietic stem cell transplantation (HSCT). Only 3 retrospective studies from single center described the clinical and microbiological features of candidemia in neutropenic patients with hematological malignancies.
Aims: A prospective, multicenter, observational study was designed to investigate the incidence, risk factors and outcomes of candidemia in neutropenic patients with hematological diseases.
Methods: This study was conducted in 11 hematological centers in China over a five-month period. From October 20, 2014 to March 20, 2015, consecutive patients of any age were included in this prospective study if they met the following criteria: (1) had hematological disease; (2) experienced at least one episodes of neutropenia during hospitalization.
Results: A total of 1139 concecutive cases were enrolled in this study. Out of 1139 neutropenic cases, 8 developed candidemia. The median time from neutropenia to diagnosis of candidemia was 18 days (range: 9-20 days). Among the 8 causative strains, 5 were *C. albicans* and other 3 were C. *Tropicalis*. The cumulative incidence of candidemia in neutropenic patients with hematological diseases was 0.00% [95% confidence interval (CI): 0.00, 0.00%] at 7 days, 0.26% (0.00, 0.65%) at 14 days, 2.24% (0.67, 3.81%) at 21 days and 2.24% (0.67, 3.81%) at 28 days after neutropenia, respectively. Among 8 casese with candidemia, 3 were from patients receiving HSCT, other 8 were from patients who had acute myeloid leukemia (AML) and receiving induction chemotherapy. The cumulative incidence of candidemia in patients with AML and receiving induction chemotherapy was also significantly higehr than that in patients receiving HSCT and other patients (5.45% *vs.* 3.1%9 *vs.* 0.00%, *P*=0.023). In addition, multivariate analysis suggested that gastrointestinal mucositis (OR=26.038; *P*=0.003), central venous catheterization (OR=12.904, *P*=0.027), duration of neutropenia >17 days (OR=11.649, *P*=.028) and diabetes (OR=12.435, *P*=0.037) were the risk factors of candidemia in neutropenic patients with hematological diseases. Based on the risk factors, 1139 cases were stratified into low-risk group (0-2 risk factors), intermediate-risk group (3 risk factors), and high-risk group (4 risk factors). The cumulative incidence of candidemia was higher in high-risk group than that in intermediate-risk group and low-risk group (100.00% *vs.* 25.84% *vs.* 0.27%, *P*<0.0001). Besides, the antifungal agents used when candidemia developed were itraconazole (3 cases), voriconazole (2 cases), caspofungin (2 cases), and amphotericin B (1 case). All patients were treated effectively and no one died of candidemia.
Summary/Conclusions: This study provided a description for the epidemiological study of candidemia in neutropenic patients with hemotological diseases. This study defined the risk factors associated with candidemia in these patients, and confirmed that based on the risk factors, risk-stratification could identify the patients with a high-risk of candidemia.

E1145

BRONCHOALVEOLAR LAVAGE AS SYSTEMATIC APPROACH FOR EARLY DIAGNOSIS OF LUNG INFILTRATES AND INVASIVE PULMONARY ASPERGILLOSIS IN HEMATOLOGIC PATIENTS: A PROSPECTIVE SINGLE INSTITUTION STUDY

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Background: The best diagnostic approach of lung infiltrates (LI) remains to be established. Despite bronchoscopy with bronchoalveolar lavage (BAL) appears to be useful for LI diagnosis, hematologists and thoracic surgeons often have qualms in performing bronchoscopy in this difficult patient population at high-risk of procedure-related complications. A proper diagnostic approach at LI seems to be particularly relevant in neutropenic patients and/or in patients with an unfavorable clinical response to broad-spectrum antibiotics, in which the cause of LI are often filamentous fungi, as *Aspergillus* spp.To date, there is not a general consensus regarding the diagnostic panel to apply in hematologic patients undergoing bronchoscopy for LI.
Aims: To evaluate the feasibility of bronchoscopy with BAL as systematic diagnostic approach at LI in hematologic patients, focusing on its role to diagnose invasive pulmonary aspergillosis (IPA).
Methods: Bronchoscopy was performed in all hospitalized patient with diagnosis of acute leukemia and LI at onset of disease before therapy start, and in any other hematologic patient in any phase of disease with LI requiring hospitalization because of concomitant febrile neutropenia and/or respiratory distress not responding to broad-spectrum antibiotics. Criteria for not response to broad-spectrum antibiotics were defined as persistent (> 48 h) fever and/or persistent respiratory distress. In all cases we performed the same diagnostic work-up including blood-swabs cultures, serum galactomannan (GM) assessment (in three consecutive checks), serum beta-D-glucan, serum PCR for CMV; BAL

fluid was studied by bacterial and fungal cultures, GM and PCR for *Streptococcus pneumoniae*, *Legionella pneumophila*, *Chlamidophila pneumoniae*, *Mycoplasma pneumoniae*, *Bordetella pertussis*, *Bordetella parapertussis*, *Haemophilus influenziae*, respiratory virus including CMV, *Pneumocystis jiroveci*, *Mycobacterium tuberculosis* complex*, Nocardia spp.*, *Lysteria monocitogenes and Aspergillus spp*. Available commercial kits were used according to manufacturer's instructions.
Results: Out of 769 patients consecutively admitted in our ward, 85 had LI and 47 of them underwent BAL (total amount: 51 procedures). A causal agent of LI was detected in 33 cases (65%) allowing to modify the ongoing anti-microbial treatment in 25 of these ones (76%). Twelve cases of probable IPA, according to standard criteria, were diagnosed. Seven cases of LI fulfilling the radiologic criteria for IPA, though presenting only a positive *Aspergillus* PCR on BAL, were detected and treated as probable IPA. One life-threatening post-procedure complication was observed.
Summary/Conclusions: BAL seems a safe approach for an early diagnosis of LI in hematologic patients. The assessment of a broad diagnostic panel allowed the detection of a putative agent in 65% of cases. Assessment of *Aspergillus* by PCR on BAL proved useful for probable IPA diagnosis.

E1146

ESCAPE DRUG-RESISTANT INFECTIONS IN HEMATOLOGICAL MALIGNANCIES. DARE TO REVIEW!

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Background: Patients with hematological cancers are at a high risk for increasingly resistant and severe infections. The Infectious Diseases Society of America has defined commonly resistant bacteria as ESKAPE (Enterococcus, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter, Pseudomonas aeruginosa, Enterobacter). As suggested in recent literature, other common and difficult-to-treat infections such as Clostridium difficile and Enterobacteriaceae organisms (E. coli, Proteus) can be added to this group and change the acronym from ESKAPE to ESCAPE.
Aims: We performed a retrospective review of the rate of ESCAPE infections, resistance profile, and outcomes in patients with various hematological malignancies at the Houston Methodist Hospital from 2006 to 2015.
Methods: The patient data was obtained from METEOR (Methodist Environment for Translational Enhancement and Outcomes Research), a clinical data warehouse that contains records dating back to January 1, 2006, with over 3 million patients and over 10 million unique patient encounters. We queried for leukemia (AML, CML, ALL, CLL), amyloidosis and myelodysplastic syndrome (MDS) along with hospitalizations due to bacterial infections. Baseline demographics and overall outcomes were also obtained.

Table 1.

Culture Results[1]	ALL	AML	CLL	CML	AMYLOIDOSIS	MDS	Unspecified	Total
Enterobacter	4	10	15	0	20	21	1	71 (4.5%)
Staphylococcus aureus	4	79	37	14	36	116	6	292 (18.5%)
Klebsiella	5	59	21	7	23	90	0	205 (12.3%)
Acinetobacter	0	14	3	0	12	12	0	41 (2.6%)
Pseudomonas	8	51	42	12	50	104	0	267 (16.9%)
Enterococcus	13	115	39	18	44	138	4	371 (23.4%)
Escherichia coli	8	34	26	14	20	100	7	209 (13.2%)
Proteus	2	17	22	4	2	38	1	86 (5.4%)
Clostridium difficile[2]	2	21	5	1	4	8	0	41 (2.6%)
Total	46 (2.9%)	400 (25.3%)	210 (13.3%)	70 (4.4%)	211 (13.3%)	627 (39.6%)	19 (1.2%)	

1. Cultures included blood cultures, urine cultures, respiratory secretion cultures and tissue cultures
2. Results obtained from positive PCR tests for clostridium difficile toxin A and toxin B

Results: Out of 6017 patients with Hematological Malignancies, 660 patients with 684 malignant diagnoses were found; 235 had MDS, 174 had AML, 105 had CLL, 77 had amyloidosis, 44 had CML, 39 had ALL, and 10 had an unspecified hematological cancer. Of 1132 infectious events, 62% were ESCAPE infections. The bacteria most frequently isolated were Enterococcus (23.4%), Staphylococcus aureus (18.5%) and Pseudomonas (16.9%). Bacteremia was the most predominant type of infection (41.9%) followed up by urinary tract infections (38.2%). Patients with MDS (39.6%) and AML (25.3%) were mainly affected. A prevalent resistance to levofloxacin was detected in gram positives and gram-negative organisms (29-54%). Pseudomonas, E. coli, Proteus and Klebsiella pneumoniae showed a significant resistance to broad-spectrum antibiotics including aztreonam (23-34%), cefepime (7-23%), and imipenem (22%). Proteus had the highest mortality rate (45.2%), followed by Enterococcus (44.2%), and Pseudomonas (36.7%).
Summary/Conclusions: Hematological cancers with risk for neutropenia such as MDS and AML were the most affected by ESCAPE. Bacteremia was frequently seen. Gram-negative pathogens had an increased resistance to broad-spectrum antibiotics and higher mortality rates. A significant resistance to levofloxacin, a prophylactic antibiotic, was also noted. New strategies for reducing ESCAPE in MDS and AML are required. Further statistical review of this data set will be presented at the EHA Meeting, Madrid 2017.

E1147

PROPOSED PEGIFLGRASTIM BIOSIMILAR CHS-1701 DEMONSTRATES PHARMACOKINETIC AND PHARMACODYNAMIC SIMILARITY TO MARKETED PEGFILGRASTIM IN A RAT NEUTROPENIA MODEL AND IN HEALTHY SUBJECTS

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Background: CHS-1701, a proposed biosimilar of pegfilgrastim, is being developed to decrease infection in patients receiving myelosuppressive anticancer drugs associated with febrile neutropenia**.**
Aims: The aim of the preclinical study was to compare pharmacokinetic (PK) and pharmacodynamic (PD) effects of CHS 1701 and marketed pegfilgrastim (MP) in a rat model of cyclophosphamide (CPA)-induced neutropenia. Since pegfilgrastim has the same mechanism of action in humans and rats, preclinical models of CPA-induced neutropenia are considered to be pharmacologically and clinically relevant models of chemotherapy-induced neutropenia in cancer patients. The aim of the clinical program was to demonstrate the PK and PD bioequivalence of CHS-1701 to MP in a multi-center, randomized, single-blind, 3-sequence, 3-period, crossover study in healthy subjects.
Methods: In the rat model, a single SC dose of CHS-1701 or MP was administered at 24 hours after CPA administration, when the peripheral neutrophil counts had been reduced by ~60-70% from baseline. Doses from 30 to 1000 µg/kg were evaluated in order to provide a broad range of exposures to pegfilgrastim and allow for the comparison of CHS-1701 and MP dose response in a steep part of the PD dose response curve. The PD response was evaluated in the blood by analyzing time-dependent changes in absolute neutrophil counts (ANC) and calculating ANC $AUC_{0\text{-last}}$ and in the bone marrow by analyzing pegfilgrastim-induced expansion of myeloid cells and calculating the myeloid to erythroid cell (M:E) ratio. In the clinical PK/PD study, healthy subjects (n=122) were randomized to 1 of 3 treatment sequences; each included 1 therapeutic dose of CHS-1701 (6-mg) and 2 doses of MP (6-mg) separated by ≥28 days. Bioequivalence of CHS-1701 to pegfilgrastim was demonstrated for PK (C_{max}, $AUC_{0\text{-}\infty}$) if the 90% confidence interval (CI) for the geometric mean ratio (GMR) was within 80-125% and for PD (ANC_{max} and ANC $AUC_{0\text{-last}}$) if the 90% CI for the GMR was within 80-125%.
Results: In the rat model, CHS-1701 and MP demonstrated similar time- and dose-dependent changes in the number of peripheral neutrophils and in the proliferative response in the bone marrow. No differences between CHS-1701 and MP in PD (Fig. 1) or PK were observed across the tested dose range.
In the clinical study, PK bioequivalence criteria were met for C_{max} (GMR=105.0; 90% CI 95.5, 115.4) and $AUC_{0\text{-}\infty}$ (GMR=97.5; 90% CI 88.6, 107.2). Pre-specified PD bioequivalence criteria (90% CI) and more stringent criteria (95% CI) were met for ANC_{max} (GMR=99.6; 90% CI: 96.2, 103.2; 95% CI: 95.5, 103.9) and ANC $AUC_{0\text{-last}}$ (GMR=96.7; 90% CI: 92.2, 101.4; 95% CI: 91.4, 102.4). The two treatments displayed similar safety profiles. Investigator-designated treatment-related AEs occurred in 71.9%, 71.2%, and 62.8% of subjects during the CHS-1701, first MP, and second MP dosing periods, respectively, and most commonly included back pain (46.9%, 42.3%, 30.8%), headache (29.2%, 36.9%, 29.5%), and arthralgia (8.3%, 13.5%, 7.7%). There were no treatment-related serious AEs.

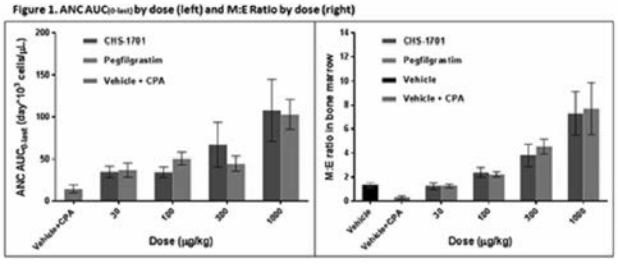

Figure 1.

Summary/Conclusions: The dose-dependent changes in the neutropenia model were consistent with the PD effects of pegfilgrastim in humans and demonstrate that CHS-1701 results in comparable neutrophil recovery and time course compared to marketed pegfilgrastim. The clinical study demonstrates highly similar PK, PD, and safety profiles in humans for CHS-1701 and marketed pegfilgrastim. Overall, preclinical and clinical results suggest that CHS-1701 would provide similar PK, PD, safety, and efficacy to marketed pegfilgrastim in patients with chemotherapy-induced neutropenia.

E1148

A RETROSPECTIVE REVIEW IDENTIFYING RESISTANT MICROBIAL STRAINS, ANTIMICROBIAL SENSITIVITIES AND RISK STRATIFICATION OF FIRST LINE ANTIBIOTIC USE IN ADULT CANCER PATIENTS WITH NEUTROPENIC SEPSIS

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Background: Neutropenic sepsis remains a leading cause of morbidity and mortality in both haemato-oncology and general oncology patients on cytotoxic treatment. In 2012, UK NICE published guidelines on the empirical use of beta lactam monotherapy in suspected neutropenic sepsis, discouraging the use of aminoglycosides, in view of potential toxicities. Increasingly in clinical practice it becomes evident that our patient population is incredibly heterogeneous and with the emergence of multi drug resistant strains of micro-organisms, high-risk individuals need to be identified early and first line antimicrobial treatment regimens tailored according to patient factors alongside local antibiogram.
Aims: To retrospectively review appropriate antibiotic use, microbial identifications and antibiotic sensitivities amongst adult cancer patients with neutropenic sepsis. To identify if any patient or disease characteristics are associated with adverse outcomes, that would support the upfront usage of aminoglycoside containing antibiotic treatment regimes.
Methods: A retrospective review of patients treated for neutropenic sepsis was conducted for the period between 1/4/2015 to 11/10/2016. Analysis of potential risk factors including primary disease, age, sex, treatment regimen, albumin, neutrophil and lymphocyte count to assess potential association with adverse outcomes.
Results: There were 116 episodes of neutropenic sepsis in 92 patients in this period. Of these, 61 were haemato-oncology patients and 31 general oncology. 42 of 76 positive cultures identified gram-negative organisms. 40 patients received single agent Tazocin and 71 patients (61.2%) received Tazocin and an aminoglycoside as first line antimicrobial treatment. Fourteen isolates demonstrated resistance, including 2 cases of stenotrophomonas maltophilia and 12 cases of enterobacteriaceae. 13 of the 14 resistant isolates were found in haemato-oncology patients. Nine of these cases were resistant to single agent Tazocin but sensitive to an aminoglycoside. The mean age of cases with resistant bacteria isolated was 54.2 years (range 25-84 years). There was no difference in sex or degree of neutropenia/lymphopenia in the cases that contracted resistant bacterial strains compared to those that were culture negative. Of the 4 fatal cases with resistant bacteria, 3 had low albumin (mean 25.5g/L cf. mean of 34g/L in resistant bacteria cases surviving).
Summary/Conclusions: This retrospective analysis supports the use of combination antimicrobials up front as first line treatment in high-risk patients with neutropenic sepsis. The study has demonstrated that the patient cohort most at risk of developing drug resistant bacteraemia are patients with high-risk or relapsed haemato-oncological disorders like AML or high-grade lymphoma, requiring multiple cycles of intensive chemotherapy. Of the patients who isolate resistant bacteria, identifying low albumin early may be a potential marker for adverse outcome in terms of morbidity and mortality. Of interest only one oncology patient isolated a resistant strain of bacteria, furthermore only 25% of general oncology patients treated with neutropenic sepsis had positive cultures compared to 79.8% of haemato-oncology patients. When comparing these findings to UK NICE recommendations it is clear that first line use of Tazocin in general oncology patients may well suffice in initial treatment of neutropenic sepsis. However with haemato-oncology patients early or up front consideration for the additional usage of an aminoglycoside is essential to optimize favourable outcomes in high-risk patients. From this small study, the proposed risk factors of isolating resistant strains of bacteria leading to adverse outcomes would be aggressive haematological malignancies, receiving more intensive cytotoxic therapy, multiple lines of treatment and low albumin. Further analysis in a multi centre prospective study in this patient population alongside close collaboration between clinicians and microbiologists is essential in providing optimal antimicrobial therapy algorithms in neutropenic patients.

E1149

PRELIMINARY RESULTS FROM A LONG-TERM REPEAT DOSE TOXICITY AND TOXICOKINETIC STUDY OF ANF-RHO, A NOVEL ANTI-NEUTROPENIC FACTOR

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Background: ANF-Rho is a novel polyethylene glycol-modified granulocyte colony stimulating factor that has biophysical and biological properties that produce a prolonged pharmacokinetic and pharmacodynamic profile as compared to pegfilgrastim (Neulasta®). As such, it has potential applications in chemotherapy induced neutropenia and chronic idiopathic neutropenia. These disorders require prolonged administration of G-CSF agents to treat neutropenia. Therefore, long term toxicology, genotoxicity and Juvenile studies were conducted with ANF-Rho.
Aims: A 13-week study was conducted in Sprague Dawley rats and cynomolgus primates to assess various safety and pharmacokinetics of ANF-Rho as compared to Neulasta® (pegfilgrastim).
Methods: The study design used 288 rats, divided into 5 dosage groups: control, 100, 300, 1000 (high) and 1000 (positive) µg/kg. A total of 58 monkeys were also divided into 5 dosage groups: control, 75, 250, 750 (high dose) and 750 (positive) µg/kg of ANF-Rho. Doses were administered by weekly subcutaneous injections on Day 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85 and 92 at a dose volume of 5 mL/kg. Genotoxicity assessments were evaluated using *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay, rodent blood micronucleus assay and chromosomal aberration assay. Toxicology assessment included clinical observations, body weight change, food consumption, ophthalmic examination, function observational battery (motor activity, behavioral changes, coordination and sensory/motor reflex response), organ weight, bioanalytical and toxicokinetic analysis, immunogenicity, gross necropsy and histopathology.
Results: No observed clinical signs seemed to be related to ANF-Rho administration. There were no related effects in body weight changes or food consumption. Observed ophthalmic effects were considered procedural related due to low incidence. No biologically meaningful findings were noted during the function observational battery assessment. Preliminary analysis showed a dose related increase in spleen weight in rats and a dose dependent decrease in kidney weight in primates. Genotoxicity studies found no signs of mutagenicity, clastogenicity or cytotoxicity.
Summary/Conclusions: The results from this preliminary toxicology studies are unremarkable and consistent with those of an earlier 28-day study. Results from the 28-day rat neutropenia dosage model found that the blood pharmacodynamics parameters of ANF-Rho were significantly superior to PEG-filgrastim. Both PK and PD data demonstrate relatively predictable systemic exposures and activity following SC or IV dose levels in both rat and primate. It is anticipated that this long terms 13-week study will provide evidence of safety sufficient to support advancement of ANF-Rho into Phase II clinical studies in chemotherapy-induced neutropenia and chronic idiopathic neutropenia in Europe, USA and India.

E1150

USE OF MICAFUNGIN IN PROPHYLAXIS IN ONCO-HEMATOLOGY: RESULTS OF AN OBSERVATIONAL, MULTICENTER, PROSPECTIVE FRENCH STUDY (OLYMPE)

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Background: Antifungal prophylaxis is being used increasingly.
Aims: The therapeutic arsenal is extensive and requires a better understanding of micafungin use in onco-hematology where most-at risk patients of invasive fungal infections (IFI) are managed.
Methods: This observational study was conducted in 18 onco-hematology units in adult patients and children treated with micafungin in prophylaxis with a 3-months follow-up period.
Results: 150 patients (95 adults, 55 children) were included and represent the analysis population. In total, 15 patients (10%) presented an IFI during micafungin treatment. Among them, 11 presented a probable or proven IFI. The rate of IFI was higher in children (15%, n=8) than in adults (7%, n=7) and seem to have been influenced by the type of hemopathy and if the patient was allografted or not: 13% (n=8) in allografted patients, 9% (n=4) in patients with AML or SMD and 7% (n=3) in other patients. Median time to infection was 24 days (1 to 68 days) and was longer in adults (25 days, 4 to 68 days) than in children (16.5 days, 1 to 68 days). Twelve patients (8 children and 4 adults) presented at least one clinical or radiological sign of suspected IFI. Fungus was identified in 8 patients (62%), mostly in blood cultures (50%, n=4): candidiasis in 4 patients, aspergillosis in 3 patients and infection related to Rhisopus in 1 patient. Incidence rate of IFI (10%, 5 patients) was inferior to prophylaxis failure rate (23%, 34 patients). Prophylaxis failure rate takes in account patients who switched to empirical treatment besides patients who switched to preemptive or curative treatment. After the end of prophylaxis, 4 patients (3%, 3 adults and 1 child) presented a proven IFI. Median time to infection after the end of treatment was 10,5 days in adults (7 to 24 days) and 52 days in children. Micafungin was overall well tolerated: only 10 patients (7%, mostly children) presented grade 1 to 4 adverse events related to micafungin, including 5 patients (3%à with grade 3 or 4 adverse events.
Summary/Conclusions: Effectiveness and safety profile of micafungin in prophylaxis are similar to what was observed in previous studies. Incidence IFI rate of 10% confirms the clinical effectiveness of micafungin in prophylaxis in high risk patients. The low rate of serious adverse events confirms micafungin safety profile, in children included.

E1151

OUTBREAK OF MULTI-DRUG RESISTANT PSEUDOMONAS AERUGINOSA (MPA) IN A HAEMATOLOGY WARD (HW): MANAGEMENT AND INFECTION CONTROL MEASURES

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Background: *Pseudomonas Aeruginosa* (PA) is a gram negative, ubiquitous, opportunistic pathogen. Its intrinsic resistance to many antibiotics and the selective pressure exerted by empiric antimicrobial use, led to the emergence of MPA in hematologic units, with high mortality and morbidity rates among infected immunocompromised patients (pts). Considering our MPA incidence of 9% in 2007/08, an outbreak developed at the HW of "Campus Bio-Medico" University Hospital of Rome, from 2009, despite the measures employed from the previous 2 years (health personnel sensitization, regular air and water filters changing, isolation precautions).
Aims: To describe the MPA outbreak occurred between 2009 and 2013.
Methods: Our HW, opened in 2007, is composed by 7 rooms, each with a private WC: 2 single, 1 double and 4 single, positive pressure, each with a filtered-zone, dedicated to stem cell transplanted pts. *Retrospective study*: From 01/2009 to 04/2013 we hospitalized 415 adult pts; of these, 106, at high infectious risk (HIR) for severe and prolonged expected post-chemotherapy neutropenia, have been routinely screened at admission with microbiological samples (nasal, pharyngeal and rectal swabs) and additional tests when clinically indicated. Because, during this period, we observed a dramatic incidence of MPA isolates, we fulfilled specific sequential measures, to assess potential reservoirs and breaks in infection control and to manage the outbreak, summarized by the following 4 phases: *phase A*: closing of HW from 29/04/2013 to 09/06/2013; *phase B*: serial pre and post-disinfection environmental sampling from each room: swabs from sink filters, bidet filter, shower filter, flush button, infusion pump, TV remote control, 70% ethylic-alcohol gel bottle, floor sink, bedpan and water samples from bedpan automatic washers (BAW); *phase C*: room environmental disinfection and microbial decontamination with nebulized H_2O_2 solution added with silver cations; *phase D*: disposal of BAW, introducing the sole use of disposable bedpans and planning an environmental sampling and disinfection program.
Results: On 04/2013 we revised *retrospective study* data: 82 pts carried bacterial isolates; of these, 48 (59%) had MPA, classified as colonisation in 13 pts (mainly detected on rectal swabs) and true infection in 35: 10 pneumonias (29%), 6 anorectal/perineal (17%), 5 urinary tract (14%), 14 bloodstream infections (40%). Ten pts died of MPA related infection, with a mortality of 53% (10 on 19 pts) and case-fatality rate of 29% (10 on 35 pts). *Phase B* defined a prevalence of PA isolates in 6 out of 7 rooms in different sample types, with 4 MPA isolates identified in 3 different BAW and 1 bedpan after washing cycle. After *phase C*, a new *phase B* demonstrated sterilization of 3 out of 6 PA and 3 out of 4 MPA isolation sites. As a main corrective action, after 41 days we resumed admissions and approached *phase D,* resulting into a prompt and maintained decrease in isolates (Table 1).

Table 1. MPA isolates and mortality rate after phase D.

	2013 (second half)	*2014*	*2015*	*2016*
HIR pts	**35**	**38**	**51**	**52**
Colonisation (%)	**0**	**1**(3)	**0**	**0**
Infection (%)	**2**(6)	**1**(3)	**1**(2)	**0**
Mortality (%)	**0**	**0**	**0**	**0**

Summary/Conclusions: We identified the contaminated water residue from BAW as the main source of MPA spread in our HW, getting a full outbreak control by improving environmental measures. Pseudomonas contaminates and survives on many ecological niches, being continuously reintroduced in nosocomial settings. Our experience highlights the value of environmental and personal hygiene measures on MPA infections control.

E1152

MONITORING VORICONAZOLE PHARMACOGENOMICS AND PLASMA CONCENTRATIONS IN THE TREATMENT AND PREVENTION OF INVASIVE FUNGAL DISEASE FOR HEMATOLOGICAL PATIENTS A SINGLE CENTER EXPERIENCE

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Background: Voriconazole has been widly used in treatment and prevention invasive fungal disease for immunodeficiency hematological patients. And the voriconazole plasma drug levels were associated with its efficacy and toxicity. The hepatic cytochrome P450 isoenzyme 2C19 plays a important role in voriconazole metabolism. However if CYP2C19 genetic polymorphism can result in voriconazole metabolism and drug plasma level in setting of Asian population especially in hematologic patients is unknown.
Aims: To evaluate the effect of CYP2C19 polymorphism on the voriconazole (VCZ) plasma concentration of patients with hematological disease and the value of serial monitoring voriconazole plasma concentrations in the treatment and prevention of invasive fungal disease(IFD).
Methods: Between January to August 2016, 76 hematological patients who received voriconazole for the treatment or prevention of invasive fungal disease were enrolled in this study. The population CYP2C19 polymorphism of voriconazole were performed using PCR-Pyrosequencing. The trough plasma concentrations of vriconazole (C_{trough}) was determined using high-performance liquid chromatography (HPLC).
Results: Genotyping for CYP2C19 polymorphic isoenzyme variations showed that 32 subjects (43.42%) for the CYP2C19 wild-type, 43 (56.58%) for the CYP2C19 no-wild-type. 2. 45 of 76 patients received voriconazole intravenous administration, Based on the genotype analysis, 45 subjects were identified as extensive metabolizers' group for EMs (CYP2C19*1/*1), poor metabolizers' group for IMs+PMs (CYP2C19 * *1/*2,*1/*3,*2/*3,*2/*2,*3/*3)□and there was a significant difference between C_{trough} values in the two groups (1.66±1.86ug/ml *vs* 3.30±2.35ug/ml, p=0.00). 3. The C_{trough} of the 45 patients were detected for 119 times totally. The medium of the C_{trough} 45 hematological patients were described. Lack of response to therapy was more frequent in patients with voriconazole levels <1.5mg/L (23.5%) than in those with voriconazole levels >1.5mg/L (21.4%])(p=0.87). And the risk of adverse events was more frequent in patients with voriconazole levels >5.5mg/L (40.0%) than in those with voriconazole levels <5.5mg/L (25.0%) (p=0.49). Furthermore, the C_{trough} values of patients with adverse events is higher than the others(3.21±2.46ug/ml *vs* 2.17±2.14 ug/ml, p=0.042).
Summary/Conclusions: The single-center study showed that the mutation of CYP2C19 was quite common in Chinese hematological patients. Patients with CYP2C19 wild-type phenotype are extensive metabolizers, their C_{trough} of voriconazole are significantly lower than patients with CYP2C19 non-wild-type phenotype (poor metabolizers). Appropriate concentrations of vriconazole can improve the effiency of therapy and safety outcome.

E1153

BACTEREMIA AND SEPSIS FOLLOWING INTENSIVE CHEMOTHERAPY OF ADULT ONCOHEMATOLOGICAL PATIENTS

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Background: Intensive cytostatic chemotherapy is a standard strategy for leukemia treatment. Meanwhile, such treatment causes negative effects, including lymphopenia, granulocytopenia and damage to tissue barriers associated with significant risks of infectious complications, especially, bacterial sepsis and viremia.
Aims: Our study was aimed for identification of bacteremia in oncohematological patients following intensive chemotherapy, and assessment of potential modifying role of herpesvirus infections.
Methods: Retrospective review of positive bacterial isolates of blood between January 1991- December 2015. Prospective study the cases of bacteremia and sepsis in cohort of 64 patients with hematologic malignancies. Diagnostics of septic conditions was based on clinical data, bacteremia and systemic inflammatory reaction syndrome (SIRS) (registration of, at least, 2 of 4 clinical symptoms of SIRS). Bacteriological analyses and identification of micromycetes were performed by uniform technique over the entire study period, according to the valid guidelines. For DNA-diagnostics, we used gene-specific PCR with real-time registration. DNA was extracted from peripheral blood leukocytes The herpesvirus panel included Herpes Simplex type 1 and 2 (HSV); Cytomegalovirus (CMV); Epstein-Barr virus (EBV), and Human Herpesvirus type 6 (HHV6). PCR techniques were performed according to manufacturer instructions.
Results: Based on the study 4923 blood samples it was shown that the frequency of detection of bacteria was 11.0%. The predominance of Gram-positive bacteria was demonstrated among pathogens detected in the bloodstream. However, the ratio of detectable Gram-negative flora was found to be increased from 23.1% to 39.6% between 2002 and 2015 (p<0.05). Coagulase-negative staphylococci (CoNS) prevailed among Gram-positive microorganisms, in particular, *S. epidermidis*, whereas *Enterobacteriaceae*, especially, *E.coli*, dominated among the Gram-negative bacteria. It is shown that the development of bacteremia were significantly more frequently occurs on the background of the detection of Cytomegalovirus and the Epstein-Barr virus genomes. In recent years there has been increase the frequency of micromycetes detection in the blood of patients with hematological malignancies. In present study, antibacterial therapy started with β-lactame antibiotics combined with fluoroquinolones, aminoglycosides, metronidazole. If required, the antimicrobial strategy was revised 48 to72 hours later as based on clinical and microbiological data, applying carbapenems also combined with other anti-infectious drugs. When detecting herpesviruses (EBV, HSC-1/2, HHV6, or CMV) the therapy was accomplished by antiviral drugs, *i.e.*, Acyclovir or Valacyclovir (for CMV treatment). Sepsis in accordance with the criteria (SIRS and bacteremia) was diagnosed in 33 of 64 patients. Mortality was higher in patients with Gram-negative sepsis – 8 of 14 patients (57%) whereas in Gram-positive sepsis 2 of 19 patients (11%).
Summary/Conclusions: Our data support a general viewpoint on regular monitoring of infectious pathogens upon intensive chemotherapy of oncohematological patients prone to both bacterial and viral infections. Severe bloodstream infectious complications are often associated with fungal invasions, and herpesvirus reactivation. In particular, our results suggest that herpesviruses, may cause immunosuppression, or may serve as additional immunodeficiency markers predictive for bacterial infections at later terms.

Iron metabolism, deficiency and overload

E1154

GLYCOSYLATED FERRITIN MEASURING SIGNIFICANCE FOR SECONDARY HEMOPHAGOCYTIC SYNDROME DIAGNOSTICS

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Background: Hemophagocytic syndrome (HPS) is a clinicopathologic condition characterized by systemic inflammatory reaction with cytopenia and tissue damage. The HPS may be primary (genetic associated) or secondary (SHPS), caused by different systemic disorders (immune, infectious, neoplastic). The overall clinical symptoms are similar to sepsis, so it could be difficult to differentiate among these entities. Ferritin levels are high in both cases, but the glycosylated/nonglycosylated ferritin fractions ratio is seems to be indicative.
Aims: The estimation of the ferritin fractions ratio and biochemical profile in patients with sepsis and SHPS.
Methods: The data from 64 patients were analyzed: 40 pts with diagnosed SHPS (median age 57, range 8-74 years) and 24 with lethal septic shock (median age 57.5, range 18-82 years). SHPS in patients with persistent fever refractory to antibacterial therapy and/or prolonged cytopenia and/or organ (lungs, CNS) involvement was established after the other conditions had been excluded. Sepsis diagnostics was based on the confirmed infection site and systemic inflammation with multiorgan failure. The following serum values were analyzed: alkaline phosphatase (AlPh), alanine aminotransferase (ALAT), asparagine aminotransferase (ASAT), lactate dehydrogenase (LDH), bilirubin, creatinine, INR, C-reactive protein (CRP), procalcitonin (PCT), total ferritin, and glycosylated ferritin percentage. Mann-Whitney U test and ROC-analysis were used for statistical analyses.
Results: No differences were found in sepsis and SHPS for ALAT, ASAT, AlPh, LDH, and bilirubin levels. The difference of INR, CRP, PCT, creatinine levels was significant (p<0.01). The most substantinal difference in SHPS and sepsis groups had serum concentrations of ferritin, triglycerides, level of ferritin glycosylation (p<0.01) (Table 1). According to ROC-analysis, the area under the curve for ferritin, triglycerides and percentage of ferritin glycosylation were 0.78, 0.82, and 0.92, respectively.

Table 1.

	Dimension	median	25 quartile	75 quartile
Ferritin-SPHS	Ng/ml	7635	2863	13559
Ferritin –sepsis	Ng/ml	2163	1094.7	3940.5
Glycosylation ferritin – SPHS	%	21	10	33
Glycosylation ferritin - sepsis	%	40.1	33.7	55.9
Creatinine – SPHS	Mcmol/l	90	72	142
Creatinine – sepsis	Mcmol/l	186	126.5	302.5
Triglycerides – SPHS	Mmol/l	3.1	2.2	4.1
Triglycerides – sepsis	Mmol/l	1.38	0.75	2.37
C-reactive protein – SPHS	Mg/l	80.6	25.3	183
C-reactive protein – sepsis	Mg/l	214.5	185.9	287.5
Procalcitonin – SPHS	Ng/l	1.65	0.71	2.29
Procalcitonin – sepsis	Ng/l	55.9	38.9	198.35
International Normalized Ratio - SPHS		2.37	1.02	2.03
International Normalized Ratio - sepsis		1.73	1.47	2.4

Table 1. Significant laboratory differences (p<0.01), SPHS – secondary hemophagocytic syndrome.

Summary/Conclusions: The most difference between sepsis and SHPS was observed for triglycerides, ferritin and percentage of glycosylated ferritin. Percentage of glycosylated ferritin fraction seems to be the most indicative, which may make it useful for SHPS diagnostics and its differentiation from sepsis.

E1155

SERUM HEPCIDIN QUANTIFICATION IN INFLAMMATORY BOWEL DISEASES

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Background: Inflammatory bowel diseases (IBD) includes different intestinal pathologies, most common among them are Colitis Ulcerosa (CU) and Crohn's Disease (CD). Pathogenesis of IBD is still unclear, however they are multifactor diseases, with genetic and autoimmune compounds, in combination of environmental factors. One of IBD symptoms is iron deficiency anemia.
Aims: We aimed to search for connection between serum hepcidin quantification and anemia in IBD.
Methods: We included 64 patients with IBD - 29 with Colitis Ulcerosa (CU), and 35 with Crohn's Disease (CD). They were diagnosed in University "Aleksandrovska" hospital in Clinic of Gastroenterology. Their results were compared to age and gender matched healthy controls. Laboratory assessments were analyzed for included groups – iron, ferritin, CRP, IL-6 and hepcidin. AAS, nephelometric, ELISA and statistical methods were used during analyzes and obtained results interpretation.
Results: 53 from our patients had with iron deficiency anemia (IDA) and low hepcidin concentrations (5.9±1.1 µg/L) compared to control group (19.9±2.8 µg/L); *P*<0.001. 11 of included cases had combination of IDA and anemia of chronic disease (ACD). Their hepcidin levels were increased (59.9±6.4 µg/L) in comparison to healthy controls (19.9±2.8 µg/L); *P*<0.001. In patients with ACD/IDA, quantified serum hepcidin correlates positively to increased IL-6 (r=0.758, *P*<0.005) and CRP concentrations (r=0.899, *P*<0.001).
Summary/Conclusions: Quantification of serum hepcidin levels in IBD patients might be a key element in diagnosis and treatment of anemia in these patients. Serum hepcidin levels are useful marker for differential diagnosis between iron deficiency anemia and combination iron deficiency anemia/ anemia of chronic disease.

E1156

MUTATIONS IN YARS2 CAUSE CONGENITAL SIDEROBLASTIC ANEMIA WITHOUT SHOWING EVIDENCES OF MYOPATHY AND LACTIC ACIDOSIS

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Background: Mutations in the gene *YARS2* encoding mitochondrial tyrosyl-tRNA synthetase have previously been identified as a cause of MLASA2, a mitochondrial respiratory chain disorder presenting with myopathy, lactic acidosis and congenital sideroblastic anemia (OMIM #610957, ORPHA 2598). Up to date in the literature it has been reported 9 families with 11 affected individuals with mutations in *YARS2* gene and affected from MLASA2.
Aims: Here we report a new case with a different clinical presentation.
Methods: We have identified two novel variations in *YARS2* gene using Next Generation Sequencing (NGS) panel containing 10 genes involved in congenital and acquired sideroblastic anemia.
Results: The proband is a young woman aged 24 where we have identified 2 novel variations in *YARS2* gene. One pathogenic splicing mutation NM.001040436.2 c.[1104-1G>A], and a missense variation NM.001040436.2 c.608 G>T; NP_001035526.1: p. Ser203Ile located in the C-core catalytic domain of the mitochondrial tyrosyl-tRNAsynthetase. None of these two variations were previously reported in public databases (ExAC, NCBI SNP, Ensembl). Clinical data from the patient showed marked sideroblastic anemia (Hb 91 g/L, 32% ring sideroblasts), but not signs of muscle weakness or myopathy and no lactic acidosis (lactic acid levels were 1.8mmol/L, normal range: 0.5 - 2.2 mmol/L; creatine kinase 23 UI/L, normal range: 23-170 UI/L), as could be expected due to previously reported cases in the literature. Functional assays are on-going to confirm pathogenicity of the novel missense variation.
Summary/Conclusions: Here, we reported a patient with mutation in *YARS2* gene showing congenital sideroblastic anemia but presenting neither lactic acidosis nor myopathy. Therefore, patients with defect in *YARS2* gene may present with a less severe clinical manifestations only involving congenital sideroblastic anemia without other extra-hematopoietic defects. MLASA2 must be consider in patients presenting with only congenital sideroblastic anemia since early diagnosis and supportive therapy will be important to prevent complications.

E1157

IRON CHELATION DATA OF CONGENITAL DYSERYTHROPOIETIC ANEMIA PATIENTS: A SINGLE CENTER EXPERIENCE

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Background: Congenital dyserythropoietic anemia (CDA) is a rare, genetically heterogenous disorder characterized with ineffective erythropoiesis, and congenital malformations in certain types. Patients present with varying degrees of anemia and some of the patients may have mild disorder wheras others may be transfusion dependent. The ineffective erythropoiesis and the transfusional iron load puts these patients at risk for iron overloading and there is very scarce data on the iron loading and chelation types in these patients.
Aims: We aimed to summarize the chelation results of our patients with CDA from a single center.
Methods: Of the 33 patients with CDA, 11 were initiated iron chelation treatment either for receiving more than 20 packed RBC transfusions previously or for having serum ferritin levels above 1000ng/ml.

Results: Of these 11 patients, 7 were CDA type II. The median age of diagnosis was 12 months (3-144) months and male to female ratio was 7/4. Median transfusion requirement per year at previous year prior to initiaition of chelation was 12 times (0-17). All of the patients were on chronic transfusion programme at initiation of iron chelation except for 2 (one receives occasional transfusion, and the other patient was on chronic transfusion programme but became treansfusion independent after splenectomy). The median age at last visit was 70 months (32m-40 years). The median value of serum ferritin at initiation of iron chelators was 822 ng/ml. All of the patients were initiated deferasirox for iron chelation at a median dose of 24mg/kg/day (10-40) and the median chelation follow-up duration was 27 months (2-54 months). Three of the patients were evaluated with cardiac and hepatic T2* assesment prior to and by the end of 1 year of chelation aand none of the patients were found to have cardiac iron loading at chelation initiation, wheras 2 had severe and 1 had moderate LIC values. In the subsequent assesment under chelation of these 3 patients all still had cardiac T2* values above 20 ms, wheras 1 had mild and 2 had moderate LIC values. Serum ferritin levels prior to chelation initiation and by the end of 1 year were compared and the difference was found statistically insignificant.
Summary/Conclusions: Patients with CDA are at risk for iron loading and they need to be screened for the iron loading periodically. The prompt chelation in these patients prevent organ failure risks at long term including cardiac failure, cirrhosis and endocrinopathies.

E1158

ORAL IRON CHELATION FOR TREATMENT OF HEREDITARY HEMOCHROMATOSIS IN CHILDREN

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Background: Hereditary hemochromatosis (HH) very rarely presents during childhood. The most common form of HH in children is Juvenile Hemochromatosis (JH), a rare genetic disorder inherited with an autosomal recessive manner, resulting from mutations in either the hemojuvelin (HJV) (type 2A) or the hepcidin (HAMP) gene (type 2 B). Early diagnosis and closely monitoring of iron overload indexes, namely, serum ferritin levels, transferrin saturation and tissue iron measurement by magnetic resonance imaging (MRI) are essential in order to prevent permanent organ damage and potentially life threatening complications (cirrhosis, diabetes mellitus, cardiac dysfunction, and hypogonadism). Therapeutic intervention in children with HH may be problematic, as eryhtrapheresis is invasive and may not be well tolerated in young children. Iron chelation therapy can be implemented as an alternative treatment to erythrocytapheresis.
Aims: The scope of this study was to evaluate the use of an oral iron chelation therapy in young children with HH.
Methods: 3 children (2 females and 1 male) were diagnosed with HH at the aged of 4, 6 and 8 years old, respectively, based on increased ferritin and transferrin saturation levels and exclusion of other not-iron-overload related causes of hyperferritinemia. Genetic analysis were performed in all 3 patients and showed positive results in 2 of them, while on the 3rd no genetic changes could be identified. All patients were at pre-symptomatic stage of the disease and they were referred for evaluation after hyperferritinemia was discovered on a routine screening examination. Liver iron concentration (LIC) and cardiac iron concentration were evaluated by MRI (table 1). Iron chelation therapy with deferasirox (DFX) at low dose (of 10mg/kg/24h) was initiated, after evaluation was completed and permission from regulatory authorities obtained.

Table 1. Clinical characteristics of the patients.

	Age (years) at treatment	Genotype	Ferritin at treatment (ng/dl)	Transferrin saturation (%)	LIC (mcgr Fe / g.d.w.tissue)	Cardiac Iron-T2* (msec)
1	7	HJV:G320V/N HFE:S65C/N	728	90%	12.6	32.9
2	6	HJV:G320V/G320V	584	71%	10.4	30.7
3	10	unknown	309	55%	1.1	18.5

Results: All 3 patients responded promptly to therapy and showed decreased levels of ferritin, LIC and cardiac iron concentration. Gastrointestinal disturbances were noted in 1 patient, which resolved with H2-blockers and with changing the treatment to 5d/wk (patient 2). Mild increase in serum creatinine (>33% from baseline but within normal range for her age) was observed in patient 3, which resolved with temporary cessation of the chelation therapy.
Summary/Conclusions: HH is very rare disorder in children, most frequently due to JH. Timely initiation of treatment to prevent iron overload consequences is essential. Chelation therapy with deferasirox is efficacious with a manageable toxicity profile and it can be considered as an alternative therapeutic option to chronic erythrocytapheresis for the treatment of JH.

E1159

NEUTROPHIL HYPERSEGMENTATION IN ADULTS WITH IRON DEFICIENCY: A CASE-CONTROL STUDY

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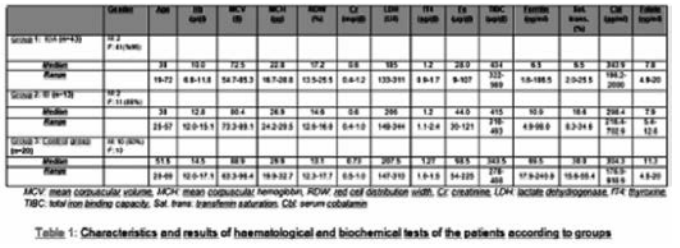
Background: Neutrophil hypersegmentation (NH) has been accepted as a hallmark of the macrocytic anaemias associated with the deficiency of cobalamin or folate. However, there are a small number of reports stating that NH might accompany iron deficiency anaemia. The aim of the present study was to determine the association of NH with iron deficiency (with or without anaemia).
Aims: The aim of the present study was to determine the association of NH with iron deficiency (with or without anaemia) in adults and also to compare neuthrophil segmentation status in anemia group before and after oral or parenteral iron treatment .
Methods: Fifty-six patients with iron deficiency and 20 age and sex matched controls were included in this prospective, single blind, case-control study between February-November 2016. Subjects were included if they were ≥ 18 years of age, and had normal serum vitamin B_{12} and folate levels, liver, thyroid and renal function tests. Pregnant women and patients with a history of blood transfusion within last 3 months and/or those with acute renal failure, anaemia of chronic disease, hypothyroidism, additional cytopenias and infection were excluded. Patients with iron deficiency were divided into 2 groups being with iron deficiency anemia (IDA) and iron deficiency without anaemia (ID). Those with anaemia were further evaluated prior and after iron replacement. Results of the study groups were compared to age and sex matched healthy controls. Blinded peripheral blood smear slides were evaluated by a haematologist by counting 200 neutrophils. Hypersegmentation was defined as reported by Bain et al.. Iron deficiency was diagnosed based on the findings of iron parameters including serum iron, total iron binding capacity, and ferritin. Anaemia was defined according to the WHO recommendation. Cohort characteristics were given in Table 1.

Table 1.



MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, RDW: red cell distribution width, Cr: creatinine, LDH: lactate dehydrogenase, FT4: thyroxine, TIBC: total iron binding capacity, Sat. trans: transferrin saturation, Cbl: serum cobalamin

Table 1: **Characteristics and results of haematological and biochemical tests of the patients according to groups**

Results: Hypersegmentation was detected in 25 individuals with iron deficiency (45%) and 1 healthy control (5%). It was significantly more frequent in the IDA group (48.8%) than in the ID group (30.7%) [p<0.001]. After iron treatment 3 IDA patients' peripheral blood smear were checked and with normalization of iron parameters and hemoglobin, hypersegmentation was undetectable. The study is still ongoing and rest of the IDA group are still on treatment, their peripheral blood smears are to be examined after iron treatment is over.

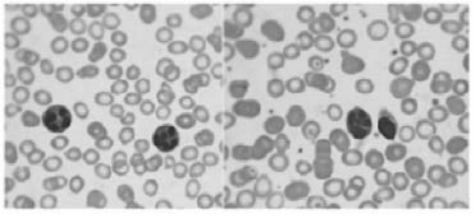


Figure 1: **Hypersegmentation of neutrophils in two different patients with IDA**

Figure 1.

Summary/Conclusions: Although the mechanism of neutrophil hypersegmentation in iron deficiency anaemia is not clear, it is thought that iron acts as a cofactor in folate metabolism and / or DNA synthesis in granulocytes. There are a limited number of studies dealing with NH associated with IDA in the literature. However most of these studies were observational and did not include controls or were not blinded. Our study is the first to demostrate the association of NH with iron deficiency anaemia in adults in the absence of megaloblastic anemia.

E1160

M-TOR INHIBITORS-ASSOCIATED MICROCYTIC ANEMIA AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Immunosuppression with mTOR inhibitors (sirolimus or everolimus) has been associated with development of microcytic anemia after solid organ transplantation. The prevalence reaches 27 to 57% in the case of kidney transplantation. This anemia has been attributed to hepcidin increase induced by the inhibition of mTOR protein[1,2].
Aims: To evaluate the prevalence of microcytic anemia after allogeneic hematopoietic stem cell transplantation in patients receiving mTOR inhibitors.
Methods: 61 consecutive allogeneic stem cell reduced intensity conditioning (alloRIC) recipients were analyzed. In all cases, a non-related donor was used. Baseline disease was: 23 acute leukemia (37.7%), 12 non-Hodgkin lymphomas (19.7%), 10 myelodysplastic syndromes (16.4%), 7 Hodgkin lymphomas 7 (11.4%), 4 multiple myelomas (6,5%),3 chronic lymphocytic leukemia (4.9%), and 2 myelofibrosis (3.2%). All of them received Fludarabine-based conditioning treatment and the combination sirolimus (mTOR inhibitor)-tacrolimus (calcineurin inhibitor) as GVHD prophylaxis. Drug d*oses were adjusted* according to blood levels and renal function. Levels of Hb, MCV and iron parameters were systematically evaluated after alloRIC. Microcytosis was considered when MCV was below 80 fl.
Results: At 6 months 56 out of 61 (92%) were alive. Anemia was observed in 30 (49%) of them, with only 8 cases (13.1%) showing Hb level below 100 g/l. Microcytic anemia was diagnosed in 2 of them (3.3%). One patient showed an iron deficiency anemia due to gastrointestinal bleeding (Hb 94 g/l, MCV 69 fl, serum ferritin 21 µg/l). However, the second one, a 61-year old male with an acute leukemia, had a microcytic anemia with iron parameter changes similar to those observed in kidney transplantation and associated with increased hepcidin, (see table).Anemia progressively improved with sirolimus tappering.

Table 1.

	Pre-inh mTOR	Inh mTOR	Post inh m-TOR
Hb g/l	113	95	127
MCV fl	87	74	79
Platelets x 109/l	169	217	220
Leucocytes x 109/l	6.8	6,4	6,3
Fe/TIBC /% SAT µmol/l (NV: 8-26/ 44-73/15-45)	5/43/12	3/42/7	-
Serum ferritin µg/l (NV:20-350)	1467	1177	2349
Serum transferrin receptor mg/l (NV: 1.-3.5)	-	8.6	
Hepcidin (NV 1-39 mg/ml)	-	40.5	-
BM Macrophage iron % sideroblasts	-	Increased 6%	-

Inh: inhibitors. Hb: hemoglobin. MCV: mean corpuscular volume. Fe: iron. TIBC: total iron binding capacity. %SAT.% transferrin saturation. BM: bone marrow. NV: normal values.

Summary/Conclusions: In contrast to kidney transplantation, microcytic anemia related to immunosuppression with mTOR inhibitors was seldom observed in alloRIC recipients. However, this association should be taken in account in this setting, as a rare cause of anemia. In case of microcytic anemia, the evaluation of iron parameters and hepcidin provides the diagnosis of this rare type of anemia.

E1161

IRON METABOLISM IN PATIENTS WITH PAROXISMAL NOCTURNAL HEMOGLOBINURIA

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired clonal non-malignant hematological disorder that is associated with hemolytic anemia, bone marrow failure, thrombosis. At the onset the condition is often interpreted as iron deficiency anemia that leads to the prescription of ferrotherapy.
Aims: Study iron metabolism in patients with PNH.
Methods: The study group included 19 patients (11 men and 8 women aged from 20 to 64 years, median age 43 years) with a diagnosis of PNH, followed up in our Center between 2014 and 2017. The median hemoglobin level was 8,1 g/dl. The erythrocyte PNH clone size ranged from 17 to 99%, median - 47%. Granulocyte and monocyte PNH clone sizes were 85% and 89%, respectively. The following parameters were studied to characterize iron metabolism: ferritin, transferrin, iron concentration, total iron binding capacity (TIBC), transferrin saturation. Ten of 19 patients undergone magnetic resonance imaging (MRI) of the liver and kidneys to determine iron overload. Five of 10 patients received treatment with eculizumab.
Results: Iron metabolism parameters varied in wide limits. Iron deficiency was detected in 4 (21%) patients, 8 (42%) patients had laboratory signs of iron overload, remaining 7 (37%) patients had normal parameters of iron metabolism. Ferritin rates ranged from 6 to 3050 ng/ml (median – 544ng/ml), transferrin – from 75 to 452mg/l (median – 238mg/l), TIBC –from 21 to 95 umol/l (median – 55umol/l), transferrin saturation – from 9 to 92% (median – 43%), iron concentration – from 5 to 50 umol/l (median – 22umol/l). MRI (including T2*-weighted images) revealed signs of liver hemosiderosis varying from mild to severe degree in 7 out of 10 studied patients. Signal intensity of renal cortex was decreased in 9 out of 10 patients, presumably due to renal hemosiderosis. In 1 patient there were no pathological findings on MRI. Laboratory signs of iron deficiency were revealed in 2 out of 9 patients with renal hemosiderosis. Signs of renal hemosiderosis were absent only in 1 out of 10 examined patients. This patient has been treated with eculizumab for 15 months.
Summary/Conclusions: Evaluation of laboratory parameters of iron metabolism is not sufficient to identify tissue iron overload in transfusion-dependent patients with PNH. MRI (including T2*-weighted images) revealed signs of hemosiderosis of liver and kidneys in 7 (37%) and 9 (47%) patients respectively, indicating the feasibility of this method for diagnosis of post-transfusion iron overload in patients with PNH and assessment of the need for chelation therapy. Described case of the patient who has no signs of renal hemosiderosis and receives treatment with eculizumab fortifies the results of clinical studies showing the efficacy of eculizumab in the prevention of renal failure in patients with severe PNH.

E1162

ORAL IRON ELEVATES SERUM IRON AND CONSEQUENTLY CHANGES IRON DISTRIBUTION IN LIVER AND ERYTHROCYTES

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Background: For renal anemia patients, there are several therapeutic options including erythropoiesis-stimulating agents (ESAs), intravenous and oral iron supplementations. In terms of iron absorption, ESAs were known to activate iron absorption via down-regulation of hepcidin, a key mediator of iron metabolism, and consequent up-regulation of duodenal iron transporters divalent metal transporter 1 (DMT1) and ferroportin (FPN). On the other hand, in our previous study, intravenous iron was demonstrated to deactivate iron absorption system via hepcidin elevation. However, iron absorption under oral iron supplementation have not fully evaluated yet.
Aims: In this study, we investigated the activity of iron absorption under oral iron supplementation in mice as well as under intravenous iron supplementation. In addition, we also analyzed iron distribution under intravenous and oral iron supplementation.
Methods: To load iron orally, a diet including 200 ppm of iron was used as control and a diet including approximately 5000 ppm of ferric citrate was used as iron-rich diet. 6-week-old male C57BL/6NCrl mice were divided into 3 groups; control group, intravenous iron (IV iron) group, and oral iron (Oral iron) group (n=5). Mice in IV iron group were fed a control diet from days 0 and intravenously administered 0.4mg/mouse of iron-dextran on days 9. Mice in Oral iron group were fed an iron-rich diet from days 0 and intravenously administered 0.4mg/mouse of dextran as vehicle on days 9. Mice in control group were fed a control diet from days 0 and intravenously administered 0.4mg/mouse of dextran on days 9. All mice were euthanized by exsanguination under anesthesia with isoflurane on days 14. For analyses of iron absorption, serum hepcidin and iron were measured and expression of duodenal DMT1 and FPN were evaluated immunohistochemically. For analyses of iron distribution, berlin blue staining for liver and hematological indices were evaluated.
Results: Serum hepcidin levels in IV and Oral iron groups were significantly higher compared with control group. However, serum iron levels were elevated only in oral iron group. In immunohistochemical analyses, expression levels of duodenal DMT1 were not detected in all groups and expression levels of duodenal FPN in IV and Oral iron groups were significantly lower than control group. As for iron distribution in liver, iron was accumulated in reticuloendothelial cells in IV iron group, on the other hand, in Oral iron group iron was accumulated in parenchyma. In hematological analyses, although red blood cell and reticulocyte count were not significantly different among all groups, Ret-He and MCH in Oral iron group were higher than IV iron groups.
Summary/Conclusions: It was demonstrated in this study that serum iron levels were elevated in spite of high hepcidin levels and down-regulation of duodenal iron transporters under oral iron supplementation. Furthermore, iron was distributed in liver parenchyma and hemoglobin contents in each reticulocyte and erythrocyte were up-regulated only under oral iron supplementation. We speculated that high serum iron lead to excess iron uptake into tissues and erythrocyte fraction. These data might provide an opportunity to rethink the importance of proper use of iron supplementations.

E1163

DEFERASIROX FOR SEVERE ANAEMIAS IN YOUNG CHILDREN

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Background: Children with haemoglobinopathy and rare anaemias often require regular red cell transfusions at some stage of their lives. Iron overload is therefore inevitable and iron chelation is a key component of therapy for children in this group. However its use has not been validated especially in children under two years of age. Deferasirox (Exjade®; Novartis Pharma AG, Basel, Switzerland) is an iron chelator that is conclusively proven to be effective and safe in transfusional anaemia such as haemoglobinopathies.
Aims: We aim to look at the efficacy and safety of Deferasirox in children with severe anemias.
Methods: We present a case report of 6 children with severe anemias treated with Deferasirox in a tertiary pediatric hematology centre in London, UK.
Results: Here we report 6 cases where deferasirox has been used in young children with rare anaemias and sickle cell disease. Patients 1 and 2 presented within the first year of life with pancytopaenia requiring regular transfusion and were diagnosed with Pearson syndrome. Deferasirox was started at the age of 30 months and 4 months respectively. Patients 3 and 4 presented with neonatal anaemia requiring regular transfusion and were diagnosed with Pyruvate Kinase deficiency. Deferasirox was started at 12 and 19 months consecutively. Patient 5 presented with pure red cell aplasia at the age of 3 months and was diagnosed with Diamond Blackfan anaemia. He was initially treated with steroid but became resistant at around 40 months of age. He was then started on regular transfusion and was started at deferasirox at 4 years of age. Patient 6 was diagnosed at birth with sickle cell anaemia. He suffered from stroke at the age of 8 months and was started on chronic transfusion program. Deferasirox was started at around at the age of 1. He had a successful maternal haplo-identical haemopoietic stem cell transplant at the age of 3 years old. Transfusion and deferasirox were subsequently stopped.

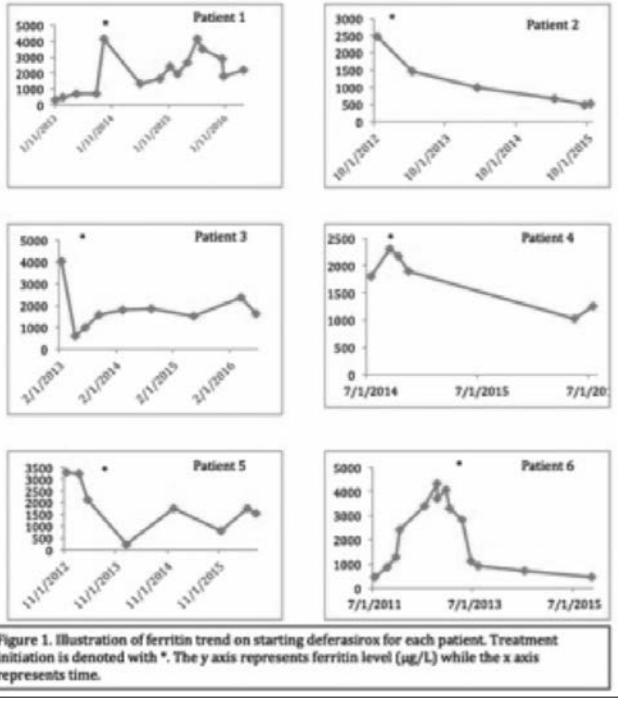

Figure 1. Illustration of ferritin trend on starting deferasirox for each patient. Treatment initiation is denoted with *. The y axis represents ferritin level (µg/L) while the x axis represents time.

Figure 1.

Summary/Conclusions: All of these children had stabilization or improvement of ferritin values after initiation of deferasirox as shown in figure 1. Deferasirox is licensed in Europe to be used in children with thalassaemia older than 6 years of age or older than 2 year of age when desferoxamine therapy is inappropriate or inadequate. Deferasirox is preferable in severe anemias due to better side effect profile on the bone marrow compared to deferiprone; the use of which can cause agranulocytosis or neutropaenia. Furthermore, its oral administration improved compliance compared to desferrioxamine that required prolonged subcutaneous administrations. Deferasirox has been associated with renal impairment. However, none of patients developed renal or liver impairment during the use of deferasirox. Furthermore, it is crucial to conduct eye and ear screening tests both before and after the commencement of deferasirox. None of our patients had neurological side effects. Three of these children had deferasirox started at younger than 2 years of age. Hence, we have shown that deferasirox is safe and efficacious in treating iron overload in very young children with rare anaemias and sickle cell disease where evidence is sparse.

E1164

MONITORING ORAL IRON THERAPY IN CHILDREN WITH IRON DEFICIENCY ANEMIA. AN OBSERVATIONAL, PROSPECTIVE, MULTICENTRIC STUDY

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Background: Iron deficiency anemia (IDA) is the most common hematological disease in infancy and childhood. Oral iron administration is a well-established, effective, and widely accepted treatment for anemia because of its efficacy, safety, and cost-effectiveness. Recently new preparations of oral iron compounds were launched, including droplet formulations, *i.e.* liposomal preparations and bis-glycinate iron; little is known on their effectiveness in real clinical practice.
Aims: To evaluate the efficiency of different oral iron preparations in children with IDA.
Methods: This observational study collected clinical and hematological data from 12 AIEOP (Associazione Italiana di Ematologia ed Oncologia Pediatrica) centers. Inclusion conditions for patient enrollment were age 3 months-12 years, diagnosis of IDA; exclusion criteria were all conditions interfering with iron absorption such as celiac disease, gastro-intestinal disorders and other chronic conditions. Local Physicians were free to prescribe any oral iron formulation, according to their standard practice. A calendar of laboratory test was suggested, including basal assessment of whole blood count, reticulocytes, iron status, with subsequent checkpoints at 3 days (WBC and retyculocytes only), 2 weeks, 8 weeks, 6 months. Clinical data regarding compliance to therapy, unwanted effects, final outcome were recorded.
Results: 112 (M 58) patients were enrolled. Ethnic distribution was: Caucasian 74, African 23, Asian 10, Other 5. The median age at diagnosis of IDA was 1.5 years, with a bimodal distribution with frequency peaks at age 0-3 and 12-14 years. Sixty-eight patients received bis-glycinate ferrous iron 0.45mg/kg, 19 elemental iron (ferrous gluconate/sulfate) 2mg/kg, 12 liposomal iron 0.7-1.4mg/kg, and 15 other preparations. Eating habits were reported as normal in 48 patients, inadequate weaning in 21, meat/fish restriction in 32, other in 11. Gastro-intestinal side effects were reported in 9/68 (13%) of the bis-glycinate iron group, in 3/19 (16%) of the elemental iron group, and in 0/12 of the liposomal iron group. Suspension of therapy due to side effects was needed only in 5 patients, 4 in the bis-glycinate and 1 in the elemental iron group, respectively. Final outcome was available for 77 patients; it was recorded as solved IDA, persistent IDA, or lost at follow up. Solved cases were 40/53 (75%) in the bis-glycinate iron group, 4/11 (36%) in the elemental iron group, and 8/13 (62%) in the liposomal iron group. Persistent cases were 8/53 (15%) in the bis-glycinate iron group, 6/11 (55%) in the elemental iron group, and 1/13 (8%) in the liposomal iron group. Lost at follow up were 5/53 (9%) in the bis-glycinate iron group, 1/11 (9%) in the elemental iron group and 4/13 (31%) in the liposomal iron group.
Summary/Conclusions: The collected data show that both bis-glycinate and liposomal iron formulations have a good efficacy/safety profile and offer a sustainable alternative to classic elemental iron preparations.

E1165

AN INVESTIGATION ABOUT WEIGHT GAIN WITH TREATMENT OF IRON DEFICIENCY ANEMIA: CHANGES OF GHRELIN AND HEPCIDIN LEVELS WITH TREATMENT

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Background: Iron deficiency anemia (IDA) is a global health problem and problems in compliance with oral iron therapy are frequently seen. It has been shown that medications are not used regularly or discontinued due to weight gain during the treatment process.
Aims: We investigated ghrelin, known as appetite hormone and its relationship with hepcidin, the homeostatic regulator of intestinal iron absorption, in order to explain some symptoms of IDA and weight gain during iron treatment.
Methods: A hundred and twenty adult IDA patients, referred to our clinic between October 2015 and October 2016 were included in the study. The study was completed with 87 patients, who gave the informed content and a control group consisted of 50 healthy people. Information about age, gender, weight, height, body mass index (BMI), waist-hip circumference and blood samples were taken from the patient and control groups. The treatment of IDA was done according to the dose and method recommended by the responsible physician, the researchers did not have any effect on the treatment. Measurements and blood tests were repeated in the patient group after normalization of the anemia parameters, not before the third month of treatment. Hepcidin and ghrelin levels

were examined once in the control group and twice in the patient group, before and after treatment.
Results: When the patient and control groups were compared, there was no significant difference in terms of age, sex, height, weight, BMI, waist and hip circumference. The pretreatment plasma hepcidin and ghrelin levels of the patient group were significantly lower than those of the control group (80±21 ng/ml *vs* 179 ng/ml p <0.001 for hepcidine, 152±119 pq / ml *vs* 213±167 for ghrelin, p=0.026). There was a significant increase in terms of weight (mean 1.15 kg, p <0.001), BMI (25.86 kg/m^2 *vs* 26.33 kg/m^2, p <0.001), waist and hip circumference measurements (mean 0.81cm in both, p <0.001) after treatment in the patient group. After treatment, the levels of hepcidine was significantly increased compared to the pre-treatment levels (80±21 ng/dl *vs* 92±13 ng/dl, p<0.001). Although an increase in the plasma ghrelin levels was encountered after treatment, it was not statistically significant (152±119 pq/ml *vs* 164±150 pq/ml, p=0.589). When correlations of individual increases in ghrelin levels were examined, a weak positive correlation was found between increase in ghrelin levels and weight gain.
Summary/Conclusions: In our study, ghrelin was significantly lower than the control group in the IDA group, suggesting that it may be the cause of loss of appetite. Ghrelin is also detected in neurons of hypothalamic arcuat nucleus, which regulates appetite. The deficiency of iron may cause deficiencies in enzymatic activities of iron depended enzymes and it may disturb the function of these neurons. But the increase with treatment did not reach statistical significance. This may be due to physiological suppression of levels by weight gain. When patient-based weight gains were examined, there was a positive but weak correlation with the increase in ghrelin levels of those people. Hepcidin was significantly lower in the iron deficiency group than in the control group and showed a significant increase with treatment, but it was not associated with weight gain and change of ghrelin levels. More extensive and controlled studies should be designed in this regard.

Myelodysplastic syndromes - Biology

E1166

SOMATIC MUTATION DYNAMICS IN HIGH-RISK MDS PATIENTS TREATED WITH AZACITIDINE IDENTIFIED VIA SERIAL SAMPLING

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Background: Azacatidine (AZA) is a standard therapy for MDS patients with higher risk of AML transformation and not eligible to undergo transplantation. While AZA is well tolerated the responses occuring in upto two thirds of patients are not durable. Somatic mutations were previously associated with pathogenesis of MDS, some of them also with prognosis. Several studies suggested that MDS patients as they progress may evolve new mutations and loose some of the clonal architecture detected at preceding stages (Pellagatti, Roy *et al.* 2016). In addition, there exist gene mutations that are detected in patients subsequently responding to hypomethylating agents (Bejar, Lord *et al.* 2014), which implies that there exist variants-bearing clones that persist upon AZA as well as those that do not.
Aims: To identify variants either persisting or not upon the AZA therapy we tracked BM samples during AZA treatment. Next, we were interested in deciphering their relationship of the dynamics in somatic variants to clinical course of the analyzed MDS patients.
Methods: Massive parallel sequencing with high accuracy utilized duplicate libraries from myeloid cells and included the non- tumorous T-cell controls to identify somatic mutations in the serial samples before and during AZA therapy. The tool for detecting the dynamics of somatic mutations was the TruSight Myeloid Panel that contains 54 gene regions with previously documented mutation recurrence in 439 patients (Bejar, Stevenson *et al.* 2011). Indeed, 92% of our MDS cohort bore at least one somatic mutation with mostly 4 mutations per patient (range 1-9), which indicated that the MDS patients were already at relatively progressed state (Papaemmanuil, Gerstung *et al.* 2013).
Results: Analysis of 38 patients treated with AZA (reaching median OS 24 months (Mo) with 60% hematology improvement) revealed 125 somatic variants with VAF over 5%. Adverse effects of variants in cooperating regulators of DNA damage and cell cycle were confirmed: *TP53* (OS on AZA 14.8 Mo), *CDKN2A* (12.3 Mo), *EZH2* (11 Mo). Besides the stable variant's allele frequency (50%<VAF <200%) there existed four additional VAF profiles. Stable variants' dynamics precluded putative AZA-resistant clones associated with shorter survival (19 Mo). In contrast, the patients bearing variants with decreasing VAF, which supposedly were inhibited by AZA, lived longer (31 Mo). Interestingly, small group of highly dynamic variants upon AZA therapy formed a subgroup with longer-lasting complete remissions.
Summary/Conclusions: Our work support the importance of catalogization of somatic variants to delineate pathogenesis of MDS with a focus on molecular AZA responsiveness. Several types of variant dynamics during the AZA therapy were noted by using the massive parallel sequencing approach of the duplicate libraries per MDS BM samples also utilizing non-tumorous controls and serial sampling. Stable dynamics was found in variants previously recorded by COSMIC and targetting the adverse outcome genes such as *TP53*, *BCORL1*, *ASXL1*, and *EZH2* as well as their combinations with *TET2* that may potentially mediate clonal selection of additional variants mediating progression during AZA therapy.

E1167

WHOLE GENOME MBD-SEQ REVEALS DIFFERENT CPG METHYLATION PATTERNS IN AZACYTIDINE-TREATED JUVENILE MYELOMONOCYTIC LEUKEMIA (JMML) PATIENTS

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Background: Juvenile Myelomonocytic Leukemia (JMML) is a rare and aggressive leukemia of early childhood. Allogeneic hematopoietic stem cell transplant (HSCT) is the only available curative treatment, but, since disease recurrence is responsible for treatment failure in at least one third of transplanted patients, developing alternative therapeutic approaches is desirable. Aberrant DNA methylation is a key molecular feature of JMML, suggesting an important role of epigenetic events in the pathophysiology of the disease. Azacytidine (AZA), a molecule that inhibits DNA methylation in human cells, is under investigation in JMML.
Aims: Here we report, for the first time, a global evaluation of DNA methylation status of CD34$^+$ cells deriving from JMML patients before and after AZA treatment and compared the results with those of healthy controls. Identifying differentially methylated CpG islands linked to various genes will help us describe

an epigenetic aberrant paradigm possibly involving transcriptional and translational regulation in JMML.
Methods: CD34+ cells isolated from 3 JMML patients samples collected at diagnosis (t0) and after the third cycle of AZA (t1) were evaluated together with those of 3 healthy donors (HD). JMML patients have been treated with AZA on a compassionate use basis after obtaining signed informed consent. DNA samples were processed and Ion fragment libraries were prepared. MBD-seq, bioinformatics and statistical analysis were performed by Genomnia srl (Bresso, Italy).
Results: First, we compared t0 JMML cells with HD cells, finding 987 different transcriptional units corresponding to 714 coding and 273 non-coding sequences. We also compared DNA methylation between t1 and HD cells. In this comparison, 644 unique transcriptional units, including 468 coding and 175 non-coding sequences, were found. Hypermethylation in JMML samples compared to HD was detected, but, unexpectedly, t0 *vs* t1 methylation analysis did not show any significant result, suggesting a likely unspecific patient-related pharmacological effect. Notably, 453 coding and 165 non-coding differentially methylated regions are shared between t0 *vs* HD and t1 *vs* HD sets. More in detail, 261 and 15 coding regions and 107 and 10 non-coding regions were uniquely found in t0 *vs* HD and t1 *vs* HD sets, respectively. However, 439 coding and 161 non-coding genomic regions preserve their hypermethylated status, probably due to a mechanism of resistance to AZA treatment. Among non-coding elements, we found different RNA species, such as microRNAs, splicing RNAs, lincRNAs/antisense transcripts (AS) and other unknown RNAs. Retrotransposons, belonging to LINEs and SINEs families, were also screened. We identified 13 sequences with a significant differential methylation profile in both t0 and t1 *vs* HD. Again, a comparison between t0 and t1 groups did not show any significant difference. Eleven hypermethylated common LINEs were evident between t0 *vs* HD and t1 *vs* HD sets. Two retrotransposons with opposite methylation patterns were found in t0 *vs* HD and t1 *vs* HD sets; while in the first comparison they included LINEs, in the second one they are 1 hypermethylated LINE and 1 hypomethylated SINE.

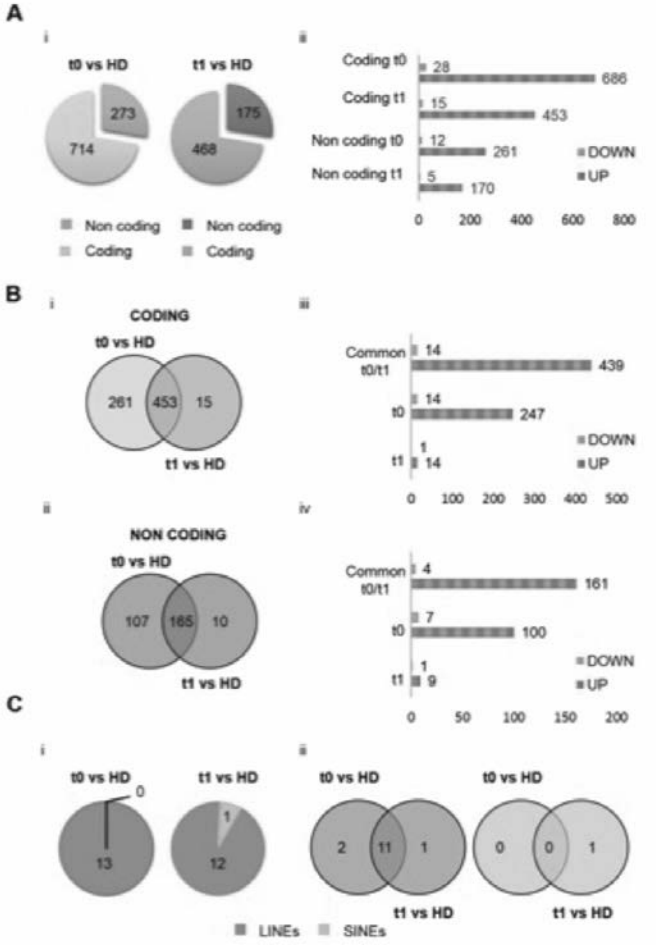

Figure 1.

Summary/Conclusions: In conclusion, the whole genome MBD-seq performed for the first time on JMML CD34+ bone marrow derived cells, showed a broad genomic hypermethylation both in pre- and post-AZA samples compared to HD, suggesting a patient-specific AZA-effect. Transcription and translation processes of coding and non-coding genes could be deregulated in multiple ways, due to heterogeneity of sequences involved in CpG islands hypermethylation. Moreover, due to their known ability to insert random mutations in the genome, retrotransposons should be candidate for further studies in JMML pathogenesis.

E1168

RESPONSE MONITORING IN MDS WITH DEL(5Q) USING DIFFERENT FLOW CYTOMETRIC (FCM)-SCORES IN COMPARISON TO CYTOGENETICS AN ELNET IMDS-FLOW EXPERIENCE

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Background: Flow cytometry (FCM) is one part of integrated MDS diagnostics. Different well established FCM-scores are applied, as FCSS (Wells *et al.* 2003), Ogata-score (Ogata *et al.* 2012), new iFS (Cremers *et al.* 2017), and del(5q)-FCM-score (Oelschlaegel *et al.* 2015).
Aims: The aim of this prospective study was to test, which of the mentioned FCM-scores fits best for response monitoring in del(5q) MDS in comparison to cytogenetics.
Methods: Overall, 245 FCM investigations were performed in 61 patients with MDS and del(5q) (IPSS-R very low/low: n=26, int: n=13, high/very high n=22) including 42 patients with isolated del(5q) or one additional cytogenetic abnormality. The majority of analyses were performed in patients receiving lenalidomide or azacitidine (n=29 and n=22 patients), or in patients receiving chemotherapy and/or allogeneic transplantation or growth factors (n=10). Standardized FCM (lyse-stain-wash) and cytogenetics/FISH procedures were performed according to ELN guidelines at the TU of Dresden, VUMC of Amsterdam, UH of Guadalajara and UH of Bristol. Cytogenetics/FISH analysis was considered the gold standard. All of the applied FCM-scores were propagated by the ELNet iMDS working group. Additionally, hematological improvement of the erythroid lineage (HI-E) was evaluated (Cheson *et al.* 2006).
Results: The del(5q)-FCM-score reflected best the disappearance / presence of the cytogenetic abnormality del(5q) with a sensitivity of 98% and a specificity of 82%. This was confirmed if only MDS with del(5q) as a single abnormality or only MDS treated with Lenalidomide were evaluated separately (sensitivity: 98% and 100%; specificity: 85% and 75%). The use of the Ogata-score considering almost only abnormalities of the myeloid progenitors, ended up with a slightly lower sensitivity (86%) and specificity (81%). The new iFS analyzing progenitor cells, granulo-, mono-, and erythropoiesis showed a comparably high specificity (83%) but a slightly impaired sensitivity (72%). FCSS, analyzing dyspoiesis of multiple cell lineages, showed a response in less than the half of all investigations being in cytogenetic CR (sensitivity: 41%), but revealed a high specificity (91%). The analysis of HI-E was high sensitive (81%) but not as specific (62%). Next, we investigated the potential prognostic impact of response monitoring using various FCM-scores compared to cytogenetics. Considering all del(5q) MDS patients as well as only those patients with del(5q) as a single abnormality, cytogenetics and all tested FCM-scores showed a significantly longer OS for MDS responding to therapy. The highest prognostic impact displayed the new iFS (p=0.0019) and Ogata-score (p=0.0092), respectively. Evaluating only MDS treated with lenalidomide, response monitoring using FCSS separated best the OS curves (p=0.0080). Finally, we combined the evaluation of HI-E with cytogenetics or the FCM-scores. This resulted in an even better OS for MDS fulfilling two response criteria *vs* none of the criteria with the highest prognostic impact for the combination of HI-E plus the new iFS (p=0.0010).
Summary/Conclusions: Flow cytometry might serve as a rapid tool for response monitoring during treatment with disease-modifying drugs. All established FCM-scores allowed for an at least similar correctness of response prediction. The prognostic impact of the various FCM-scores seems to be even higher than that of cytogenetic response evaluation in this MDS subgroup. One reason might be, that most of the FCM-scores reflect not only the genetic background of the MDS but dyspoietic alterations in various cell lineages of the hematopoietic system.

E1169

EVALUATION OF MUTATIONS AT RELAPSE IN MYELODYSPLASTIC SYNDROME PATIENTS RECEIVING ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: Allogeneic transplant (AlloSCT) is the only curative therapy for myelodysplastic syndromes (MDS). Unfortunately, relapse is the main cause of treatment failure. Evaluation of genetic mutations both at diagnosis and

before AlloSCT is a potent prognostic tool. However, mutational profile at relapse after AlloSCT has not been widely explored.
Aims: In this study, we evaluate mutational profile at post-AlloSCT relapse in MDS patients to determine if pre-AlloSCT mutations are still present at relapse, so we could eventually monitor them as minimal residual disease (MRD) after AlloSCT.
Methods: From a retrospective cohort of 115 patients, we selected those who relapsed post-AlloSCT (19/115, 16.5%) with available material at relapse (18 patients). We performed an in-house target-capture panel, sequencing across selected exons of 117 cancer-related genes previously related to MDS in pre-AlloSCT samples to identify genetic mutations and we checked the presence of those mutations in samples at relapse. Six patients were discarded because lack of pre-AlloSCT mutations, so we selected 12 patients for the sequential study. DNA was amplified with FastStart High Fidelity PCR System using exon-specific primers for each mutation. The indexed paired-end library was prepared with Nextera XT DNA Sample Preparation Kit (Illumina) The median coverage per base achieved was 4579 reads range 2401-8573). In a second step, we explore the possibility of evaluating mutations in both CD34 positive and the rest of bone marrow cells, to check if we could increase the sensitivity of the detection.
Results: Median age of relapsed patients was 60 (45-70). Diagnosis were RAEB 1 (n=4), RAEB 2 (4), dysplasia associated AML (2) and RCMD (2). They relapse post-AlloSCT after a median of 2.5 months (1-7), and 4 of them are alive at last follow up after a median of 22 months (9-33). Patients had a median of 2.5 mutations (range 1-4). TET2 mutations were detected in 4 (33%) of patients; U2AF1, EZH2, SRSF2, KRAS, JAK2 and RUNX1 in 2 (17%), and NRAS, TP53, ETV6, PHF6, SMC1A, ZRSR2, BCOR, DNMT3 and SF3B1 mutations in 1 (8%) (Table 1). In 10 out of 12 patients evaluated, we found same genetic mutations at relapse compared with pre-AlloSCT sample (Table 1). In addition, mutational pattern was similar for all patients except for one in which dominate mutation at relapse was SRSF2 (present in 14% of cells pre-Allo and in 3% at relapse) instead of ETV6 (51% pre-AlloSCT and 0.6% at relapse). In 2 patients, pre-AlloSCT mutations were not detected at relapse (Patient 8: BCOR and RUNX1. Patient 11: SRSF2, TET2 and RUNX1). In a second step, we searched for mutations in CD34 positive cells to check its sensitivity to detect genetic alterations. We selected CD34 positive cells in one patient with KRAS and IDH2 mutations pre-AlloSCT. KRAS and IDH2 were present in 40% and 45% of CD34 positive cells and in 37% and 48% of the bone marrow (CD34 depleted) compartment respectively in pre-AlloSCT samples. In relapse samples, mutations were present in similar percentage in CD34 positive cells compared to CD34 depleted bone marrow (KRAS 0.63% and 2.23%; IDH2 1.6% and 1.45% respectively).

Table 1.

Table 1. Mutations before and after the AlloSCT in relapsed patients

PATIENT		MUTATION	PRE-ALLOSCT MUT %		RELAPSE MUT %	
1	U2AF1	NM_001025203:p.R156H	45,46		8,54	
2	EZH2	NM_004456:p.R690H	89,12		51,25	
	NRAS	NM_002524:p.G60E	15,27		---	
	TET2	NM_001127208:p.R1216Ter	43,3		25	
	TET2	NM_001127208:p.F1901IfsTer4	40,24		26,9	
3	TP53	NM_001126115:p.R43H	34,98		72,23	
4	ETV6	NM_001987:p.R433H	51,21		0.6	
	SRSF2	NM_001195427:p.95_103del	14,69		3.56	
5	TET2	NM_001127208:p.S1286F	27,44		46,8	
	PHF6	NM_001015877:p.R129K	9,43		13,7	
	KRAS	NM_004985:p.G13R	6,01		1,61	
	U2F1	NM_001025203:p.S34F	31,2		47,4	
6	EZH2	NM_001203249:p.Q514K	71, 36		11,91	
	SMC1A	NM_006306:p.R586Q	61,11		7	
	TET2	NM_001127208:p.E1318D	32,24		6,26	
	TET2	NM_001127208:p.Q1466fs	39,69		6,28	
	ZRSR2	NM_005089:p.R295X	65,93		6,38	
7	JAK2	NM_004972:p.V617F	87,19		3,49	
8	BCOR	NM_001123384:p.R1219W	60,69		Not present	
	RUNX1	NM_001001890:p.R139K	13,3		Not present	
	RUNX1	NM_001001890:p.S114P	27,75		Not present	
9	DNMT3	NM_153759:p.F662fs	44,14		8,63	
	DNMT3	NM_001290114:p.R10Q	45,95		9,08	
	IDH2	NM_002439:p.K700E	41,33		8,91	
	SF3B1	NM_153759:p.542_543del	36,84		7,48	
10	JAK2	NM_004972:p.V617	11,69		4	
11	RUNX1	NM_001001890:p.D133E	22,49		Not present	
	SRSF2	NM_001195427:p.P95_R103del	44,04		Not present	
	TET2	NM_001127208:p.C1193Y	22,47		Not present	
12	KRAS	NM_033360:p.Gly12Val	CD34+ 40,4	CD34- 37	CD34+ 0,63	CD34- 2,23
	IDH2	NM_002168:p.Arg140Gln	45	48	1,6	1,45

Summary/Conclusions: Post-AlloSCT relapsing MDS show same genetic mutations found in pre-AlloSCT evaluation, so they would potentially be used to confirm clonality and probably MRD assessment after AlloSCT in the near future. CD34 selection does not provide additional sensitivity to whole bone marrow cellularity sample.

E1170

RIGOSERTIB COMBINED WITH AZACITIDINE EPIGENETICALLY MODULATES CHROMATIN AND HEMATOPOIETIC STEM CELL POPULATIONS IN THE MYELODYSPLASTIC SYNDROME (MDS)

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Background: Azacitidine (AZA) is the standard of care for patients (pts) with higher-risk MDS, however, only 50% of pts respond and the majority will relapse within 2 years. All pts ultimately fail treatment due to primary or secondary resistance. RIGosertib (RIG) is a "ras mimetic" agent that binds to the Ras Binding Domain of RAF kinases and inhibits the RAS-RAF-MEK and the PI3K pathways. Initial results of an ongoing Phase I/II study with RIG combined with AZA, in pts with MDS demonstrated a response rate of: 76% overall; 62% in pts following hypomethylating agent (HMA) failure and 85% in HMA naïve pts (Navada et al ASH 2016).
Aims: To Investigate the *in vitro* effects of RIG combined with AZA or vorinostat (VOR) on epigenetic and stem cell pathways on two cell lines: AML (BW90), MDS (MDS-L) and on pt bone marrow samples
Methods: We investigated the *in vitro* effects of RIG combined with AZA or vorinostat (VOR) on two cell lines: AML (BW90), MDS (MDS-L) and on pt bone marrow samples treated on the phase I/II study, obtained prior to and after one cycle of AZA and RIG.
Results: Treatment with RIG alone altered global histone post-translational modifications (PTMs) including methylation (H3K4me3, H3K4me2, H3K27me3, and H3K27me2) and acetylation (H3K9ac, & H3K18ac) levels associated with transcriptional activation or repression in both the cell lines and pt samples. Q-PCR studies demonstrated that individual treatment of BW90 and MDS-L with RIG or combined with AZA or VOR in sequential treatment (AZA/RIG, RIG/AZA, VOR/RIG or RIG/VOR) altered DNA methyl transferases (DNMT1, 3a and 3b), the class I, II and IV histone deacetylases (HDACs), and chromatin remodeler (KDM2a, SET1, JMJD3 and LRWD1) transcript levels in a cell line specific context. Sequential treatment of RIG with AZA or VOR demonstrated differential effects on the association of RNA polymerase II (Pol II) with active histone marks (H3K4me3 and H3K4me2) in both cell lines. An overall decrease in association of Pol II/H3K4me2 was observed with the combinations (AZA/RIG, VOR/RIG or vice versa) in MDS-L and BW-90, 10-33% (ANOVA, p=0.0006), 9-20% (ANOVA, p=0.0004), respectively. Significant differences were observed in association of H3K4me3/Pol II in BW-90 cells (7-30%; ANOVA, p<0.0001). Similarly, in BM from a pt with MDS after 1 cycle of RIG and AZA treatment demonstrated a decrease in association of Pol II with H3K4me2 (67%) and H3K4me3 (28%). Treatment of MDS-L cells with RIG alone or RIG/AZA failed to induce expansion of $CD34^+$ cells and yielded maximum aldehyde dehydrogenase (ALDH) activity, a marker of primitive hematopoietic stem and progenitor cells (HSCs) (ANOVA, p=0.006), but the RIG/VOR induced 1.9-fold expansion of $CD34^+$ cells (ANOVA p=0.002). A marked decrease in ALDH activity was observed in AZA or VOR or RIG/VOR, that was inversely proportion to the expansion of $CD34^+$ cells. In an MDS pt treated with RIG/AZA an expansion of primitive HPSCs expressing low levels of CD34 appeared with disappearance of a highly expressing CD34 subpopulation that co-existed prior to treatment. Expansion of $CD34^+$ cells led to ≥2 fold increase in pluripotent genes (SOX2, OCT4, NANOG and ZIC3) expression levels in the BM from MDS pts after RIG/AZA treatment and 1.7-34 fold increase in the presence of RIG or RIG/AZA or RIG/VOR in MDS-L. These findings indicate that expression of pluripotency genes is a consequence of epigenetic reprogramming that favors expansion of more primitive HSPCs in a pt.
Summary/Conclusions: RIG potentially functions as a chromatin modifying agent, in combination with AZA and may overcome HMA resistance through chromatin remodeling. RIG alone and in combinations also leads to epigenetic reprogramming of HSPC that may manifest in hematological improvements in the clinical setting.

E1171

UNEXPLAINED CYTOPENIAS IN HOSPITAL: INDICATIONS AND BENEFITS OF NEXT-GENERATION SEQUENCING

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Background: Unexplained cytopenias (UC) are common problems during hospitalisation, particulary in elderly patients. If there is no evident cause, myelodysplastic syndrom (MDS) is frequently suggested and a bone marrow aspiration is performed. Next-generation sequencing (NGS) reveals MDS-associated somatic mutations but their significance are discussed. In our centre, NGS was systematically realized in the context of unexplained cytopenias.
Aims: The objective of this study was to explore results of NGS in practical routine in the context of UC and to precise if some groups of patients could more specifically benefit of NGS.
Methods: All patients in our centre with analysis of NGS performed in blood or in bone marrow in a context of UF were included. Exclusion criteria were: patients under 18 years, monocytosis >1000/mm^3, excess of blasts, history of hematological malignancy disorder. Patients were included in group "positive NGS" if at least one significant mutation (no SNP) was found on 25 genes panel (ASXL1, CBL, DNMT3A, ETV6, EZH2, IDH1, IDH2, JAK2, KIT, KRAS, MPL, NPM1, NRAS, PHF6, PTN11, RIT1, RUNX1, STBP1, SF3B1, SRSF2, TET2, TP53, U2AF, WT1, ZRSR2). Clinical and biological criteria were reported from local database. All patients gave consent.

Results: 156 patients were included between January 2014 and December 2015 with a mean age of 68 years [65.8-70.3] and 47.4% of men. 127 patients (81.4%) had a bone marrow analysis. 53 patients (34.0%) were reported in the group "positive NGS" and 103 patients (66.0%) in the group "negative NGS". In univariate analysis, significant variable associated with "positive NGS" were age (p <10-7), no history of auto-immune disease (p=0.002), hemoglobin <12g/dL (p=0.017), platelets >150000/mm^3 (p=0.015), >10% dysplastic cells in erythroid (p=0.012) and granulocytic lineage (p=0.034). Trend test on dysplastic lineage number was significant (p=0.006). Normal karyotype (78.1%) was comparable in the two groups (p=0.352). Cirrhosis and/or portal hypertension were comparable in the two groups (14.1%, p=0.092) as well as mean serum creatinine (p=0.24). In multivariate analysis, age >70 years (p=0.0011) and platelets >150000/mm^3 (p=0.0213) remained significantly associated to positive NGS (Table 1). In "positive NGS" group, 1 (58.5%), 2 (32.1%), 3 (7.5%) or 4 (1.9%) mutation(s) were found per patient. Most frequent mutations were TET2 (25.9%), DNMT3A (17.3%), SF3B1 (12.3%), ASXL1 (12.3%), SRSF2 (8,6%), U2AF1 (4,9%), TP53 (3,7%) and ZRSR2 (3,7%). Other mutations were reported in less than 3 patients. As expected in this elderly population, if a unique mutation was found, TET2 and DNMT3A were predominant (35.5% and 25.8% respectively) but interestingly mutation R882 of DNMT3A was found in only one patient. Sideroblasts were found>15% in 46.2% of patients with a mutation of SF3B1, SRSF2, U2AF1 or ZRSR2.

Table 1.

	Total	Negative NGS	Positive NGS	OR[CI95%]	p
Effective	156	103	53		
Age(years),mean[CI95%]	68.0[65.8-70.3]	63.9[61.0-66.6]	76.2[73.3-79.1]		**<10^{-7}**
Age>70 years,n(%)	73(46.79%)	33(32.0%)	40(75.5%)	6.5[3.1-13.8]	**<10^{-4}**
Sex(male),n(%)	74(47.4%)	49(47.6%)	25(47.2%)	1.0[0.5-1.9]	0.962
Immune disease,n(%)	16(10.3%)	16(15.5%)	0(0%)		**0.002**
Cirrhosis,n(%)	22(14.1%)	18(17.5%)	4(7.5%)	0.4[0.1-1.1]	**0.092**
Creatinine(mg/L),mean[CI95%]	12.9[11.2-14.5]	13.4[11.0-15.8]	11.8[10.7-13.0]		0.24
Cytopenias					
Hemoglobin<12g/dL,n(%)	121(77.6%)	74(71.8%)	47(88.7%)	3.1[1.2-8.0]	**0.017**
Platelets<150000/mm^3,n(%)	77(49.4%)	58(56.3%)	19(35.8%)	0.4[0.2-0.9]	**0.015**
Neutrophils<1800/mm^3,n(%)	49(31.4%)	35(34.0%)	14(26.4%)	0.7[0.3-1.5]	0.335
MCV(fL),mean [CI95%]	94.8[93.5-96.1]	94.8[93.1-96.4]	94.9[92.6-97.2]		0.926
Bone marrow analysis	127(81.4%)	87(84.5%)	40(75.5%)	0.6[0.2-1.3]	0.171
Dyserythropoiesis,n(%)	74(59.2%)	42(51.2%)	32(74.4%)	2.8[1.2-6.2]	**0.012**
Megakaryocyte dysplasia,n(%)	25(20.0%)	13(15.9%)	12(27.9%)	2.1[0.8-5.0]	0.110
Dysgranulopoiesis,n(%)	40(32.0%)	21(25.6%)	19(44.2%)	2.3[1.1-5.0]	**0.034**
Absence of dysplasia,n(%)	43(34.4%)	36(43.9%)	7(16.3%)	4.0[1.6-10.1]	**0.002**
Normal karyotype,n(%)	100(78.1%)	70(80.5%)	30(73.1%)	1.5[0.6-3.6]	0.352

Table 1. Clinical and biological characteristics, univariate analysis. Variables with p<0.20 were included in multivariate analysis. Age > 70 years (p=0.0011) and platelets > 150000/mm3 (p=0.0213) remains significantly associated to positive NGS in multivariate analysis.

Summary/Conclusions: In the context of unexplained cytopenias, a third of patients had at least one MDS-associated somatic mutation. Age above 70 years and no thrombopenia seems to be good arguments to realize NGS in this context. Probably thrombopenia is frequently associated to other causes than MDS. If NGS is positive, aging genes are the most frequently mutated genes and they can reflect age-related clonal cytopenias. Even if their clinical significance is uncertain, monitoring is recommended because of an increased risk of hematologic cancer.

E1172

Abstract withdrawn.

E1173

RESISTANCE TO AZACITIDINE IS DETERMINED AT CELLULAR LEVEL BY LOWER EXPRESSION OF NUCLEOSIDE ACTIVATING ENZYMES UCK1 AND UCK2

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Background: Azacitidine is at present the standard treatment for MDS. We demonstrated that MDS patients responsive to azacitidine have significantly higher intracellular expression of the azacitidine-activating enzyme uridine-cytidine kinase-1 (UCK1) in bone marrow mononuclear cells (Valencia *et al.* Leukemia 2014). Correlation of the expression of nucleoside transporter, activating and disactivating enzymes with clinical and *in vitro* response to hypomethylating drugs azacitidine and decitabine has been suggested by several authors. Yet, the crucial role of these enzymes has to be ascertained, as well as their possible different importance in determining resistance to azacitidine.

Aims: To confirm that the cellular expression of nucleoside metabolizing enzymes plays a major role in cellular resistance and significantly impacts on clinical response to azacitidine.
Methods: Two cell lines, SKM1 sensitive (SKM1-S) and SKM1 resistant (SKM1-R) to azacitidine, were analyzed for expression of *UCK1, UCK2, hENT1, hCNT3, RRM1* and *RRM2* by quantitative PCR. Corresponding proteins were quantitated by western blotting in both cell lines. The expression of *UCK1* and *UCK2* was blunted by siRNAs in SKM1 sensitive cells to determine their role in *in vitro* sensitivity to azacitidine. For UCK1 and UCK2 silencing in SKM1-S, specific siRNAs were used (OriGene Technologies, MD, USA); cells were cultured at a density of 600x10^{5}cells/ml in 5 ml of RPMI 1940 medium. After 72 h of transfection, cells were treated for further 48h with azacitidine at the concentrations of 0,1 and 1 μM. After assessment of effective gene silencing, apoptosis and cell cycle arrest were evaluated, respectively by Annexin V test and Propidium Iodide. In parallel, the percentage of 5-methylcytosine was quantitated by ELISA assay (Global DNA Methylation LINE-1 kit ActiveMotif, CA, USA). In addition, the expression of nucleoside metabolizing enzymes was evaluated prospectively in 18 IPSS high risk MDS patients treated with azacitidine 75mg/m^2/7 days every 28 days. Furthermore, UCK1 and UCK2 expression was evaluated in 37 patients (classified as 26 responder and 29 non-responder) treated with azacitidine, by RNAseq analysis using DEXseq2.
Results: SKM1-R cells did not express *UCK1, UCK2, hENT1, hCNT3*, *RRM1* and *RRM2*. Corresponding proteins were also not expressed. A reduction of apoptosis was observed in *UCK1*-silenced SKM-1 S after azacitidine 0.1 μM treatment: 35,7%±0,77% Annexine V-positive cells *versus* 25%±0,35% (*P*=0.031) in non-silenced control SKM1-S cultures. We observed a reduction of apoptosis during *UCK2*-silencing after azacitidine 0.1 μM treatment too: 31%±0,85% Annexin V-positive cells *versus* 21%±0,35% (*P*=0.054). Hypomethylation induced by *in vitro* azacitidine treatment was also hampered by reduction of expression of *UCK1 and UCK2*. Quite surprisingly gene expression of *UCK1, UCK2, hENT1, hCNT3*, *RRM1* and *RRM2* in primary cells did not predict different clinical response to azacitidine. RNAseq analysis for UCK1 and UCK2 did not find any differences between responder and non-responder patients.
Summary/Conclusions: We demonstrated that *UCK1, UCK2, hENT1, hCNT3*, *RRM1* and *RRM2* and the corresponding proteins are absent in azacitidine-resistant cell line SKM1-R suggesting to be the determinant of the induced resistance to azacitidine. *UCK1 and UCK2* silencing induced by synthetic siRNAs significantly decreased azacitidine effects. Prospective evaluation of the predictive role of cellular expression of genes involved in azacitidine metabolism is ongoing in a larger cohort of MDS patients.

E1174

FAMILIAL TIN2 N-TERMINAL LOSS OF FUNCTION MUTATION IN TELOMERE SYNDROME

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Background: The shelterin complex protects telomeres from being processed by the DNA damage repair machinery and regulates telomerase access and activity (Frank 2015). *TINF2* (14q12) is encoding for TIN2, the central component of shelterin which interacts with other members of the complex (TRF1,TRF2 and TPP1), thus contributing to telomere length regulation and structural integrity (Frank 2015). About thirty *TINF2* mutations are known in Dyskeratosis Congenita (DC) (Savage 2008) and other telomere related phenotypes, *i.e.* aplastic anemia (AA), idiopathic pulmonary fibrosis, liver cirrhosis, myelodysplastic syndromes (MDS) and acute myeloid leukemia (Armanios 2012). All mutations were missense and heterozygous, clustering in exon 6 encoding for a highly conserved segment at the C-terminus (aa 280–291) (Frank 2015).
Aims: Precise diagnosis in AA/MDS with clinical features of telomere syndrome.
Methods: AA was diagnosed in a 69-year-old man, with a multisystem disorder, *i.e.* psoriasis, nail dystrophy, severe osteoporosis, chronic hepatopathy, mild chronic kidney failure and hypertension, suggesting a telomere syndrome. Karyotype was normal. Patient was unresponsive to immune-suppressive therapy. DNA from peripheral blood and hair bulbs was analyzed for *TERT*, *TERC* and *TINF2* using DHPLC and Sanger sequencing. Q-FISH investigated telomere length. SNPs were performed following manifacturer's instructions (Affymetrix). Paired-end libraries for Whole Exome Sequencing (WES) were generated with NimbleGen Exome Capture v3 (Roche), according to manufacturer. DNA from CD3+ population was used for germinal matching. Data were aligned to the human reference genome (GRCh38/hg38) and analyzed with the in-house CEQer2 software (Piazza 2013). Mutational analysis and telotype were performed in both proband and familial members. *TERF2* and *TINF2* coding sequences were cloned in pGem-Teasy vector and site direct mutagenesis reproduced *in vitro* the mutation. Using expression vectors, respectively pEGFP-C1 and pDsRed-Express-C1, TRF2 and TIN2 wild type or TRF2 and TIN2 mutated were co-expressed in HEK-293T cell line. Co-immunoprecipitation was performed with anti-GFP antibody and differences in TRF2 binding between TIN2wt and TIN2mut were revealed by western blotting.

Results: A new *TINF2* germinal variation at exon 2, c.254A>G p.H85A, was identified in the proband and in two brothers. Screening on 200 healty donors was negative. Significantly short telomeres were found in proband (p=0,0161) and brothers (p=0.0082 and p<0,0001), compared to age and sex matched controls. The proband had a normal SNPs profile and WES identified an additional somatic variation in *TLR1* gene (c. 1859G>A, p.R620Q). Co-immunoprecipitation experiments showed that the new *TINF2* mutation reduced TIN2 binding with TRF2 *in vitro*.
Summary/Conclusions: A new *TINF2* germinal variation at exon 2, c.254A>G p.H85A, was identified in the proband and in two brothers. Screening on 200 healty donors was negative. Significantly short telomeres were found in proband (p=0,0161) and brothers (p=0.0082 and p<0,0001), compared to age and sex matched controls. The proband had a normal SNPs profile and WES identified an additional somatic variation in *TLR1* gene (c. 1859G>A, p.R620Q). Co-immunoprecipitation experiments showed that the new *TINF2* mutation reduced TIN2 binding with TRF2 *in vitro*.

E1175

FUNCTIONAL EXPRESSION OF TIM-3 AND CLINICAL SIGNIFICANCE OF PLASMA GALECTIN-9 LEVELS IN MYELODYSPLASTIC SYNDROMES

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Background: T-cell immunoglobulin and mucin domain-3 (Tim-3) is an inhibitory immune checkpoint molecule that suppresses adaptive immunity by binding with galectin-9 (gal-9). The Tim-3−gal-9 pathway is associated with self-renewal of leukemic stem cells in acute myeloid leukemia (AML), although the function of the axis in myelodysplastic syndromes (MDS) remains unclear.
Aims: To clarify the expression and function of Tim-3 and clinical impact of its ligand gal-9 in MDS.
Methods: 1) We evaluated Tim-3 expression on CD45-gating blasts of bone marrow mononuclear cells (BMMCs) in 20 patients with MDS and AML transformed from MDS (AL-MDS), 12 healthy controls, and 4 MDS cell lines using flow cytometry (FCM). 2) To investigate Tim-3 induction, MDS cell line F-36P cells were co-cultured with the culture supernatant of human stromal cells and MDS-related cytokines. 3) To elucidate the functions of Tim-3 on MDS cells, F-36P cells were divided into Tim-3$^+$ and Tim-3$^-$ fractions with FACS sorting and their differential gene expression was determined with oligonucleotide microarray analysis. 4) To investigate the proliferative potential of Tim-3 signaling, intracellular Ki-67 expression in F-36P cells was evaluated using FCM when co-cultured with/without anti-Tim-3 blocking antibody. 5) Finally, we analyzed gal-9 concentrations in cell culture supernatants of MDS cell lines and in plasma obtained from patients with MDS (n=51) and AL-MDS (n=19), and healthy donors (n=10) using ELISA.
Results: 1) Tim-3 expression was observed on monocytes and CD45-gating blasts in MDS BMMCs and in all 4 MDS cell lines. In AL-MDS patients, Tim-3 expression levels on blasts were markedly higher than in controls and MDS patients. 2) Tim-3 expression in F-36P cells was increased by co-culture with the culture supernatant of human stromal cells and the MDS-related cytokine transforming growth factor-β1 (TGF-β1). Tim-3 cell-surface protein and mRNA expression in MDS cell lines was induced by co-culture with TGF-β1. The Tim-3 induction was abrogated by adding the TGF-β receptor I kinase inhibitor SD208. 3) Microarray analysis showed 572 upregulated genes (>2-fold difference) and 304 downregulated genes (<0.5-fold difference) in Tim-3$^+$ F-36P cells compared with Tim-3$^-$ cells, and ingenuity pathway analysis of those genes revealed upregulation associated with cell proliferation and antiapoptotic responses in Tim-3$^+$ cells. 4) The blockade by anti-Tim-3 antibody decreased intracellular Ki-67 expression in F-36P cells, suggesting that Tim-3 signaling induced MDS cell proliferation. 5) Soluble gal-9 was detected in culture supernatants of MDS cell lines and PBMCs obtained from AL-MDS patients. Soluble gal-9 levels and gal-9 mRNA expression were upregulated by MDS-related cytokines interferon-γ and tumor necrosis factor-α. Plasma gal-9 levels were higher in MDS and AL-MDS patients than in healthy controls (P<0.0001). When MDS patients were divided into high (defined as >10 ng/mL) and low (≤10ng/mL) gal-9 groups, the high group had poorer overall survival compared with the low group (P=0.001), even in refractory anemia (RA)/RA with ringed sideroblast patients alone (P=0.0029). Multivariate analysis revealed that a high gal-9 level was an independent poor prognostic factor (P=0.0017).
Summary/Conclusions: Our data suggest that Tim-3 expression and plasma gal-9 levels were upregulated in advanced-stage MDS. Tim-3 is associated with cell proliferation of MDS blasts, and higher plasma gal-9 is a poor prognostic marker in MDS. These molecules could play a key role in MDS disease progression.

E1176

PROGNOSTIC SIGNIFICANCE OF GENE MUTATIONS IN MDS DEPENDS ON THE LOCI OF GENE VARIANCES

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Background: Myelodysplastic syndromes are a collection of clonal hematopoietic disorders with a wide range of clinical manifestations and eventual outcomes. Predicting the prognosis is of great importance for defining the risk and select treatment options. Several models of risk stratification exist, all of which include genetic markers along with other clinical and paraclinical features. The Revised International Prognostic Scoring System (IPSS-R, *Greenberg et al., Blood. 2012;120(12): 2454-2465)* defines 5 risk levels based on the presence of specific chromosome abnormalities. These genome aberrations provide evidence for disease although reports of frequent driver mutations (*Papemauilie et al., Blood, 2013)* and/or structural variants detected by single nucleotide polymorphism (SNP) arrays (*Tiu et al., Blood, 2011*) have shown a potential for score criteria in the diagnosis of MDS. Recent reports of the presence such genetic aberrations in disease free individuals makes this approach problematic (*Genovese et al., N Engl J Med 2014; Lichman, Blood 2015, Kwok et al., Blood 2015*). A study of patients without evidence for MDS identified a driver mutation and/or structural gene variants in 91% of pre-diagnostic samples with the mutational spectrum mirroring that seen in MDS population (*Cargo et al. Blood, 2015*). The presence of mutations with greater median variant allele fraction (40% *vs* 9% to 10% in healthy individuals) and occurring with additional mutations (>2 mutations, 64% *vs* 8%) were shown to define a high-risk group with a shorter time to disease progression and poorer overall survival.
Aims: To compare the genomic profile of bone marrow from 145 adults, 76 of whom met the WHO criteria for MDS.
Methods: All samples were screened by chromosome G banding or molecular karyotyping using 8x60K oligonucleoitiude arrays (Agilent, USA) or screened by FISH using probes (Cytocell, UK) targeting the most common aberrations associated with MDS as per IPSS-R classification (Greenberg et al., Blood, 2013). The commercially available target gene panel TruSight on a MiSeq platform (Illumina, USA) was used to screen mutational hotspots in 54 cancer-related genes relevant in myeloid malignancy. Gene variances were reported at allele frequencies (VAF) >10% and at minimum read depth of 300 as per manufacturers criteria. We used the Catalogue of Somatic Mutations In Cancer (COSMIC), dbSNP and 1000 genome (>2%) to classify gene variants as either drivers, variants of unknown significance and germline polymorphisms (SNPs).
Results: A total of 145 bone marrow samples from 58 women and 87 men, aged from 26 to 85 suspected to have myeloid dysplasia were investigated. Of these only 76 (52%) were found to fulfill the WHO, criteria referred to as MDS positive, the rest as MDS negative. Gene variances were detected in all but 7 samples (5%). The latter appear to be void of gene mutations. We observed driver mutations as reported in myeloid malignancies in 68 (47%) samples whilst 70 (48%) were found to carry the same variances seen in disease free individuals or of unknown significance. As expected driver variances were not identified in any of the samples that failed the WHO criteria for MDS. Variances were detected in all samples for 35 of the 54 genes targeted by the TruSight myeloid panel. In order of frequency these are TET2, SRSF2, ASXL1, CUX1, DNMT3A, RUNX1, BCORL1 and HRAS, seen in more that 10% of all samples, while the rest were less frequently reported. The aberrant genes ASXL1, TET2 and SRSF2 figured prominently in both groups of samples with comparable frequencies as may be expected from published data and there were in addition a number of aberrant genes unique to the MDS group albeit at low frequency. When we examined the distribution of individual variances (rather than genes) we found a number of unique loci of the genes ASXL at 131022441, U2AF at 144524456 and TET2 at 106197285 to be associated with the MDS positive group. A more detailed analysis on the significance of these findings will be presented.
Summary/Conclusions: We compared 145 bone marrow samples from patents presenting with MDS of which 76 met the WHO criteria. There is little difference in their genomic profile when comparing the two groups on the basis of the most highly involved genes (ASXL1, TET2 and SRSF2) but if we compare the two groups by variance, 9 variances are exclusively associated with MDS positive disease.

E1177

SUPPRESSION OF DNA METHYLTRANSFERASE ENZYMES BY A NOVEL HYPOMETHYLATING AGENT, SGI-1027, IN AZACITIDINE- AND DECITABINE-RESISTANT CELL LINES

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Background: We established azacitidine- and decitabine-resistant cell lines, MOLM/AZA-1 and MOLM/DEC-5 from MOLM-13, an acute myeloid leukemia cell line (*Oncotarget* in press). DNA methyltransferase (DNMT) 3B was upregulated in the resistant cell lines.
Aims: We tried to find out clues to overcome the resistance to hypomethylating agent (HMA).

Methods: Besides azacitidine and decitabine, three other agents (SGI-1027, zebularine, and gemcitabine) are known as having hypomethylating effect. *in vitro* activities of the 5 HMA's on HMA resistant cell lines (MOLM/AZA-1 and MOLM/DEC-5) were tested by cell viability assay using luminescent-based CellTiter-Glo system. Protein and mRNA levels of DNMT enzymes (1, 3A, and 3B) were assayed before and after treatment of each HMA. Proteosomal degradation and activation of Akt were also determined to see the correlation with changes of DNMT's.
Results: Although azacitidine and decitabine could suppress DNMT1 and DNMT3A in MOLM-13, the agents could not suppress DNMT enzymes in resistant cell lines. Inhibition of proteosomal degradation by bortezomib induced accumulation of DNMT enzymes in MOLM-13, whereas it did not accumulate the enzymes in MOLM/AZA-1 and MOLM/DEC-5. Phosphorylated Akt (p-Akt) was dramatically overexpressed in MOLM/AZA-1 and MOLM/DEC-5. SGI-1027 showed the lowest IC_{50} values for MOLM/AZA-1 and MOLM/DEC-5, and it suppressed the protein levels of all three DNMT enzymes. SGI-1027 could also decrease the level of p-Akt. GDC-0941, a PI3K inhibitor, suppressed DNMT1 and DNMT3A as well as p-Akt, but it could not decrease DNMT3B in MOLM/AZA-1 and MOLM/DEC-5. Cell viability assay showed the synergistic effects of combination of GDC-0941 and Nanaomycin A, a specific DNMT3B inhibitor, in MOLM/AZA-1 and MOLM/DEC-5.
Summary/Conclusions: DNMT levels of MOLM/AZA-1 and MOLM/DEC-5 were not dependent on proteosomal degradation. DNMT1 and DNMT3A might be regulated via PI3K-Akt pathway, while regulation of DNMT3B might be different from DNMT1 and DNMT3A. SGI-1027 appears to exert inhibitory effects on MOLM/AZA-1 and MOLM/DEC-5 by inhibition of both p-Akt and DNMT3B.

E1178

MECHANISTIC HIGHLIGHTS OF IMPROVED ERYTHROPOIESIS WITH A LOW DOSE OF DEFERASIROX IN LOW RISK MYELODYSPLASTIC SYNDROMES

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Background: Myelodysplastic syndromes (MDS) are a group of heterogeneous clonal stem cell disorders leading to ineffective hematopoiesis. Anemia is a frequent cytopenia in MDS and the majority of patients requires red blood cell (RBC) transfusion resulting in the development of iron overload (IO). Deferasirox (DFX) became a standard treatment of IO in MDS and seems to have positive effects on hematopoiesis with a reduced need of RBC transfusion.
Aims: Decipher the mechanisms of the potential improvement of erythropoiesis with DFX.
Methods: We report our *in vitro* data about the proliferation, cell cycle, apoptosis, erythroid differentiation, and cell signaling pathways concerning $CD34^+$ hematopoietic stem progenitor cells from low risk MDS samples in a 2-step erythroid differentiation liquid culture with low dose DFX and iron overload.
Results: We observed a higher proliferation rate for cultures with 3µM DFX *versus* the control condition (p=0.038). In contrast, no increased proliferation was found with DFX>5µM and with other chelators used in the clinic. The higher proliferation rate with DFX 3µM was due to the combination of decreased apoptotic cells at day 10 (D10) (p=0.03) and D14 (p=0.007) and increased cycling cells at D10 (p=0.0001). Regarding clonogenic assays, there were more CFU-E colonies with DFX 3µM (p=0,04). Despite the low concentration of DFX, cells exposed to DFX 3µM had a lower intracellular iron concentration measured by ICP-MS than control cells (p=0.019). Nevertheless, this decreased iron amount was not sufficient to activate cellular iron regulation by Iron Regulatory Proteins suggesting the absence of a direct effect of low dose DFX on iron homeostasis. Moreover, low dose DFX decreased intracellular and mitochondrial reactive oxygen species (ROS) at D14 (p=0.048 and p=0.03) and decreased the level of malonaldehyde (p=0,048), a product of lipid peroxidation. Then, we have investigated which signaling pathways were sensitive to DFX 3µM. We found an increased nuclear translocation of NFκB with DFX 3µM (p=0,028) in confocal microscopy (CM). Using RT-qPCR microarrays, we have analyzed target genes of NFκB. We found 4 upregulated genes (BIRC3, CASP8, CSF3, IRAK2) and 10 downregulated genes (FASLG, IL1R1, TLR9, PSIP1, CCL2, TRAF2, LTBR, TICAM2, CD27, TLR4) suggesting an anti-apoptotic and anti-inflammatory pattern. To demonstrate a link between the likely ROS modulation effects of DFX and the NFκB activation pathway, we have engineered a cellular model of inhibition of thioredoxin (TRX) by siRNA. TRX1 and 2 contribute to the cellular antioxidant response. Inhibition of TRX1 and 2 in iron overloaded medium led to increased ROS levels (p<0.0001; p=0.006, respectively) *versus* mock-transfected cells. In this model of iron overload and in the siTRX1 condition, DFX 3µM triggered activation of NFκB detected by both CM (p=0.04) and luciferase reporter assay (p=0.03). NFκB activation was absent in the knock-down (KD) of mitochondrial TRX (siTRX2) condition. Moreover, in non-iron overloaded medium condition, the level of ROS was not increased, and DFX in the TRX1 KD condition was not associated with NFκB activation. These results suggest that NFKB activation in this model is linked to TRX1 and regulated by an extremely fine control of ROS levels with a likely threshold effect.
Summary/Conclusions: Our study describes the pro-proliferative effects of low dose of DFX on erythroid progenitors in low risk MDS patients. These results provide a biological rationale for a clinical trial which will propose low dose of DFX in MDS patients, refractory to erythropoiesis stimulating agents.

Myelodysplastic syndromes – Clinical

E1179

EVALUATING ERYTHROBLAST PAS POSITIVITY IN THE DIAGNOSTIC APPROACH OF MYELODYSPLASTIC SYNDROME

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Background: According to WHO minimal morphological criteria for myelodysplastic syndrome (MDS) diagnosis, at least 10% of bone marrow (BM) cells of at least one hematopoietic lineage must show unequivocal dysplasia to be considered as dysplastic. Morphological abnormalities of erythroid cells include cytoplasmic Periodic acid-Schiff (PAS) positivity, but the diagnostic power of this cytochemical reaction is not yet fully clear.
Aims: The aims of our study were to evaluate the diagnostic significance of erythroblast PAS positivity in MDS and to investigate a possible correlation between levels of PAS positivity and other morphological and clinical features.
Methods: We retrospectively examined the results of the cytochemical PAS staining for glycogen in BM smears from 165 patients with MDS, 116 patients with non-clonal cytopenia and 49 healthy subjects. We developed a PAS score by counting 100 nucleated cells for the erythroid lineage and classifying them according to their degree of PAS reactivity. The discriminant power of both PAS positivity rate and score for MDS identification was evaluated in comparison with that of the conventional morphological features of dyserythropoiesis; then, PAS positivity was included into the morphological scoring system we have previously defined (Leukemia 2015;29:66-75).
Results: PAS positive erythroblasts were observed in 104 (63%) MDS patients, 46 (40%) patients with non-clonal cytopenia, and 12 (24%) non-cytopenic controls, with a significant difference between MDS and non cytopenic controls ($p<0.0001$) or non-clonal cytopenias ($p=0.0001$), but not between healthy controls and non-clonal cytopenias ($p=0.09$). In MDS, both positivity rates (median 2%, range 0-33) and scores (median 2, range 0-53) were significantly higher than those in normal and pathological controls ($p=0.0001$ and $p=0.0004$ for rate, $p=0.0001$ and $p=0.0002$ for score, respectively), without significant difference in relation to excess blasts or multilineage dysplasia. MDS patients with >4% ring sideroblasts (RS) showed lower PAS positivity rates and scores than MDS patients with<4% RS ($p=0.0332$ and $p=0.0412$, respectively). In MDS-RS, erythroblast PAS positivity was not influenced by *SF3B1* mutation status. In MDS, no significant relationship was detected between erythroblast PAS positivity and percentage of BM blasts, percentage of BM erythroblasts, dyserythropoiesis grading, or Hb levels, whereas an inverse correlation was noticed between PAS score values and internuclear bridging ($r=-0.23$, $p=0.0395$). A ROC curve analysis allowed us to identify a PAS score value ≥1 (AUC=0.697, $p=0.0008$) and a PAS positive erythroblast percentage ≥1% (AUC=0.674, $p=0.0034$) as optimal cutoff to discriminate MDS patients from non-clonal cytopenias. Considering the most discriminant morphological features for dyserythropoiesis, the weight of both PAS positivity rate and score in the identification of BM dysplasia was lower than that of ring sideroblasts and megaloblastosis, but higher than that of defective hemoglobinisation, nuclear lobulation, multinuclearity, cytoplasmic fraying, pyknosis, and internuclear bridging. Integrating conventional parameters and PAS results significantly improved the sensitivity of our morphological scoring system.
Summary/Conclusions: The evaluation of BM erythroblast PAS positivity, easy to perform and inexpensive, may be useful in the work-up of patients with suspected MDS, especially if there is only unilinear dysplasia without ring sideroblasts or excess blasts.

E1180

A PHASE 3 RANDOMIZED PLACEBO (PBO)-CONTROLLED DOUBLE-BLIND TRIAL OF DARBEPOETIN ALFA IN LOW OR INTERMEDIATE-1 (INT-1) RISK MYELODYSPLASTIC SYNDROMES (MDS)

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Background: There is a lack of PBO-controlled data for erythropoiesis-stimulating agents (ESAs) in MDS.
Aims: To evaluate darbepoetin alfa (DAR) in IPSS low/int-1 risk MDS (EudraCT2009-016522-14, NCT01362140).
Methods: Patients with MDS per WHO 2008 criteria with IPSS low/int-1 risk, anemia [hemoglobin (Hb)≤10 g/dL], low transfusion burden, no previous treatment with ESAs or biologic response modifiers, and serum EPO≤500mU/mL were randomized 2:1 to 24 weeks (wk) SC DAR 500 µg or PBO every 3 wk (Q3W), stratified by IPSS, then 48 wk open label (OL) DAR; follow-up is ongoing. Doses were withheld for Hb>12g/dL and decreased if Hb increased by >1.5 g/dL in 3 wk. Key endpoints were transfusion incidence and HI-E per IWG 2006.
Results: Randomized patients [N=147] had median Hb of 9.3 (min-max:5.5-10.6) g/dL and median baseline EPO of 69 (min-max:4.3-497) mU/mL; WHO classification was RA:15%, RARS:14%, RCMD:44%, del5q:9%, RAEB-1:16%, and MDS-U/unknown:2%. Transfusion incidence wk 5-24 was significantly reduced with DAR [DAR:36.1% *vs* PBO:59.2%, $p=0.008$]. In the 48-wk OL DAR period, 50.8% of patients had transfusions. More DAR patients achieved HI-E in the double blind period [DAR:14.7% (11/75 evaluable) *vs* PBO:0% (0/35 evaluable), $p=0.016$]. In the 48-wk OL DAR period, 34.7% (34/98) of patients achieved HI-E. Improved HI-E and transfusion responses were seen with more favorable status for IPSS-R but not IPSS. In the 48-wk OL DAR period, dose frequency increased from Q3W to Q2W in 81% of patients; doses were held/reduced frequently. Safety results from this trial were consistent with the previous DAR phase 2 MDS trial, with similar AML rates in PBO and DAR arms.

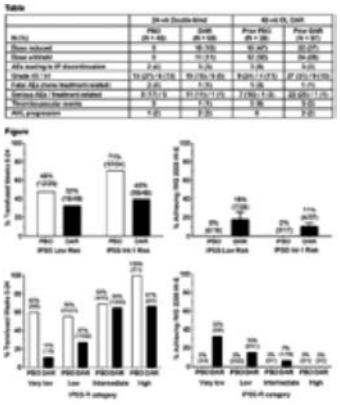

Figure 1.

Summary/Conclusions: In this phase 3, randomized, double-blind, PBO-controlled trial in anemic IPSS low/int-1 risk MDS patients, 24 wk of darbepoetin alfa Q3W significantly reduced transfusions and increased HI-E rates with no new safety signals. Most patients met criteria to change to Q2W dosing during the 48-wk OL period, suggesting that Q2W dosing may offer more benefit. The true clinical benefit of darbepoetin alfa may have been underestimated due to the nature of IWG 2006 HI-E criteria and trial design (Hb measured Q3W, dosing rules).

E1181

PRELIMINARY ANALYSIS OF EFFICACY AND SAFETY OF SINTRA-REV CLINICAL TRIAL, LENALIDOMIDE *VS* PLACEBO PHASE 3 STUDY IN LOW/INT-1 MDS PATIENTS WITH DEL(5Q) AND TRANSFUSION INDEPENDENCY

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Background: Lenalidomide (LEN) is the first choice of treatment in low risk MDS patients with isolated de(5q) (MDS-del(5q)) and transfusion dependency (TD). Most of the low risk MDS-del(5q) patients diagnosed with anemia and independent of transfusions developed TD or needed treatment for symptomatic anemia early after diagnosis (median of 20 months, *abstract 3180 ASH, 2016*). LEN directly targets the del(5q) clone improving anemia, quality of life and survival in these subset of patients. For these reasons, the use of LEN in patients with del(5q), anemia and not TD seems to be very attractive. However, data about the use of LEN in MDS 5q- patients and transfusion independency (TI) are scanty, some retrospective studies suggest a benefit with the early use of LEN in this setting, but there is not already available any prospective and randomized study to confirm this likely advantage.
Aims: Our aims were to analyze efficacy and safety at week 12 of treatment with LEN *vs* Placebo in this setting of low risk MDS del(5q) patients with anemia and not in TD at diagnosis
Methods: From 2010 to 2017, 47 patients have been included in the Sintra-Rev trial, a phase III, multicenter, randomized and double blind study with LEN (5mg/day) *vs* placebo [2:1 randomization] in Low – Int-1 risk (IPSS) MDS del(5q) patients with anemia but TI. Preliminary results of efficacy (according to the IWG 2006 criteria for erythroid [HI-ER] and cytogenetic response [CyR]) and safety has been analyzed at week 12. Progression disease (DP) in the trial was defined as the development of TD.

Table 1.

Table 1. Main clinical characteristics (N=47)	
Age median (range)	72 (37-89)
Gender M/F	7/40
Hb (g/dL) median (range)	9.7 (7.1-11.7)
Platelets median (range)	263 x 10^9/L (104–761)
Neutrophils median(range)	2.18 x 10^9/L (0.69–16)
%Blasts in BM median (range)	2 (0-9)
IPSS-R (n [%])	
• Very-low	15 (31.9%)
• Low	26 (55.3%)
• Int	2 (4.3%)
• No info	4 (8.6%)
Cytogenetics:	
• Del5q alone	39 (83%)
• Del5q + other Cy abn	2 (4.3%)
• Unknown	6 (12.8%)

Results: Main clinical characteristics are summarized in **table 1**, 85% were females, median age was 72 years (37-89) and most of patients (95%) had del(5q) as the only cytogenetic abnormality. Among 47 patients, only 38 were evaluable at week 12 (5 out of 38 discontinued the study: 3 due to DP, 1 due to toxicity and 1 for unknown reasons), 7 patients are currently receiving the first 12 weeks of treatment and 2 patients were excluded (screening failures). Regarding efficacy (w12), data from 36 patients were available. HI-ER was observed in 14/36 patients (39%), minor HI-ER (Hb increased<1.5g/dL) in 4/36 (11%), stable disease in 15/36 (42%) and PD (transfusion dependency) in 3 (8%). CyR was available in 30 patients: complete CyR was obtained in 12 (40%), partial CyR in 6 (20%) and no CyR in 12 (40%) patients. Safety information in 38 patients demonstrated that most patients (87%) developed any adverse events (AE) while only 42% of these were relevant (G3-4). Most G3-4 AE were hematological (neutropenia 38%) being non-hematological only in 5%. Seven serious AE were reported in 5 patients: vestibular neuritis, congestive heart failure, polyarthritis, arterial hypertensive crisis, carpal arthritis, respiratory infection and chronic obstructive pulmonary disease exacerbation. All SAE were not related with the drug of the study (LEN/Placebo).
Summary/Conclusions: In this study we confirm a high rate of erythroid and cytogenetic responses early after treatment with an adequate safety profile in the first 12 weeks of treatment with LEN or placebo.

E1182

MYELODYSPLASIA-RELATED MORTALITY REMAINS THE MAIN CAUSE OF DEATH ALONG DIFFERENT GROUPS OF RISKS: AN ANALYSIS FROM MDS ARGENTINEAN STUDY GROUP

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Background: Myelodysplastic syndrome (MDS) are the most frequent hematological malignancy in elderly patients. The impact of MDS burden over overall mortality remains controversial, moreover, after the incorporation of hypometilating agents in the therapeutic armamentarium.
Aims: We aimed to analyze overall mortality and causes of death in our population of patients with MDS.
Methods: A retrospective analysis of patients with MDS reported to Argentinean MDS registry and a previous study from Academia Nacional de Medicina. Causes of death were classified in: acute myeloid leukemia (AML), infections, bleeding, solid tumor, cardiovascular, transplant related mortality (MRT), others and unknown. AML, infections and bleeding were considered as MDS-related mortality. Causes of death were analyzed using cumulative competitive events curves with Gray test and Fine-Gray for proportional hazard regression was used for the multivariate analysis.
Results: From 1981 to 2016, 1040 patients with MDS were recorded; 717 out of 1040 (69%) were diagnosed after 2006. Median age of patients was 70 years (range: 14-95 years) with 588 (56%) being male. MDS was primary in 974 patients (94%). Median follow-up of 25 months (range: 1-170 months) for the surviving patients. The cumulative incidence of overall mortality was 20% at 12 months (95%CI 2-22), 37% at 24 months (95%CI 3-40) and 59% at 60 months (95%CI 5-63). The incidence of overall mortality did not significantly differ along the years of diagnosis (p=0.291) neither according to age group. Multivariate analysis for cumulative incidence of overall mortality found Charlson index (HR 1.38; p<0.001), sex (HR 1.45; p=0.014) and IPSS-R (HR 2.79; p=<0.001) as prognostic variables. The main cause of death was AML accounting for 9% at 12 months (95%CI 7-11), 16% at 24 months (14-19) and 25% at 60 months (95%CI 22-28) of mortality for all patients. Infection-mortality and bleeding-mortality were the second and the third cause of death respectively. MDS-related mortality was 16% at 12 months (95%CI 13-18), 29% at 24 months (95%CI 26-32) and 44% at 60 months (95%CI 40-48); this incidence was not different by year of diagnosis. MDS-related mortality remained the main cause of death in all IPSS-R groups and in all Charlson index categories. Multivariate analysis for cumulative incidence of MDS-mortality found Charlson index (HR 1.29; p=0.02), IPSS-R (HR 2.88; p<0.001) and sex (HR 1.47;p=0.03) as independent variable. Age (p=0.034) and IPSS-R (p<0.001) were associated with AML-related mortality. A total of 56 patients underwent allogeneic transplant; cumulative incidence of MRT for all cohort was 0.5% at 12 months (95%CI 0.2-1.2) and 1.4% at 24 months (95%CI 0.8-2.4). Only male sex was associated with a higher cumulative incidence of mortality by solid tumor (p<0.001) and a Charlson index ≥2 was associated with higher cumulative incidence of cardiovascular mortality (p=0.021).
Summary/Conclusions: In this large cohort of patient with MDS we demonstrate that MDS-related causes are the leading cause of death along all IPSS-R groups. The absence of difference in mortality along the years of diagnosis highlights the necessity of better treatments for these patients.

E1183

PROSPECTIVE STUDY OF FLOW CYTOMETRY OF BONE MARROW IN 105 CONSECUTIVE PATIENTS WITH CYTOPENIA AND SUSPICION OF MYELODYSPLASTIC SYNDROME: STRONG CORRELATION WITH RISK OF AML-EVOLUTION AND SURVIVAL

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Background: Diagnosis of myelodysplastic syndromes (MDS) remains a challenge, specially in patients with scant displastic morphology features and/or in the absence of cytogenetic changes. Multiparametric flow cytometry (MFC) findings have been recognized as a co-criterion for the diagnosis of MDS and have also demonstrated prognostic value in some studies. Nevertheless, this diagnostic tool is not fully implemented for the study of MDS in many centers and data from real life out of investigational studies are few.
Aims: To prospectively assess the value of MFC in the diagnosis of MDS in our center and correlate its findings to the clinical outcome of patients in terms of overall survival, transfusional needings, risk of hospitalization and evolution to acute leukemia.
Methods: We studied bone marrow samples from 105 consecutive patients submitted to our hospital between January 2013 and Abril 2015 because of one or more cytopenia and suspicion of MDS. Cytomorphology of every sample

was evaluated by at least two morphology experts and a consensus diagnostic of MDS-confirmed, MDS-suspected or MDS-excluded was emitted. MFC was performed applying at least five-colour staining and a numerical score was calculated for every patient following criteria defined by Ogata *et al* (Blood. 2006 Aug 1; 108(3):1037-44), with a score >=2 suggesting MDS. Conventional karyotype and FISH employing probes to detect usual 5q-, 7q-, +8, 20q- and delTP53 were performed in all cases.

Results: Median age of the patients was 73.5 y/o. Patients presented with anemia in 88 cases (84%), neutropenia in 36 (34%) and thrombopenia in 49 (47%). Cytomorphology was reported as MDS-confirmed (60 pts), MDS-excluded (22) or MDS-suspected (23). MDS subtypes were Multilineage Dysplasia (23), Unilineage Dysplasia with Ring Sideroblasts (4), Blast Excess (14), Multilineage Dysplasia with Ring Sideroblasts (9), del5q Syndrome (3) and Unclassified (2), 4 pts being diagnosed of CMML. MFC score was MDS-suggestive in 56 cases, MDS-not suggestive (36) and in 13 cases its use was precluded because of morphology findings. Considering cytomorphology as gold standard, and excluding those patients with MDS-suspected but not confirmed, MFC score sensitivity was 77%, especificity 88%, with positive and negative predictive values of 96% and 56% respectively. Furthermore, MFC-score showed a significant correlation with single morphologic findings of granulocytic (p<0.001), erythroid (p=0.001) and megakaryocytic displasia (p=0.002), and a trend to significant association with del7q by FISH (p=0.085). In the subset of patients with MDS-suspected but not confirmed by morphology, the presence of a MFC score >=2 was significantly associated with a poorer overall survival (log-rank p=0.012), with all MFC score <2 patients alive after a median follow-up of 35 months. There was also a trend to statistical association between MFC findings and overall survival in the whole series of patients (log rank p=0.053). Interestingly, there was a striking difference in risk of evolution to AML according to MFC findings (log rank=0.013), with a 100% of patients free from this complication in the group of patients with MFC score <2.

Summary/Conclusions: MFC analysis of the bone marrow provides useful information in the diagnosis of MDS which can be specially helpful in the subset of patients with inconclusive morphological findings, showing a strong correlation in this group of patients with clinical outcome in terms of risk of evolution to AML and overall survival.

E1184

ECONOMIC IMPACT AND HEALTHCARE UTILIZATION IN PATIENTS WITH HIGHER-RISK MYELODYSPLASTIC SYNDROMES (HR-MDS) IN ROUTINE CLINICAL CARE IN THE UNITED STATES (US) – A CLAIMS DATABASE STUDY

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Background: Therapy for patients with HR-MDS includes systemic chemotherapy, stem cell transplant (SCT), and supportive care aimed at improving symptoms associated with MDS-related disruption of normal hematopoiesis. However, the economic impact of these interventions over time for HR-MDS patients has not been fully examined.

Aims: We evaluated the costs and healthcare utilization (HCU) of US HR-MDS patients treated during routine care.

Methods: Newly diagnosed adult HR-MDS patients who initiated first-line therapy were identified from Optum, a large US claims database, between 1/1/08 and 10/31/15. HR status was based on ICD coding: ≥1 inpatient claim or ≥2 outpatient claims with ≥1 HR-MDS ICD-9/10 code (ICD-9 code: 238.73; ICD-10 codes: D46.20, D46.21, D46.22). The first MDS claim served as the index date. Exclusion criteria included: lack of continuous enrollment in medical/pharmacy benefits in the 12 months prior to index date (baseline period), presence of other primary cancer, or receipt of any chemotherapy or SCT during baseline period. MDS-related and non-MDS-related HCU and costs incurred during follow-up were evaluated. MDS-related HCU and costs were medical claims with a primary diagnosis of MDS or MDS-related treatment (ie, MDS chemotherapy as defined by NCCN MDS Guidelines v2.2017 or MDS-directed supportive care which included hydroxyurea, erythrocyte- and colony-stimulating- growth factors and erythrocyte/platelet transfusions) and pharmacy claims for MDS treatment. Proportions of patients with HCU were reported. Costs were calculated as per-patient-per-month (PPPM) costs adjusted to 2015 US dollars and reported as mean (standard deviation [SD]). Patients with a capitated payment plan were excluded from the cost analysis. Patients were followed until death, progression to acute myeloid leukemia (AML), end of continuous enrollment, or end of study (12/31/2015).

Results: 209 treated HR-MDS patients were identified. During the follow-up period, 69.4% of patients had ≥1 inpatient admission, but more patients had an MDS-related than non-MDS-related admission (Table 1). 56.9% of patients had ≥1 emergency room visit over the follow-up period; visits were predominately for non-MDS-related services. The majority of patients had ≥1 physician office visit (91.9%) and other outpatient visits (99.5%). Over the follow-up period, the mean PPPM cost was $17,361 (SD: $19,747) (Table 1) and was higher in Year 1 than in Year 2 ($17,337 [SD: $19,696] *vs* $12,976 [SD: $14,135]). The majority of costs overall were for MDS-related medical services ($10,327 PPPM, SD: $11,050). Between Years 1 and 2, MDS-related medical PPPM costs decreased from $10,557 (SD: $11,164) to $6,530 (SD: $7,406) while non-MDS-related medical PPPM costs remained fairly constant in both years. Chemotherapy and supportive care medical services were the main drivers of MDS-related medical costs, also decreasing from Year 1 to Year 2. Non-MDS-related costs accounted for a smaller portion of the overall medical PPPM costs ($6,124 [SD: $15,158]); and remained relatively similar in Years 1 and 2.

Table 1.

Table 1. HCU & Cost (Overall, Year 1 & 2 Post-index) for Treated HR-MDS Patients

Proportion with HCU, %[a]	Overall (N=209)	Year 1 (N=209)	Year 2 (N=92)
Inpatient Visit (≥1)	69.4	57.9	37.0
MDS-related vs non-MDS-related	57.9 vs 41.6	48.3 vs 33.5	23.9 vs 18.5
ER Visit (≥1)	56.9	51.2	34.8
MDS-related vs non-MDS-related	12.0 vs 55.0	9.1 vs 48.8	5.4 vs 32.6
Physician Office Visit (≥1)	91.9	90.4	81.5
MDS-related vs non-MDS-related	82.3 vs 89.0	81.3 vs 87.6	72.8 vs 75.0
Other Outpatient Visits (≥1)	99.5	98.6	91.3
MDS-related vs non-MDS-related	96.2 vs 99.0	93.3 vs 97.6	73.9 vs 90.2
Cost, mean $ in PPPM (SD)	**Overall (N=149)**	**Year 1 (N=149)**	**Year 2 (N=62)**
Total	17,361 (19,747)	17,337 (19,696)	12,976 (14,135)
MDS-related	10,839 (11,101)	11,068 (11,217)	6,995 (7,433)
Non- MDS-related	6,522 (15,181)	6,268 (15,093)	5,980 (11,637)
Medical	16,451 (19,203)	16,427 (19,111)	11,971 (14,104)
MDS-related	10,327 (11,050)	10,557 (11,164)	6,530 (7,406)
Non- MDS-related	6,124 (15,158)	5,870 (15,093)	5,980 (11,637)
Pharmacy	910 (2,342)	909 (2,419)	1,005 (2,320)
Selected Medical Costs Components			
Inpatient	**4,904 (14,703)**	**4,655 (14,644)**	**4,079 (11,184)**
MDS-related	1,175 (4,670)	1,205 (4,829)	873 (3,917)
Non- MDS-related	3,729 (14,003)	3,451 (13,930)	3,206 (10,272)
Physician Office	**997 (1,749)**	**1,031 (1,908)**	**736 (1,162)**
MDS-related	639 (1,483)	681 (1669)	424 (695)
Non- MDS-related	358 (490)	351 (513)	312 (679)
Other Outpatient	**3,419 (3,938)**	**3,491 (4,179)**	**3,328 (5,844)**
MDS-related	1,602 (2,281)	1,644 (2,456)	1,613 (4,635)
Non- MDS-related	1,817 (2,826)	1,847 (2,995)	1,716 (3,241)
Chemotherapy	**3,161 (2,106)**	**3,304 (2,157)**	**1,888 (2,134)**
Supportive Care	**3,700 (9,134)**	**3,678 (9,174)**	**1,652 (2,897)**

[a] MDS-related vs non-MDS-related are not mutually exclusive groups; patients could be in both categories, and percentages are relative to the overall "n" in the column as the denominator.
Key: ER – emergency room; HCU – healthcare resource utilization; HR-MDS – higher-risk myelodysplastic syndrome;; PPPM – per patient per month; SD – standard deviation

Summary/Conclusions: The economic impact of HR-MDS is considerable, with higher costs incurred within the first year of diagnosis. The decrease in cost between Year 1 and Year 2 was mainly due to decreased MDS-related medical costs. Consistent with this cost trend, healthcare utilization for MDS-related services decreased in Year 1 *vs* Year 2. As treatment of HR-MDS continues to evolve, economic impact and HCU need to be further investigated in this patient population.

E1185

INTRAVENOUS IMMUNOGLOBULIN IS AN EFFECTIVE TREATMENT FOR CYTOPENIAS ASSOCIATED TO CIRCULATING T-CELL CLONES IN MYELODYSPLASTIC SYNDROMES

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Background: Myelodysplastic syndrome (MDS) can be associated with immunologic disorders, including autoimmune cytopenias and Coombs positive or negative (C±) hemolytic anemia. Abnormally expanded T-cells can be detected in these patients, possibly contributing to both bone marrow insufficiency and peripheral cytopenia, and offer another target for therapeutic intervention.

Aims: To explore the role of intravenous immunoglobulin (IVIG) as a treatment for immune-related cytopenia in a series of 20 consecutive patients with MDS at a single institution.

Methods: T-cell clonal expansion in the peripheral blood (PB) was documented by flow cytometry and PCR. Eighteen patients had a confirmed MDS (16 IPSS lower-risk, LR). Two suspected MDS were designated as idiopathic cytopenia of uncertain significance (ICUS). Reasons for IVIG treatment were chronic hemolysis refractory to corticosteroids (16: 12 LR, 1 higher-risk (HR), 1 ICUS) or pancytopenia (2 LR and 1 HR refractory to standard therapy, 1 ICUS) associated to a T-cell clonal proliferation in the PB. Hematological response was assessed by IWG criteria 2006. Hemolysis response (HLR) included normalization (CR) or a greater than 50% improvement (PR) of LDH, reticulocytes, indirect bilirubin and haptoglobin.

Results: Clinical characteristics are shown in the Table. All patients had a clonal rearrangement of TCR by PCR (10 TCRβ, 8 TCRβ+TCRγ, 2 TCRγ). In 9 cases the clone was characterized by flow cytometry: 6 had a CD3+ T-cell and 3 had a CD3-/CD16+/CD56+ NK-cell expansion. Associated immunologic disorders were: ITP (4), neutrophil dermatosis (3), inflammatory bowel disease

(3), seronegative arthritis (2), connectivitis (2). One patient with hypoplastic MDS had LGL liver involvement. Coombs test was positive in 4/16 hemolysis cases. From Jan-'10 to Jan-'17, IVIG was administered at a dose of 500mg/kg once per week, in cycles of 1 to 4 weeks. The ORR was 75% (15/20): all patients showed an erythroid hematological improvement (HI) (100%). Platelets and neutrophil HI was seen in 50% and 80% of responsive cases, respectively. HLR occurred in 13/16 (81%: 4 CR and 9 PR). Median number of cycles and duration of treatment were 11 and 12 months (mo), respectively. The HLR-CR was stable in 7 patients; 4 relapsed from HLR but subsequently responded by shortening the intervals between administrations of IVIG; 2 were secondary refractory. Eventually, 6 responders became refractory to IVIG. Response was more durable with continuous rather than sporadic dosing. Median time to response was 1 mo. Median duration of response was 39 mo. Corticosteroids were discontinued in 5/10 patients and reduced in 5/10. Adverse events were: 1 palpitations (G1); 1 hypertension (G1). Responders had lower platelet counts ($p<0.05$), but no other clinical differences compared to non-responders. However, the 5-year OS rate was higher in the responders to IVIG: 53% compared to 30% ($p=0.08$).

Table 1.

	All (20)	Responders (15)	Non-responders (5)	P value
Age, median	71.5	73	70	ns
Male, n.	16	11	5	ns
Hemoglobin, median	7.45	7.5	7	ns
Platelets, median	58	55	308	<0.05
ANC, median	1.7	1.09	2.87	ns
Reticulocytes, median	4	4.2	3.5	ns
LDH, median	266	272	259	ns
Bilirubin indirect, median	1.2	1.4	0.9	ns
Haptoglobin, median	48	17	79	ns
Coombs test IgG ± C3D, n.	4	4 (weak)	0	ns
IPSS low-int1 (=lower-risk), n.	16	11	5	ns
IPSS int2-high (=higher-risk), n.	2	2	0	ns
Cytogenetics unfavorable, n.	5	4	1	ns
Splenomegaly, n.	8	7	1	ns
Immunologic disorders, n.	9	6	3	ns

Summary/Conclusions: Treatment with IVIG of C± hemolytic anemia and pancytopenia associated with T-cell immune-clones and MDS was safe and yielded high rates of durable response on all lineages and on hemolysis. Transfusion independency and reduction/discontinuation of corticosteroids for chronic hemolysis make this drug a valuable option not only in LR but also in HR patients, although a confirmation on larger cohorts is needed. IVIG at intermediate-high dose suppresses proliferation of T-cells and induces immune-regulation. Given the relative rarity of T-cell clones in MDS, further investigational studies are underway to define their pathogenetic role and the mechanism of action of IVIG in this specific subset of patients.

E1186

DEVELOPMENT AND EXTERNAL VALIDATION OF A NEW PATIENT-CENTERED PROGNOSTIC INDEX FOR PATIENTS WITH ADVANCED MYELODYSPLASTIC SYNDROMES

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Background: The clinical presentation of myelodysplastic syndromes (MDS) is highly variable, and the ability to accurately predict outcomes is critical. Current prognostic systems for these diseases are based on traditional clinical, pathologic and laboratory indicators.
Aims: We aimed to develop and validate a new prognostic index for advanced MDS by including self-reported fatigue severity into a well-established clinical risk classification: the International Prognostic Scoring System (IPSS).
Methods: Untreated patients (n=280) were recruited at the time of diagnosis of advanced MDS from 37 hospitals in nine countries to create the index. The index was then applied to an independent cohort including pre-treated MDS patients from the Dana-Farber Cancer Institute (DFCI) in Boston, Massachusetts (USA; n=189). Patients in both the International and DFCI cohorts were adults with newly-diagnosed intermediate-2 or high-risk MDS (advanced disease on the IPSS). Patients were enrolled regardless of age, comorbidity, performance status and progression from a lower IPSS category. All completed a baseline health-related quality of life assessment. Data from international and DFCI cohorts were independently collected and analyzed. Univariate and multivariate Cox proportional hazards (PH) regression analyses were performed to estimate hazard ratios with 95% Confidence Intervals (CIs). Discrimination and calibration were evaluated for both the development (internal validation) and independent DFCI datasets (external validation). Statistical significance for all tests was set as two-sided $\alpha=0.05$.
Results: A new risk classification was developed, namely, the fatigue (FA)-IPSS(h). Whereas use of the standard IPSS in more advanced disease discriminates between two risk categories for untreated patients, the new fatigue FA-IPSS(h) classification was able to distinguish three subgroups of patients with distinct survival outcomes. Overall survival rates at 6 months, 1 year, and 2 years were markedly different among the three groups. To illustrate, one year survival was 80.3% (95% CI, 73.4;87.8), 60.5% (95% CI, 52.3;70.0) and 37.6% (95% CI, 23.9;59.1) for patients classified into Risk-1, Risk-2 and Risk-3 respectively. Median OS in DFCI data by FA-IPSS(h) risk was similar to that of the development cohort for each of the three risk groups, indicating good external calibration. Patterns of OS through 2 years were also distinct between risk groups as in the development cohort of untreated patients, with one exception: 2–year OS was similar for FA-IPSS(h) risk-3 and risk-2. Predictive accuracy of this new index was higher than the IPSS alone in both the development cohort (C-statistic, 0.61 *vs* 0.57) as well as in the independent cohort including pre-treated patients (C-statistic, 0.58 *vs* 0.54).
Summary/Conclusions: The FA-IPSS(h) is an additional prognostic tool that might enhance clinicians' ability to provide more personalized treatment strategies both in untreated and pretreated advanced MDS patients. This analysis offers a model for integration of PROs in prognostic systems for patients with other cancers and advanced illnesses.

E1187

PROGNOSTIC AND THERAPEUTIC IMPLICATIONS OF SIGNIFICANT MARROW FIBROSIS IN COMBINATION WITH P53 OVER-EXPRESSION IN PATIENTS WITH MYELODYSPLASTIC SYNDROME: A SINGLE CENTRE STUDY

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Background: Myelodysplastic syndromes (MDS) are defined in the WHO 2016 classification as a group of clonal bone marrow neoplasms characterized by ineffective hematopoiesis, morphologic dysplasia in hematopoietic cells and peripheral cytopenia(s)[1]. They present as a diverse phenotype with some patients requiring merely observation while others require more intensive management due to significant marrow failure and the risk of development of acute leukaemia. The presence of significant marrow fibrosis has previously been shown to be a poor prognostic factor in patients with MDS, with reduced overall survival[2]. Significant marrow fibrosis has also been associated with both the presence of the TP53 gene mutation and with p53 over expression, which is a known adverse prognostic risk factors in MDS patients[3].
Aims: To assess the presence of p53 expression in patients with moderate to severe marrow fibrosis (grade 2-3), observe its effect on overall survival in patients with marrow fibrosis, and determine whether the use of azacitadine had any impact on survival.
Methods: We conducted a retrospective study utilizing a hospital database of 247 patients with MDS diagnosed in a single center between 2000 and 2014. Of these patients, 200 had bone marrow trephine samples adequate for reticulin stain analysis, which was completed using the European consensus on grading bone marrow fibrosis (grades 0-3). P53 expression was examined using immunohistochemistry staining defined according to the modified quick scoring system. We then looked for an association between degree of marrow fibrosis and p53 expression. In patients with significant marrow fibrosis and p53 expression we examined overall survival and response to treatment with azacitadine.

Results: Overall, no significant correlation was seen between expression of p53 and degree of fibrosis (p=0.25). However, degree of fibrosis predicted for overall survival in patients with p53 expression (median overall survival of 4 months in patents with both p53 over expression and significant fibrosis compared with median overall survival of 18 months in patients with p53 over expression without fibrosis, p=0.001). In patients who received azacitadine, those with both significant fibrosis and p53 expression had a significantly increased overall survival compared with those who did not receive azacitadine (4 month *versus* 1 month, p=0.002). Azacitadine treatment was not associated with increased survival in patients with p53 expression without fibrosis but these patients did have an overall increased survival compared to those with fibrosis (median survival 12 *vs* 37 months).
Summary/Conclusions: This study confirms that significant marrow fibrosis adversely affects overall survival in patients with MDS, including those with p53 over expression. Patients who received azacitadine had a significant increase in median survival. Alhough the numbers of patients who received azacitadine were small, this data suggests that patients with fibrosis may benefit from the use of azacitadine and larger and randomized studies should be considered to study this further.

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E1188

FACS PURIFICATION OF BLAST CELLS IN MDS IMPROVES THE FISH DETECTION RATE FOR DEL(5Q) AND DEL(20Q), BUT NOT FOR DEL(7Q) OR T8

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Background: Prognostication in Myelodysplastic Syndromes (MDS) using validated scores includes the detection of chromosomal aberrations by conventional karyotyping. When the latter is unavailable or unsuccessful, fluorescence in-situ hybridization (FISH) panels can be used. Although panels vary by laboratory, some of the most commonly used probes include the search for monosomy 5 or del(5q), monosomy 7 or del(7q), del(20q) and trisomy 8 (T8). In our Lab, FISH was historically performed on the whole unsorted patient sample (Full Sample); since 2015, we have primarily performed the analysis on Fluorescence Activated Cell Sorting (FACS) separated blast cells.
Aims: In this study, we aim to analyze the benefit of using purified samples of blast cells for FISH analysis in MDS, when compared to full mixed cellularity samples.
Methods: We reviewed all samples analyzed in our laboratory between January 1st 2011 and February 28th 2017 in which a FISH panel was performed due to a suspicion of myelodysplasia, using probes for del(5q), del(7q), del(20q) and T8. The proportion of patients positive for the test, as well as the proportion of positive cells within a positive sample, were compared.
Results: We obtained valid results for 328 samples during the relevant timeframe, 39.6% of which were collected from female patients. FISH was performed after FACS in one third of samples (35.1%, n=115), starting in 2015. Considering the overall cohort, nearly a quarter of samples (23.8%) had at least one aberration in the four probes tested in this study. This proportion of abnormal findings was nearly double in FACS patients (33.0%) compared to full sample patients (18.8%, p=0.004). **Del(5q)** was present in 5.6% of the cohort; however, positivity was 8-fold higher in FACS patients, compared to full sample patients (12.3% *vs* 1.6%, p<0.001). Considering the percentage of positive cells in each sample, it doubled from 38.7±29.9% in the full sample to 71.8±28.1% after FACS, p=0.08. **Del(7q)** was similarly present in 5.7% of the cohort; however, in contrast, there were no relevant differences between FACS patients, 4.2% of whom had del(7q), and full sample patients (8.1%, p=NS). There were, however, differences in the percentage of positive cells within the sample, doubling from 32.1±11.2% in the full sample to 77.6±17.8% after FACS, p<0.001. **Del(20q)** was identified in 7.0% of the overall tested cohort; the asymmetry in results was marked, with a 36-fold higher proportion of positive findings after FACS (18.7%) compared to full samples (0.5%, p<0.001). The percentage of positive cells doubled from 15% in the single positive test in the full sample cohort, to an average of 35.5±22.2% after FACS. Finally, **T8** was found in 10.2% of patients, a result which was uninfluenced by the type of sample used (10.2% in both full and FACS samples, p=NS). The percentage of positive cells once again doubled from 25.5±14.7% in the full sample to 53.3±28.1% after FACS (p=0.0008).
Summary/Conclusions: We found that one quarter of all patients who underwent a FISH panel workup for a suspected diagnosis of MDS presented with aberrations in at least one of the four selected probes, a proportion which was significantly lower (one fifth) when a full sample was analyzed, and significantly higher (one third) in FACS purified blast cells. Although the purification of the sample through FACS doubled the percentage of positive cells within each sample for all four probes, the likelihood of obtaining a positive result for del(7q) and T8 in the cohort was unaffected by the methodology used. In contrast, the use of a sorted sample greatly increased the proportion of positive findings in del(5q) and, especially, in del(20q), the two probes for which the basal positivity in full samples were lowest. The clinical value of this increased rate of detection of del(5q) and del(20q) remains unclear, since their prognostic utility has only been established for levels detectable by conventional karyotyping of a full sample.

E1189

COUNTING BONE MARROW BLASTS AS A PERCENTAGE OF NON-ERYTHROID CELLS PROVIDES SUPERIOR RISK STRATIFICATION FOR MDS PATIENTS WITH ERYTHROID PREDOMINANCE

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Background: Patients with erythroid predominance (≥50% erythroblasts, MDS-erythroid) compose a significant proportion of patients with MDS. The erythroid/myeloid subtype was divided from the AML category into MDS-erythroid by the 2016 WHO classification of myeloid neoplasms. At that time, there was no consensus on a more appropriate way of enumerating bone marrow (BM) blasts from TNCs or NECs in MDS-erythroid patients.
Aims: To clarify these questions, 1283 MDS patients were retrospectively analyzed in our center.
Methods: MDS-erythroid was observed in 27.0% of patients (346/1283), and these patients had similar clinico-pathological features and overall survival, with 937 cases of MDS with <50% ENCs.
Results: By calculating the percentage of BM blasts from NECs, 73 of 200 patients (36.5%) with MDS-erythroid who were diagnosed within WHO subtypes without excess blasts (EB) were moved into higher-risk categories and showed shorter OS than those who remained in the initial categories (P=0.041). Recalculating the International Prognostic Scoring System-Revised (IPSS-R) by enumerating blasts from NECs, 40 of 168 (23.8%) MDS-erythroid patients with relatively lower risk were re-classified as higher-risk and had significantly poorer survival than those who remained in the lower-risk category (P=0.030). This was especially true for the intermediate risk group that was stratified by IPSS-R (unchanged patients *vs* shifted patients, P=0.007). However, the impact of enumerating BM blasts from NECs on classification and prognostication was not evident in all MDS patients.
Summary/Conclusions: In conclusion, our results suggested that enumerating the percentage of BM blasts from NECs significantly improved the prognostic assessment of MDS-erythroid, especially for patients within the intermediate risk group stratified by IPSS-R.

E1190

SUCCESSFUL TREATMENT WITH DANAZOL FOR MYELODYSPLASTIC SYNDROMES AND APLASTIC ANEMIA REFRACTORY OR INELIGIBLE TO STANDARD THERAPY

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Background: The discovery of danazol potential activity on telomere elongation in bone marrow failure has renewed interest in this drug. The treatment of cytopenia in myelodysplastic syndromes (MDS) and aplastic anemia (AA) patients who fail or are ineligible to standard therapies is an unmet medical need; however only dated reports on danazol use in this setting are available.
Aims: We report the results of treatment with danazol in patients with MDS and AA at a single institution.
Methods: From Jun-'11 to May-'15, danazol was administered to 31 consecutive patients (20 MDS and 11 AA). Criteria for treatment were non-severe AA (8), severe AA ineligible/refractory to immunosuppressive therapy or allogeneic transplantation (3), transfusion dependent (TD) lower risk MDS refractory to erythropoietin (11), MDS with isolated thrombocytopenia <50x10^9/L (6) or with bone marrow hypoplasia and bicytopenia (3). Diagnosis was defined by WHO 2008 for MDS and according to Camitta (Blood 1975) for AA; response was assessed by IWG 2006 criteria.
Results: The characteristics of the patients are shown in the Table. All MDS patients had low-risk disease according to IPSS and IPSS-R, except 2 and 3 patients respectively. Nineteen patients (12 MDS, 7 AA) received danazol at full dose (600mg daily). A 400mg daily dose was given to 12 patients, due to toxicity (4 MDS, 4 AA) or comorbidities (4 MDS). Median duration of treatment

was 19 months (mo) (1-66) in AA and 6 mo (1-60) in MDS. ORR was 73% and 50%, respectively. Age and hemoglobin levels impacted on response in AA. Hematological improvement was seen on all lineages in 92% of cases, with a median time to best response of 3-5 mo on platelets and neutrophils and of 8-12 mo on hemoglobin. Interestingly, duration of response in MDS patients was significantly longer with a danazol dose of 600mg than with 400mg (p=0.001). Conversely, dosing did not impact on response to danazol in AA patients. Grade 2-3 toxicity was significantly higher in AA patients (p<0.05), 60% pretreated with IST. Adverse events included: hepatotoxicity (3 G1, 1 G2, 3 G3), muscle pain/CPK elevation (3 G1, 2 G2), transient renal impairment (1 G1), hyporexia (1 G1). Responders to danazol had a better survival in terms of OS and EFS in both groups (Figure 1).

Table 1.

	AA				MDS			
	TOT	R	NR	*p-value*	TOT	R	NR	*p-value*
Total	**11**	**8**	**3**	-	**20**	**10**	**10**	-
Male	6	4	2	ns	11	4	7	ns
Age	57	48	74	**<0.05**	70	69.5	73.5	ns
Hb g/dL	9.35	11.5	7.4	**<0.01**	8.9	8.6	8.9	ns
Plts x10⁹/L	21	24	6	ns	27	31	25	ns
ANC x10⁹/L	0.98	1.27	0.7	ns	1.7	1.7	1.7	ns
Cellularity%	20	20	30	ns	40	28	74	ns
TD%	5	2	3	ns	13	6	7	ns
Toxicity (G≥2)	5	-	-	-	2	-	-	**<0.05**

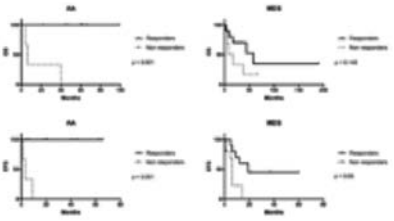

Figure 1.

Summary/Conclusions: Danazol was proved both effective and safe as treatment of cytopenia in MDS and AA patients refractory or ineligible to standard therapies. The daily dose of 600mg was more effective for MDS patients, whereas a lower dose of 400mg may have a better risk/benefit ratio in AA. Younger AA patients with less severe anemia were more likely to respond. Danazol use is particularly attractive in thrombocytopenic patients, where responses were rapid, but delayed responses may be expected also on anemia by using danazol for prolonged periods, when tolerated. Response to danazol is also potentially associated to a survival advantage, although these data should be confirmed by larger prospective studies.

E1191

SURVIVAL OUTCOMES IN PATIENTS WITH HIGHER-RISK MYELODYSPLASTIC SYNDROMES (HR-MDS) IN ROUTINE CLINICAL CARE IN THE UNITED STATES (US) – A CLAIMS DATABASE STUDY

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Background: MDS is composed of multiple and rare hematological stem-cell disorders, resulting in cytopenias and disease-related complications and deaths. There are no robust trial data comparing the available treatment options for HR-MDS patients; and of the approved drugs, only azacitidine has demonstrated a statistically significant, but modest clinical impact on overall survival (OS).
Aims: We evaluated first-line treatment (1LT) choice and survival outcomes in a US cohort of HR-MDS patients engaged in routine care.
Methods: Newly diagnosed HR-MDS patients who were ≥18 years old and who had initiated 1LT were retrospectively identified from Optum, a large US claims database, between 1/1/2008 and 10/31/2015. HR status was based on ICD coding: ≥1 inpatient claim with an HR-MDS ICD-9/10 code (ICD-9 code: 238.73; ICD-10 codes: D46.20, D46.21, D46.22), or ≥2 outpatient claims with ≥1 HR-MDS ICD-9/10 code. The first MDS claim served as the index diagnosis date. Exclusion criteria included: absence of continuous care for 12 months prior to index date (baseline period), presence of other primary cancer, or receipt of any chemotherapy or stem-cell transplant (SCT) during the baseline period. 1LT was defined as MDS-specific treatment (MDS-Tx) (NCCN MDS Guidelines v2.2017)[1] and initiated after first HR-MDS claim (index date). Follow-up ended upon death, end of continuous enrollment, end of study, or progression to acute myeloid leukemia (AML) (except for OS). Transfusion independence (defined as ≥60 days with no evidence of erythrocyte [RBC] or platelet [PLT] transfusions) was evaluated in patients with ≥60 days of follow-up. Median progression-free survival (PFS) (defined as progression to AML, initiation of second-line therapy, or death) and OS, along with PFS and OS rates at 2 years following initiation of 1LT, were evaluated using unadjusted Kaplan-Meier analyses.
Results: 209 newly diagnosed HR-MDS patients initiating 1LT MDS-Tx were identified; mean age was 73 years (standard deviation [SD]: 10.1) and 61.2% were male. In the 12 months prior to diagnosis, 27.3% of patients used MDS-directed supportive care (ie, colony stimulating-, erythrocyte-, or thrombopoietic growth factors; RBC or PLT transfusions; or hydroxyurea). 1LT with hypomethylating agents (HMAs) predominated in 89.5% of patients (azacitidine, 68.9% and decitabine, 20.6%); 8.6% of patients received an immunomodulator monotherapy; and 8.6% of patients underwent SCT during follow-up. Of the 169 treated HR-MDS patients with ≥60 days of follow-up on 1LT, 51% achieved transfusion independence. For all treated HR-MDS patients, median PFS and 2-year PFS rates were 12.5 months (95% confidence interval [CI]: 9.1, 14.9) and 27.9%, respectively. OS rate at 2 years was 59.1%. Patients who achieved transfusion independence had a higher rate of 2-year OS (65.2% *vs* 53.8%) and PFS (36.3% *vs* 25.7%), but neither were statistically significant.

Table 1.

Table 1. PFS and OS Outcomes in Treated HR-MDS Patients in Routine Care

Outcome	Overall N=205[a]	Transfusion Independent N=87[b]	Transfusion Dependent N=82[b]
PFS from initiation of 1LT			
Median (95% CI), months	12.5 (9.1, 14.9)	16.5 (11.3, 23.8)	13.4 (8.9, 15.1)
		P=0.08	
1-Year Rate, %	51.5	61.7	57.0
		P=0.34	
2-Year Rate, %	27.9	36.3	25.7
		P=0.10	
OS from initiation of 1LT			
1-Year Rate, %	81.9	84.9	87.8
		P=0.73	
2-Year Rate, %	59.1	65.2	53.8
		P=0.61	

[a] Excludes the 4 patients treated with stem-cell transplant only.

[b] Includes only those treated in 1LT with ≥60 days of follow-up post-1LT initiation.

Key: 1LT – first-line therapy; CI – confidence interval; HR-MDS – higher-risk myelodysplastic syndromes; PFS – progression-free survival; OS – overall survival.

References:
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Summary/Conclusions: Survival outcomes in routine clinical care were higher than reported in clinical trials, specifically in HR-MDS trials with azacitidine.[2] Among patients able to achieve transfusion independence, a trend toward increased 2-year PFS and OS rates was observed, although statistical significance was not reached. Characteristics that contribute to variations in PFS and OS outcomes within the HR-MDS population need further investigation.

E1192

DOSE-CONFIRMATION PK/PD STUDY OF ORAL ASTX727, A COMBINATION OF ORAL DECITABINE WITH A CYTIDINE DEAMINASE INHIBITOR (CDAI) E7727, IN SUBJECTS WITH MYELODYSPLASTIC SYNDROMES (MDS)

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Background: We have previously shown that ASTX727, a combination of oral decitabine and the oral CDAi E7727, emulates the pharmacokinetics of a one hour intravenous decitabine infusion (IV-DAC) in a dose-escalation phase 1 study. (Garcia-Manero. Blood 2016 128:114)
Aims: To confirm pharmacokinetic (PK) and pharmacodynamic (PD) comparability of 20mg/m^2 IV-DAC administered D1-5 of a 28 day cycle with an entire cycle of ASTX727 given at the selected dose from phase 1 (35mg decitabine and 100mg of E7727).
Methods: Adult patients with Int-1/Int-2 or HR MDS or Chronic Myelomonocytic Leukemia (CMML) were enrolled in a randomized cross-over Phase 2 study. Patients were randomized 1:1 to receive in the first 28 day cycle, either 5 days of IV-DAC or 5 days of ASTX727, followed by a cross-over to the other in Cycle

2. Cycles 3 forward were with ASTX727. PD were assessed with LINE-1 methylation measured on blood cells at baseline and days 8, 15, 21 and 28 in cycles 1 and 2. Full PK assessments of ASTX727 were performed on Days 1, 2 and 5 with sparse sampling on Days 3 and 4 and on Day 1 of IV-DAC. Modeling of 5 day exposures of ASTX727 and IV-DAC was created for each patient. Safety and clinical response were assessed on all patients.
Results: 50 patients were randomized, of whom 43 had matched PK and 46 had matched PD sample sets for the first 2 cycles. No significant differences were seen when comparing the randomized sequences for any parameters, so all assessments comparing ASTX727 and IV-DAC were performed independent of sequence. The geometric mean maximum demethylation was 9.9% for ASTX727 and 11.2% for IV-DAC [Ratio of oral over IV=0.89, with a lower limit of 90%CI of 0.804]. The geometric mean AUC for IV-DAC was 161 ng*h/mL. The 5 day total geometric mean of the AUC (ng*h/mL) was 769 for ASTX727 and 805 for IV-DAC [(Ratio of oral over IV=0.96, with a lower limit of 90%CI of 0.806]. Decitabine C_{max} was higher for IV-DAC (189 ng/mL) than after ASTX727, Day 2 (109ng/mL) and Day 5 (121ng/mL). The most common adverse events regardless of grade or causality were febrile neutropenia 34%, neutropenia 28%, thrombocytopenia 16%, fatigue 16%, and hypomagnesemia 16%. There were no reported GI Adverse Events greater than Grade 2 with ASTX727 regardless of relationship to treatment.
Summary/Conclusions: Fixed dose oral administration of 35mg decitabine and 100mg E7727 (ASTX727) emulates the AUC of IV-DAC over the 5-day treatment cycle and induces a similar degree of demethylation of LINE-1 sequences in blood cells compared to IV decitabine at 20mg/m^2 dose. The preliminary safety profile is similar to what has been reported for IV-DAC and no increase in GI side effects has been observed. Clinical responses continue to be evaluated as the study data mature. This combination will be tested further as an alternative to parenteral administration of decitabine.

E1193

FACTORS PREDICTIVE FOR INFECTION IN PATIENTS WITH HIGHER- RISK MYELODYSPLASTIC SYNDROMES, CHRONIC MYELOMONOCYTIC LEUKEMIA AND ACUTE MYELOID LEUKEMIA TREATED WITH AZACITIDINE
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Background: Hypomethylating agents (HMAs) are the current standard treatment of higher-risk myelodysplastic syndrome (MDS), chronic myelomonocytic leukemia (CMML) and older acute myeloid leukemia (AML) patients. Severe infectious complications are common during HMAs therapy and have been recognized as one of the most important reasons of morbidity and mortality in MDS, CMML and AML patients especially at the beginning of treatment.
Aims: To evaluate the incidence of and predisposing risk factors for infections during the first three azacitidine (AZA) cycles treatment
Methods: In this retrospective study we analyzed 282 consecutive patients with higher- risk MDS (174), CMML (34) and AML with bone marrow blasts <30% (74) treated with azacitidine in 10 Polish hematologic centers between the years 2009-2016. Patients who did not complete three AZA cycles and also did not experience infection were excluded (50 cases).
Results: Median age was 68 (28-93). Median number of AZA cycles was 6 (1-43). Infectious episodes during the first three cycles of AZA were reported in 94 out of 232 (40%) eligible patients - in 32% of MDS, 30% of CMML and 63% of AML patients (p<0.05). Among patients with infection, most of them had their first infection episode during the first cycle -53%, during the second cycle - 29% and during the third AZA cycle -18%. It was found that low neutrophil count (0,6x10^9/Lvs 1,2x10^9/L), low lymphocyte count (1,1x10^9/L *vs* 1,3x10^9/L), low platelet count (37x10^9/L *vs* 63x10^9/L), high blood ferritin level (1101ng/ml *vs* 569 ng/ml), low albumin concentration (3,6 g/dl *vs* 4,0 g/dl), high bone marrow blasts percentage (15% *vs* 12%), higher IPSS, IPSS-R and WPSS score, red blood cell transfusion dependency and worse WHO performance status prior therapy were significantly predictive factors for infection (p<0.05). Factors like age, gender, hemoglobin concentration, iron, immunoglobulin and creatinine blood level, time from diagnosis to beginning of AZA treatment, IPSS and IPSS- R cytogenetics, coexistence of diabetes mellitus, heart failure, chronic obstructive pulmonary disease, second cancer, autoimmune disease and corticosteroids treatment did not have significant impact on infection risk (p>0.05). Antibacterial, antifungal or antiviral prophylaxis did not significantly reduce the risk of infection (p>0.05). Median overall survival patients with infection was 5 months and patients without infection 12 months.
Summary/Conclusions: Patients treated with azaciditine (especially AML patients) are at high risk of infection during the first three AZA cycles. All important infection predictive factors should be assessed before therapy. Patients possessing factors predictive for infection require special approach and predictive infection model should be developed in further analysis.

E1194

OVERALL SURVIVAL, INITIAL TREATMENT AND TREATMENT DURATION OF PATIENTS WITH MYELODYSPLASTIC SYNDROME, A DETAILED POPULATION BASED STUDY
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Background: Population-based studies on myelodysplastic syndrome (MDS) containing detailed clinical information of patient characteristics, treatment and follow-up of the disease are scarce. Since 2005, all patients diagnosed with hematological malignancies in Friesland, a province in the Netherlands, are prospectively registered and followed by their clinicians in a population-based registry, the HemoBase. The registry provides representative population-based data on diagnosis, treatment and outcomes in an era where low-intensity treatment such as hypomethylating agents have become available for the elderly.
Aims: The objectives of this study were to determine the overall survival (OS) of patients with MDS and the effect of the variables gender, age, comorbidities, IPSS(-R) score and MDS subtype according to WHO 2016 classification. Furthermore, the leukemia free survival (LFS), the initial treatment and the duration of first-line treatment were analyzed.
Methods: An observational, population-based study was performed using the HemoBase registry. The bone marrow biopsies and aspirates of all MDS patients diagnosed between 01-01-2005 and 31-12-2013 were independently and blindly reviewed by both the hematologist and hemato-pathologist and classified according to the WHO 2016. Treatment categories were defined as intensive chemotherapy (IC) either combined or not combined with allogeneic stem cell transplantation, the hypomethylating agent azacytidine, the immunomodalatory agent lenalidomide, hydroxyurea or best supportive care (BSC) (blood transfusions, erythropoiesis-stimulating agents). Approval was obtained from the Medical Ethics Review Committee from Medical Centre Leeuwarden. Statistical analyses were performed with SPSS 19; survival analyses were presented using Kaplan-Meier estimates.
Results: 217 patients (72.4% male, 66.8% >70 years old, median age 75 years, 27.2% Charlson Comorbidity Index (CCI) score ≥3 at diagnosis) were included with a median follow-up duration of 70.2 months. 15.7% of the population had an IPSS score of ≥1.5 and 12.4% of the population had an IPSS-R score of ≥4.5. In 41.5% no cytogenetic information was available. MDS-RS, MDS-SLD/-MLD, MDS-EB, MDS-U and CMML were diagnosed in 11.5%, 14.7%, 36.4%, 27.2% and 10,1% of the population respectively. 18.4% showed progression towards acute myeloid leukemia (AML). IC, azacytidine, lenalidomide, hydroxyurea and BSC were the initial treatment in 5.1%, 13.8%, 1.4%, 9.7% and 66.4% of the patients respectively. Within 12 months 78.1% of all treated patients terminated their first-line therapy because of death (20.0%), refractory to treatment (18.3%) or disease progression (16.7%). A second treatment was initiated in 10.1% of patients. The median LFS was 18.2 months (95% CI: 12.6-23.8). The median OS of MDS patients in Friesland was 22.5 months (95% CI: 15.2-29.7). Univariate analysis showed an association between lower OS and male gender (HR for women: 0.54, p=0.008, 95% CI: 0.34-0.85), age >80 years (HR: 2.7, p<0.0005, 95% CI: 1.6-4.6), CCI score ≥3 (HR: 2.0, p<0.001, 95% CI: 1.3-3.0), IPSS score ≥1,5 (HR: 2.3, p=0,004, 95% CI: 1.3-4.1), IPSS-R score ≥4,5 (HR: 5.7, p<0.0005, 95% CI: 2.4-2.4) and MDS subtype MDS-EB (HR: 1.8. p=0.016, 95% CI: 1.1-2.9).
Summary/Conclusions: This study provided complete and representative population-based data on overall survival and treatment of patients with MDS in an era of new treatment modalities. Despite the median age of 75 years and significant comorbidity in this population, a third of the patients received treatment in addition to BSC.

E1195

DANAZOL TREATMENT FOR THROMBOCYTOPENIA IN LOWER-RISK MYELODYSPLASTIC SYNDROMES: A REAL LIFE EXPERIENCE
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Background: Severe thrombocytopenia is an uncommon event in lower-risk MDS patients, but it may significantly affect the prognosis. In fact, when it occurs, major bleeding may be a life-threatening complication. No licensed pharmacologic approach is nowadays available yet for this unmet need in Europe. Eltrombopag seems to be a very interesting product, but its efficacy and safety still need to be better demonstrated. Even romiplostim could be

suitable, but, at present, its safety is questioned in MDS patients. Furthermore, in clinical practice, danazol, an attenuated androgen, has been reported to have some ability to increase the platelet count in this context (Wattel 1994; Chan 2002).

Aims: To asses efficacy and toxicity of danazol employed to improve severe thrombocytopenia in lower-risk MDS setting.

Methods: We retrospectively reviewed twenty-four patients affected by MDS and treated with danazol for thrombocytopenia. The initial dose was 600mg/day for all patients. The IWG criteria of response (Cheson 2006) were adopted. The outcome was observed every 3 months till 12th month. The overall response rate and the average platelet count or each time of observation were described. Progression free survival was estimated with the Kaplan-Meier product limit method, followed by the logrank test and by the Cox proportional-hazard regression.

Results: Of the 24 patients, 3 patients had a therapy-related MDS. At the starting time of danazol therapy, the IPSS was "low" in 9, "int-1" in 13 and "int-2" in 2 cases respectively; the IPSS-R was "very low" in 2, "low" in 11, "intermediate" in 7 and "high" or "very high" in 4 cases. At baseline in 14 patients the platelet count was lower than 20x10^3/mL, the average was 20x10^3/mL and the maximum value was 38x10^3/mL. The median dose was 600mg (range 200-600) also maintained at least up to 3 months (range 400-600). At 6 and 12 months the median dose therapy was 400mg (range 400-600 and 200-600 respectively). The response rate was 79.1% (19 responders on 24 treated). The average count increased as shown in Figure 1, over 60x10^3/mL after 6 months from the beginning of therapy and so maintained after one year. Only 3 patients lost the response at 187, 600 and 633 days respectively. The median survival was not reached in the presented series, and the probability to maintain the response is over 75% after two years from the beginning therapy in the responder patients (Figure 2). Adverse events recorded were as follows: moderate (grade 1 and 2) increase in transaminases in 4 cases (with reduction of danazolo to 400mg/day in 2 of these); 1 case of severe but reversible liver toxicity (grade 3) (with subsequently drug suspension); severe (grade 3) but reversible renal failure in 1 case (the drug was stopped); moderate (grade 1 and 2) increasing of serum creatinine in 6 case (with reduction of danazolo to 400mg/day in 2 of these); reversible cutaneous rush in 3 cases; amenorrhea in 1 case (the only fertile woman in the series); weight loss and loss of appetite in 1 case, weight gain in 1 case.

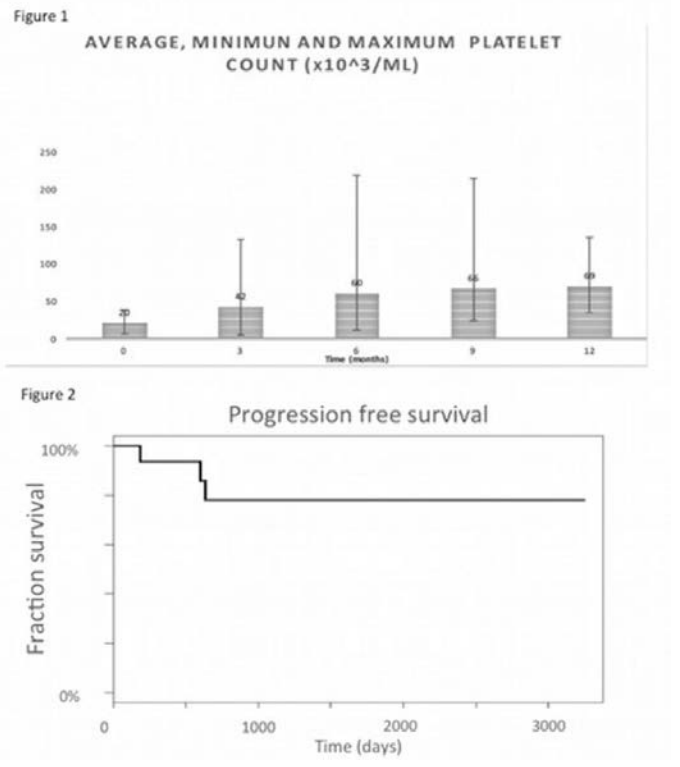

Figure 1.

Summary/Conclusions: This series confirms the efficacy of danazol to improve platelet count in the most of patients with severe thrombocytopenia due to lower-risk MDS. In all patients with increased platelet count, the response was clinically significant. The median dose of 600mg should be maintained for at least 3 months to properly assess the effectiveness of therapy and then adjusted according to response and toxicity. The response may not be immediate, but seem to be reachable after 3-6 months of treatment. A responsive patients have short probability to loss the response, that may last for very long time. The toxicity profile of this drug is low. The mechanism of action of danazol in MDS patients remains unclear. Waiting for more information on the efficacy and safety of eltrombopag from the clinical trials in progress, danazol may be a good therapeutic option for these patients.

E1196

TREATMENT PATTERNS IN PATIENTS WITH HIGHER-RISK MYELODYSPLASTIC SYNDROMES (HR-MDS) IN ROUTINE CLINICAL CARE IN THE UNITED STATES (US) – A CLAIMS DATABASE STUDY

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Background: Treatment of patients with HR-MDS includes hypomethylating agents (HMAs) (azacitidine and decitabine), high-intensity induction chemotherapy (IC), and stem cell transplant (SCT). Given the rarity of disease, information available on how these treatments are applied in practice is limited.

Aims: We evaluated the treatment patterns of HR-MDS patients engaged in routine care within the US.

Methods: Newly diagnosed HR-MDS patients who were ≥18 years old were retrospectively identified from Optum, a large US claims database between 1/1/2008 and 10/31/2015. HR status was based on ICD coding: ≥1 inpatient claim or ≥2 outpatient claims with an HR-MDS ICD-9/10 code (ICD-9 code: 238.73; ICD-10 codes: D46.20, D46.21, D46.22). The first MDS claim served as the index date. Exclusion criteria included: absence of continuous enrollment in medical and pharmacy benefits for 12 months prior to index date (baseline period), presence of other primary cancer, or receipt of any chemotherapy or SCT during the baseline period. First-line therapy (1LT) was defined as an MDS-specific treatment (as defined by NCCN MDS Guidelines v2.2017)[1] initiated on or after the index date. Patients were followed until death, end of continuous enrollment, or end of study (12/31/2015). For patients with progression to acute myeloid leukemia (AML), treatment pattern evaluation stopped at AML diagnosis.

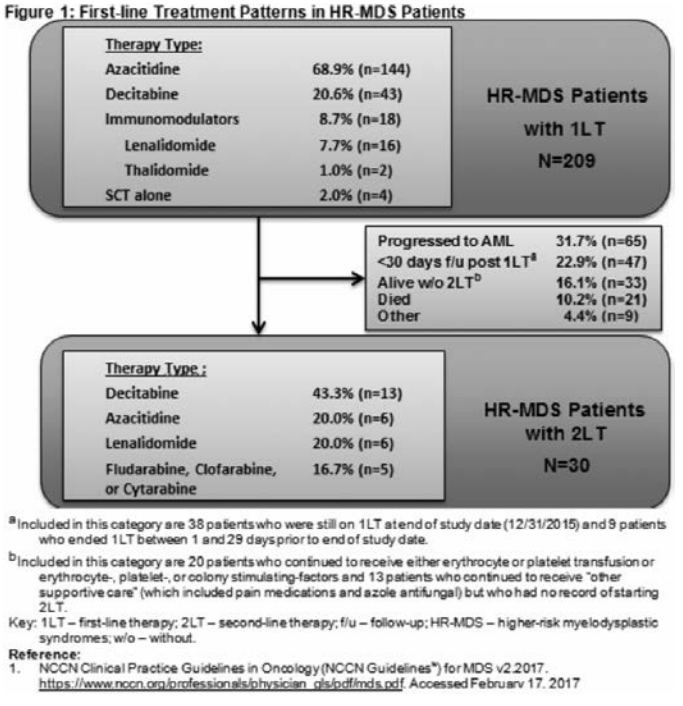

Figure 1.

Results: 335 newly diagnosed HR-MDS patients were identified; 209 (62.4%) were treated with 1LT with treatment initiated within 1 month of diagnosis (median: 17 days, interquartile range [IQR]: 9, 35). A higher proportion of untreated patients (n=126) was ≥75 years of age (71.4% *vs* 53.1%) and had certain comorbidities at baseline (congestive heart failure, 23.0% *vs* 16.3%; renal disease, 24.6% *vs* 16.3%; diabetes 31.0% *vs* 23.4%, diabetes with end organ failure, 16.7% *vs* 8.1%) than treated patients. For treated patients, 1LT with azacitidine predominated in 68.9% of patients (n=144), followed by decitabine in 20.6% of patients (n=43), and immunomodulators (lenalidomide or thalidomide) in 8.7% of patients (n=18) (Figure 1). 4 patients had only SCT and an additional 14 had SCT at some point during follow-up. With regard to HMA therapy, median duration was 4.5 months (IQR: 2.6, 9.5) for azacitidine and 4.8 months (IQR: 2.1, 11.6) for decitabine. A greater proportion of decitabine-treated patients

received supportive care with colony-stimulating factors (CSFs) (39.5% *vs* 28.5%) and either erythrocyte or platelet transfusions (69.8% *vs* 57.6%) during 1LT *vs* azacitidine-treated patients. Second-line therapy (2LT) was administered to 30 (14.4%) patients; the HMAs again predominated in 63.3% of patients (n=19). Of patients not receiving 2LT, 65 (31.7%) progressed to AML, 47 (22.9%) had <30 days of follow-up due to proximity to end of study (38 [80.9%] of these were on 1LT at end of study date), 33 (16.1%) continued to receive some supportive care and, 21 (10.2%) died.

Summary/Conclusions: Most HR-MDS patients treated in routine care are treated according to guidelines, with the HMA, azacitidine, predominating. Underlying comorbidities and older age may influence whether or not to treat HR-MDS patients with 1LT. For treated HR-MDS patients, duration of 1LT did not differ with azacitidine and decitabine. However, use of certain MDS-related supportive care treatments varied by choice of HMA, with more decitabine-treated patients receiving CSFs and transfusions. Further research is needed to determine how these factors influence both clinical outcomes in a HR-MDS population.

E1197

APPRECI8: A PIPELINE FOR PRECISE VARIANT CALLING INTEGRATING 8 TOOLS

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Background: For the use of next-generation sequencing in clinical routine valid variant calling results are crucial. However, evaluation of eight open-source variant calling tools on two different real- and simulated data sets has pointed out that variant calling - even of single nucleotide variants and short indels - remains challenging. Perfect results could not be obtained with any of the considered tools. High sensitivity was always accompanied by low positive predictive value (PPV).

Aims: We aimed at developing a variant calling pipeline with both, high sensitivity and high PPV.

Methods: We developed *appreci8*, a variant calling pipeline combining the output of eight open-source variant calling tools: GATK HaplotypeCaller, Platypus, VarScan, LoFreq, FreeBayes, SNVer, SAMtools and VarDict. The pipeline performs several steps of filtration, including a final automatic characterization of all reported calls as artifacts, likely polymorphisms and likely mutations. To train our pipeline, we analyzed two data sets covering data of 54 myelodysplastic syndrome (MDS) patients, sequenced on Illumina HiSeq, and 111 MDS patients, sequenced on Illumina NextSeq. Subsequently, two independent test sets were analyzed. The first test set covered 237 MDS patients, sequenced on Illumina HiSeq. The second test set covered 89 MDS patients, sequenced on Roche 454. In all cases the same target region consisting of 19 genes (42,322bp) was analyzed. Validation was performed by re-sequencing on the same platform, on a different platform and expert-based review.

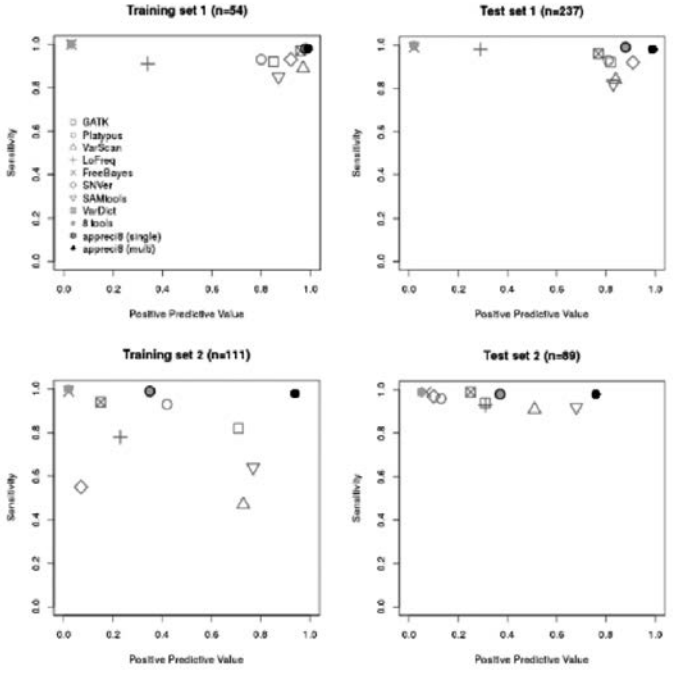

Figure 1.

Results: When analyzing the training sets with only one of the eight variant calling tools and considering all variants -pathogenic as well as polymorphisms-, sensitivity ranges between 0.85 and 1.00 in case of set 1 and 0.47 and 0.99 in case of set 2. Although FreeBayes features highest sensitivity regarding both sets, it consistently features lowest PPV as well (set 1: 0.03, set 2: 0.02). Combining the output of all variant calling tools leads to perfect sensitivity, while PPV is 0.03 for set 1 and 0.02 for set 2. Application of our *appreci8* pipeline leads to a minor decrease in sensitivity (set 1 and set 2: 0.98), while PPV is significantly increased (set 1: 0.99, set 2: 0.94). The PPV of the *appreci8* output for both training sets is higher compared to each of the individual tools. Analysis of the independent test set 1 leads to comparable results. Sensitivity of the individual tools ranges between 0.82 and 0.99, while PPV ranges between 0.02 and 0.91. Combining the output of all variant calling tools leads to sensitivity of 1.00 and PPV of 0.02. However, application of *appreci8* leads to variant calling results with sensitivity of 0.98 and PPV of 0.99. To test the robustness of our approach, we analyzed Roche 454 data, although the pipeline was exclusively trained on Illumina data. Regarding the individual tools sensitivity ranges between 0.91 and 0.99, while PPV ranges between 0.07 and 0.68. By combining the output of all variant calling tools, sensitivity increases to 0.99, while PPV is 0.05. Application of *appreci8* leads to sensitivity of 0.98 and PPV of 0.76.

Summary/Conclusions: To consider variant calling results in clinical routine, it does not seem appropriate to rely on the output of a single tool only. Instead, combining the output of several tools and applying a set of filters as it is done by our appreci8 pipeline leads to results with both high sensitivity and PPV. Nonetheless, variant calling results should, especially at allelic frequencies below 20%, always be viewed with criticism.

E1198

COMPARISON OF ADMINISTRATION OF HYPOMETHYLATING AGENTS WITH EFFICIENCY OF ALLOGENEIC SCT IN ELDERLY PATIENTS WITH ADVANCED MDS

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Background: Hypomethylating agents (HMA) have been introduced as a promising agent in the treatment of elderly patients with advanced myelodysplastic syndromes (MDS) leading to a response in approximately 50% of patients. However, most of the patients relapse and estimated years survival is below 10%. Stem cell transplantation (SCT) still represents the only curative treatment even in elderly patients with advanced MDS and it is connected with long-term survival in 35-40% despite relatively high risk of transplant related mortality (25-30%).

Aims: The aim of the study was a retrospective analysis of results of the treatment of 59 elderly patients (50 years of age or older) with MDS RAEB-2 or with acute myeloid leukemia with multilineage dysplasia with less than 30% of bone marrow blasts (MDS RAEB-T according to the FAB classification) who received either HMA or underwent allogeneic SCT.

Methods: In the HMA group, 34 out of total 38 patients received azacytidine (Vidaza®) in the dose of 75mg/m^{2}x7 every 28 days and 4 patients were treated with decitabine (Dacogen®) in the dose of 20mg/m^2 x5 every 28 days. Median number of cycles administered was 10.4 (range 3-31). An age and diagnosis matched transplanted group consisted of 21 patients, 9 patients were transplanted upfront, 12 patients were pretretated either with combination chemotherapy (10 patients) or with HMA (2 patients) and achieved CR prior to SCT. Ten patients received myeloablative conditioning and 11 patients were transplanted after reduced conditioning regimen.

Results: A hematologic response to HMA (CR,PR, hematologic improvement) was observed in 22 out of 38 patients in HMA group (57.9%), CR was achieved in 10 patients (31.8%). In SCT group, engraftment was reached in 20 out of 21 patients, 11 patients died after SCT (6 on complications related to SCT, 5 patients relapsed). No difference was observed between both the groups in 2 years estimated overall survival (OS), (42% for SCT *vs* 36% for HMA), a significant difference in favour of SCT was present in estimated 3 years and 5 years OS (42% and 38% for SCT *vs* 9% and 4% in HMA group, P=0.001). Median OS was 18.7 months in HMA treated group compared to 42.6 months in SCT group (P=0.02). In a recent analysis performed at 48 months after starting the treatment, 2 patients treated with HMA (5,3%) and 9 transplanted patients (42.8%) were alive, 23 patients in HMA group and 6 patients in SCT group relapsed. No significant differences in results and adverse effects of treatment were observed between patients aged 50-60 years and those older than 60 years in both HMA and SCT groups.

Summary/Conclusions: Our results confirm previous observations showing that despite a promising effect of HMA resulting in hematologic response in more than 50% of elderly patients with advanced MDS, allogeneic SCT still represents the only potentially curative treatment connected with long-term survival in a significant number of patients even in elderly MDS patients.

E1199

A MULTICENTER, OPEN-LABEL, PHASE I CLINICAL STUDY: SAFETY, EFFICACY, AND PHARMACOKINETICS OF ORAL RIGOSERTIB IN JAPANESE PATIENTS WITH RECURRENT/RELAPSED OR REFRACTORY MYELODYSPLASTIC SYNDROMES

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Background: Rigosertib, a novel phosphoinositide 3 kinase pathway inhibitor, induces G2/M arrest leading to the apoptosis of cancer cells and myeloblasts and is safe for and well tolerated by pts with low, intermediate-1, intermediate-2, or high-risk myelodysplastic syndromes (MDS).
Aims: The aims of the study were to assess the safety, efficacy, and pharmacokinetics of oral rigosertib and to determine the recommended dose (RD) for a Phase II clinical study in Japanese pts with recurrent/relapsed or refractory MDS.
Methods: We conducted a multicenter, open-label, Phase I clinical study of oral rigosertib. The key eligibility criteria were as follows: recurrent/relapsed or refractory MDS; age: 20 or older; ECOG PS of 0 to 2; and no major organ dysfunctions. Rigosertib (280 and 560mg BID) was administered orally in one 21-day cycle (up to cycle 6) that consisted of the 14-day, twice-daily, oral administration term, followed by 7-day monitoring. The primary endpoint was dose-limiting toxicity (DLT). The secondary endpoints were 1) safety as assessed with adverse events (AEs) and laboratory results, 2) efficacy as assessed with the International Working Group 2006 criteria, and 3) pharmacokinetics.
Results: Between March 2013 and November 2014, 6 male and 3 female pts (median age: 70; range 52-80) were enrolled. ECOG PS was 0 in 7 pts and was 1 in 2 pts, and 3 and 6 pts were eventually assigned to the 280 and 560mg BID arms, respectively. According to the FAB classification, 4, 2, 2, and 1 pts were categorized to RAEB, RARS, RA, and RAEB-t, respectively. The prognostic factor according to IPSS was Int-1 risk in 4 pts (1 and 3 pts in the 280 and 560mg BID arms, respectively) and was Int-2 in 5 pts (2 and 3 pts in the 280 and 560mg BID arms, respectively). DLT occurred in 1 pt in the 280mg BID arm and in 2 pts in the 560mg BID arm: the former consisted of type 2 diabetes and grade 4 delirium, and the latter grade 5 urinary tract infection and grade 3 prolonged QT interval. Therefore, the RD for a Phase II clinical study in Japanese pts was determined to be 560mg BID. On day 11 of treatment, 1 pt in the 560mg BID arm died of grade 5 urinary infection whose relationship with the investigational drug was rated to “Definite”. The presumed cause of death for this patient was septic shock caused by urinary tract infection. The mean counts of leukocytes, neutrophils, lymphocytes, and reticulocytes in the 280mg BID arm did not decrease along with increases in the number of cycles delivered but decreased slightly in the 560mg BID arm. Any changes of note were not found in other hematological items. One case of grade 3 neutropenia developed in the 280mg BID arm, and 1 case each of grade 3 laboratory abnormalities—increased alanine aminotransferase, increased aspartate aminotransferase, prolonged QT interval, neutropenia, and decreased hemoglobin—occurred in the 560 BID arm. The hematological remission rate was 11.1% (1 marrow CR; 1/9 pts), and the hematological improvement rate was 11.1% (1 HI-P; 1/9 pts). Among the PK parameters, inter-individual variability was observed in the C_{max} and AUC. However, changes suggesting the accumulation of rigosertib during repeated oral administration (e.g., consistent increases in the C_{max} and AUC) were not found.
Summary/Conclusions: The present chemotherapy regimen of oral rigosertib was well tolerated. Our study indicates that the RD for a Phase II clinical study is 560mg BID in Japanese patients with recurrent/relapsed or refractory MDS.

Myeloma and other monoclonal gammopathies - Biology

E1200

NON-OVERLAPPING PROMOTER AND SUPERENHANCER DRIVEN PROCESSES SUPPORT MYELOMA CELL GROWTH AND SURVIVAL VIA DISTINCT REGULATORY AXES

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Background: We have previously reported that E2F1 and its heterodimerization partner DP1 promote MM tumor proliferation both *in vitro* and in vivo; and observed an inverse correlation between their expression and patient survival suggesting a role in MM pathogenesis. Moreover, E2F functional impairment by a dimerization inhibiting stapled peptide significantly affects myeloma tumor cell growth while sparing effect on normal components of bone marrow as well as normal plasma cells, suggesting an E2F dependency in MM cells.
Aims: In this study, our aim was to defined the regolatory landscape of E2F in MM to better understand how E2F1 and DP1 drive myeloma cell proliferation; and to define the relationship between promoter proximal transcription factor-associated gene expression and super-enhancer-driven transcriptional programs.
Methods: We integrated genetic perturbation with functional omics to define E2F role in MM. Global occupancy of E2F1 and DP1 in MM was evaluated by ChIP-seq analysis. E2F1 and DP1 genomic localizations were then integrated to MM reference epigenome. Enhancers and super-enhancers were mapped using ROSE2 (github.com/bradnerlab/pipeline). Read densities were calculated using bamliquidator (github.com/BradnerLab/pipeline/wiki/bamliquidator).
Results: Integration of E2F1 and DP1 genomic localization to MM reference epigenome revealed specific co-occupancy of the factors at promoters of active genes marked by H3K4me3, with a strong positive correlation between E2F and RNA Polymerase II (RNA Pol II) binding at transcription start sites. In contrast, active enhancers, as defined by promoter distal Mediator (MED1) peaks and marked by H3K27ac and BRD4, showed virtually no E2F binding. Prompt by these observations, we explored the transcriptional and functional interrelationship between E2F and BETs to identify their individual contribution to eventual functional effect in MM. Unbiased hierarchical clustering revealed distinct regulatory axes for E2F and BETs, with E2F predominantly localized to active gene promoters of growth/proliferation genes and BETs disproportionately at enhancer- regulated tissue specific genes confirming that these factors establish distinct target gene programs. At the extremes, we found less than 10% of genes were among the top 500 in BRD4 enhancer signal (*i.e.* SE-regulated) and top 500 E2F promoter signal. We hypothesized that the presence of BETs and E2F in distinct regulatory axes divides active genes in MM into those that can be selectively influenced by BET inhibition or E2F perturbation, but not both. In line with this we have observed that dual E2F and BET inhibition is synergistic for MM cell growth, both *in vitro* and in vivo.
Summary/Conclusions: In conclusions, our results highlight the existence of non-overlapping promoter and super-enhancer-associated dependencies in multiple myeloma, suggesting a sequestered molecular control that may be perturbed in cancer with potential for development of a promising therapeutic strategy.

E1201

ANALYSIS OF THE GENOMIC LANDSCAPE OF MULTIPLE MYELOMA HIGHLIGHTS NOVEL CANDIDATE PROGNOSTIC MARKERS AND DISEASE SUBGROUPS

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Background: In multiple myeloma (MM), next generation sequencing (NGS) has expanded our knowledge of genomic lesions, and highlighted a dynamic and heterogeneous composition. Despite a growing number of cases sequenced, the full potential of NGS studies has not been exploited so far.

Aims: We used a custom target pulldown (TPD) approach on a large cohort of MM samples at diagnosis, with homogeneous treatment and long follow-up, to further our understanding of the landscape of driver lesions in MM and how this can be used to improve prognostication and disease classification.
Methods: We used a custom-designed SureSelect pulldown strategy (Agilent Biotechnologies) to target 246 genes implicated in MM or cancer in general; 2538 single nucleotide polymorphisms; the immunoglobulin heavy chain (IGH) locus. We sequenced unmatched DNA from CD138-purified plasma cells from 418 patients with a median follow-up of 5.4 years using Illumina Hiseq2000 machines. We applied algorithms developed in-house to detect driver genomic events, filtering out potential artifacts and germline variants. We then ranked each mutation on its likelihood of being oncogenic.
Results: We identified 197 driver events including gene mutations, aneuploidies and IGH translocations (IGH-Tx), median of 6 per patient. Gene mutations where found in >99% of patients. At least one oncogenic mutation of a known driver gene previously identified (*KRAS, NRAS, TP53, FAM46C, BRAF, DIS3, TRAF3, SP140, IRF4*) was found in 64%, with a long tail of infrequently mutated genes with uncertain significance. Karyotypic class was assigned in 80% of patients, with 9% of hyperdiploid cases also showing an IGH-Tx (mostly t(4;14)). IGH-Tx and aneuploidies dominated the MM genomic landscape, KRAS and NRAS being the only point mutations present in the 15 most frequent driver events. Multivariate analysis by sparse Cox regression highlighted only four driver events with significant prognostic impact for both progression-free (PFS) and overall survival (OS): t(4;14) (HR 1.88, CI 1.25-1.84), amp(1q) (HR2.63, CI 1.92-3.59), del(17p) (HR2.55, CI 1.66-3.92), and rare mutations of *ATP13A4* (HR 0.08, CI 0.01-0.65, mutated in 1.4% of patients). We found a significantly worse prognosis for increasing numbers of driver lesions in each patient (median OS 8.2 *vs* 3.5 years for <5 and>8 driver events, respectively). This was only partially explained by instances of additive effect or interactions between variables, which were very informative but not frequent. To better investigate these findings in the context of the genomic landscape of each case, we applied Bayesian clustering algorithms. The large number of driver events screened led to the identification of three groups: in the largest one, some hyperdiploid and IGH-Tx cases clustered together, suggesting that secondary mutations and CNAs required for tumor progression are often shared between these two subgroups. We then identified two clusters both characterized by significantly lower number of mutations, but with opposing features. One was enriched for IGH-Tx, had the highest number of CNAs overall, showed higher prevalence of amp(1q), del(13), del(17p), *TP53* mutations, and had a shorter median OS of 5.3 years. The other was mostly composed of hyperdiploid cases and showed fewest CNAs and mutations, with a good prognosis (median OS not reached).

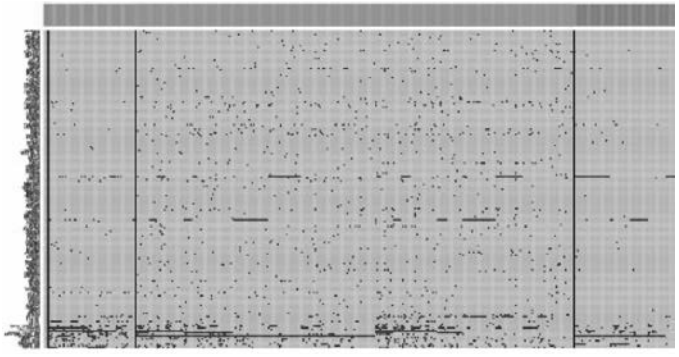

Figure 1.

Summary/Conclusions: We report on the first attempt towards the use of extended tumor genotype for a genomic classification of MM using innovative clustering algorithms. Despite the heterogeneity of the disease, we could identify disease subgroups with a distinct spectrum and number of driver events carrying different prognosis, supporting the introduction of genomics in the clinical approach to MM

E1202

A NOVEL METHOD FOR GENOME-WIDE COPY NUMBER ASSESSMENT FROM TARGETED SEQUENCING DATA AND CLINICAL APPLICATION IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Assessment of gene mutations by next generation sequencing is now standard in patients with haematological malignancy. However, larger chromosomal aberrations (e.g. exon, gene and chromosome level gains and losses) also serve as critical prognostic indicators that guide therapeutic decision making. These larger genetic lesions are typically detected using a separate methodology such as conventional cytogenetics/FISH.
Aims: We aimed to develop and clinically validate a novel method for assessing genome-wide copy number changes using an existing hybridisation-based targeted sequencing panel in order to provide further critical prognostic information in addition to variant-level data without the need for a separate assay.
Methods: A custom Agilent SureSelect capture panel targeting 313 genes of relevance in myeloid and lymphoid malignancies was sequenced on an Illumina NextSeq (paired end 75bp reads) to a mean depth of 700x. An in-house bioinformatics pipeline was created to analyse probe counts from on-target and off-target reads, which also corrects for biases introduced during DNA enrichment and sequencing by normalisation to a pooled reference comprising 10 normal controls. Three metrics for copy number calling were tested including a per-mutation-based statistic from circular binary segmentation, weighted mean and variance for the bins in each segmented region, and an MLPA-like test using read count ratios compared to controls. An interactive web-based graphical user interface was developed to visualise both large-scale and exon level amplification and deletions.
Results: We validated the approach on 45 samples from patients with multiple myeloma (predominantly advanced disease) with known copy number status as determined by conventional cytogenetics, FISH and MLPA. Our novel method detected numerous copy number changes that were outside the targeted region (through genome-wide mapping and analysis of off-target reads) such as del(1p) in 12 patients, gain(1q) in 15 patients and MYC amplification in 5 patients. Moreover our method was able to interrogate and resolve the complexity of changes on del(1p) including isolated deletions of FAM46C, CDKN2C and FAF1. Of 25 patients with a TP53 mutation, 20 had concomitant del(17p) detected by our assay, while 1 case had a del(17p) without mutation; both monoallelic and biallelic TP53 aberration was associated with poor survival. Other findings in this cohort include frequent DIS3 mutations in patients with monosomy 13 and novel oncogenic copy number changes such as the high level amplification of KRAS in 1 case.
Summary/Conclusions: We have developed and demonstrated utility of a reliable workflow for genome-wide copy number assessment that can be implemented using existing targeted short read sequencing data, greatly extending the utility of this technology beyond the identification of mutations in patients with haematological malignancy. In the context of myeloma this can be used to report clinically relevant changes including deletions of 1p and 17p, and gains of 1q and 8q, as well as novel numerical chromosome aberrations.

E1203

THE MULTIPLE MYELOMA GENOME PROJECT: DEVELOPMENT OF A MOLECULAR SEGMENTATION STRATEGY FOR RISK STRATIFICATION OF MULTIPLE MYELOMA

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Background: Segmenting multiple myeloma (MM) into subgroups with distinct pathogenesis and clinical behavior is critical to implement a targeted therapy approach and improve prognosis for patients. Current technologies have elucidated major translocation groups and recurrent copy number changes with varying effects on prognosis. However, minor translocation and mutational groups remain poorly described due to limited sample numbers and small datasets. The availability of multiple sets of high quality genomic data associated with clinical information, cytogenetics, and outcomes provides an opportunity to create an integrative genomic predictor using mutational, chromosomal, and gene expression alterations to develop a classification system to segment MM into therapeutically meaningful subgroups.
Aims: The Multiple Myeloma Genome Project (MGP) is a global collaborative research initiative that aims to develop a molecular segmentation strategy for MM to inform development and deployment of clinically relevant tests that could improve diagnosis, prognosis, and treatment of patients with MM.
Methods: We have established a dataset representing 1766 MM patients for which whole exome sequencing (WES; n=1367), Whole Genome Sequencing (WGS; n=779), and expression data from RNA-Seq and Expression arrays (n=1059) were available. Data were derived from the Myeloma XI trial, Dana-Faber Cancer Institute/Intergroupe Francophone du Myeloma, The UAMS Myeloma Institute and the Multiple Myeloma Research Foundation (IA1 – IA9). Data were investigated for genetic abnormalities following preprocessing with state of the art methods and algorithms.
Results: Our analysis is focused on data from newly-diagnosed MM patients (n=1751), which is the majority of our dataset. We have begun to integrate genomic dataset with various correlates. Based on our data, we have at least

80% power to detect gene expression changes and genomic variants associated in >2% of the study population. WES data identified the main cytogenetic groups, somatic variants, and significantly mutated genes. 28 significantly mutated genes were present in newly diagnosed samples (17 genes in >2% of samples). The main recurrent mutations included *KRAS* and *NRAS*, and negative regulators of the NF-κB pathway; however, novel genes were also identified. Distinct mutational patterns, proportions, and sites between translocation subgroups were found and will be presented. In addition, we detected recurrent copy number abnormalities and examined the interaction with mutations and fusion gene expression from RNASeq. Preliminary analysis with an integrative model developed with machine learning methods/approaches using CN, SNV and structural variants predicted a subset of high-risk patients. Unsupervised molecular classification is in progress to integrate genomic data and define subgroups, which will be presented.

Summary/Conclusions: We have established the largest repository of molecular profiling data in MM associated with clinical outcomes. Integrated analyses are enabling generation of clinically meaningful disease segments associated with differing risk that will inform development of clinical tests. ThemgP intends to build a global network by expanding collaboration with global MM centers to incorporate additional datasets through current and new collaborations.

**The first 6 authors share co-first authorship. The last 3 authors share co-senior authorship.*

E1204

ALVOCIDIB SYNERGIZES WITH VENETOCLAX IN PRECLINICAL MODELS OF MULTIPLE MYELOMA

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Background: With over 30,000 new cases expected in 2016 (US), new treatments are desperately needed for the treatment of multiple myeloma (MM). Major developments in the treatment of MM have included introduction of agents such as lenalidomide, thalidomide, or bortezomib. Bortezomib, an inhibitor of the proteasome, reduces the degradation of many proteins, including the pro-apoptotic protein NOXA. However, high levels of MCL-1 and/or low basal levels of NOXA have been implicated in bortezomib resistance and negative patient outcomes. The BCL-2-specific BH3 mimetic, venetoclax (ABT-199), is also being explored in multiple hematologic malignancies, including multiple myeloma. However, intrinsic resistance to venetoclax treatment observed in MM patient samples has been attributed to a low BCL-2-to-MCL-1 gene expression ratio, suggesting a central role for MCL-1 in cell survival in this context as well. NOXA functions to sequester the anti-apoptotic BCL-2 family member, MCL-1. Increased MCL-1 expression is a known resistance mechanism to venetoclax treatment in a variety of cell types including chronic lymphocytic leukemia and lymphomas. Considering the central role of MCL-1 to treatment efficacy in MM, we investigated the ability of an MCL-1-lowering agent, namely the CDK9 inhibitor alvocidib, to potentiate the activity of venetoclax in MM. Alvocidib suppresses MCL-1 expression via CDK9-mediated regulation of RNA polymerase II. Alvocidib has demonstrated robust improvements in the clinical response rates of high-risk, newly diagnosed acute myeloid leukemia (AML) patients as part of the time-sequential ACM regimen (alvocidib + cytarabine + mitoxantrone).

Aims: We hypothesize that alvocidib would potentiate the activity of venetoclax in MM through an MCL-1-dependent mechanism.

Methods: CellTiter-Glo and Caspase-Glo were used for cell viability and apoptosis assays interrogating alvocidib and venetoclax in cell lines. We performed RT-PCR to measure mRNA levels of MCL-1 and other genes following treatment. Protein levels were interrogated using standard immunoblotting techniques. To determine the efficacy of an alvocidib/venetoclax combination on tumor growth *in vivo*, we performed a mouse study in the OPM-2 xenograft model.

Results: In this report, we demonstrate that alvocidib inhibited the protein expression of MCL-1 in MM cells in a time-dependent fashion, up to 96 hours. In cell viability assays, the addition of up to 100 nM venetoclax resulted in a 2.8-fold reduction in the IC_{50} of alvocidib in the cultured OPM-2 cell line. Conversely, the potentiation of venetoclax activity with the addition of alvocidib resulted in a more than 500-fold decrease in IC_{50} in the relatively venetoclax-resistant OPM-2 cells. Additional studies are currently underway to investigate the efficacy of alvocidib and venetoclax in the context of bortezomib resistance where low NOXA may contribute to enhanced cell survival via MCL-1.

Summary/Conclusions: Taken together, our data suggest that the combination of alvocidib with venetoclax may constitute a novel therapeutic regimen in the treatment of MM. Further, it suggests that CDK9-mediated targeting of MCL-1 may offer a clinical route to addressing intrinsic resistance in MM patients.

E1205

NOVEL COMPOUND, OSSL_325096, INDUCES APOPTOSIS IN MULTIPLE MYELOMA CELLS THROUGH VCP INHIBITION

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Background: VCP (p97) is an ER-associated protein that belongs to the AAA ATPase family. It has a variety of cellular functions including ER-associated protein degradation, autophagy, and aggresome formation. Recent studies have elucidated emerging roles of VCP and its potential as a therapeutic target in several cancer subtypes including multiple myeloma (MM).

Aims: We screened approximately 2,000 small molecular compounds to find out novel small compounds that suppress growth of MM cell lines, and found that OSSL_325096 has strong anti-proliferative activity on MM cell lines (IC50 100-500nM). In this study, we evaluated anti-MM activity of OSSL_325096 through VCP inhibition, in an ATP-competitive manner.

Methods: OSSL_325096 were purchased from Princeton BioMolecular Research, Inc. (Princeton, NJ, USA). His-tagged human *VCP* (*hVCP*) cDNA was cloned and utilized to generate hVCP protein *in vitro* as previously described [Chou *et al., PNAS,* 2011, vol. 108(12): 4834-4839] to evaluate the VCP inhibition by OSSL_325096. For *in vivo* analysis, MM xenograft model mice were intraperitoneally administrated with vehicle or 50mg/kg of OSSL_325096 twice a week.

Results: OSSL_325096 inhibited proliferation of MM cell lines, including one bortezomib-resistant cell line (Figure 1). OSSL_325096 induces apoptosis in these MM cell lines and primary MM cells purified from patients but not in PBMCs from healthy donors. OSSL_325096 treatment leads to accumulation of poly-ubiquitinated proteins, cleavage of caspase-3, and up-regulation of CHOP in MM cell lines (Figure 2), suggesting this compound induces caspase-mediated apoptosis and ER-stress in MM cells. OSSL_325096 has a chemical structure similar to several known VCP inhibitors. Therefore, to evaluate the role of VCP in MM cell lines, we next performed knockdown of VCP. Knock-down of VCP induced apoptosis in MM cell lines, accompanied with accumulation of poly-ubiquitinated protein. In-silico protein-drug binding simulation suggests possible binding of OSSL_325096 to the ATP binding site in VCP's D2 domain. Indeed, in the cell-free ATPase assay, OSSL_325096 showed dose-dependent inhibition of VCP's ATPase activity (Figure 3). The IC50 of OSSL_325096 on ATPase activity was 7-10μM, while IC50 of cell survival in MM cells was 0.1-0.8μM, suggesting that OSSL_325096 may have other anti-myeloma function in addition to VCP inhibition. RNA-sequencing of MM cells treated with OSSL_325096 revealed that several pathways including mTROC1 signaling, TNFα signaling, and unfold protein response, were activated by OSSL_325096. Finally, OSSL_325096 was administered to xenograft mice with MM cell tumors and inhibited the tumor growth *in vivo* (Figure 4).

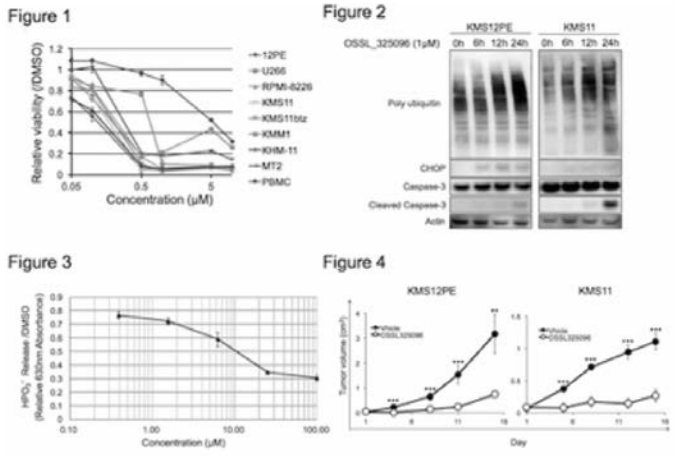

Figure 1.

Summary/Conclusions: The present data suggest that OSSL_325096 may be novel anti-myeloma drug candidate partially through its direct inhibition activity of VCP.

E1206

A NOVEL PREDICTIVE MODEL COMBINING LINCRNAS AND PROTEIN CODING GENES IN MULTIPLE MYELOMA

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Background: RNA has diverse sets of regulatory functions and a recent analysis of RNA repertoire has identified a large numbers of non-coding transcripts. One of which, long intergenic non-coding RNA (lincRNA) with transcripts longer than 200 nucleotides, are located between the protein coding genes and do not overlap exons of either protein-coding or other non-lincRNA genes. lincRNAs have been considered to provide regulatory functions, however, their precise role in cellular biology remains unclear.
Aims: Here, we have studied lincRNAs using uniformly treated patients to show their impact on survival outcome in MM.
Methods: We performed RNA-seq on CD138+ MM cells from 360 newly-diagnosed patients and 18 normal plasma cells (NPM) and analyzed for lincRNA and protein coding genes. MM patient data for clinical characteristics, cytogenetic and FISH as well as clinical survival outcomes were also analyzed and correlated with lincRNA data.
Results: Using only the expressed lincRNAs, we developed a risk prediction signature. The Kaplan-Meier estimates of EFS at 4 years were 53.3% (95% CI, 45.1% to 63.1%) and 32.6% (95% CI, 25.1% to 42.2%), and OS at 4 years were 93.2% (95% CI, 88.9% to 97.6%) and 71.1% (95% CI, 62.9% to 80.3%) in our patients having a low or high risk score. We then combined lincRNA signature with known expression signatures and improved the risk prediction for known expression signatures dramatically. We validated our results on independent large cohort with newly diagnosed MM RNAseq data. When applied to patient cohort separated by other risk categorization including minimal residual disease status (MRD), cytogenetic risk status (del17p, t(4;14) and t(14;16)) and International Staging System (ISS), lincRNA signature was able to further identify patients with significant differential survival outcomes.
Summary/Conclusions: In summary, we report that lincRNAs have an independent effect on survival outcome in MM and provides rational for its use in risk stratification as well as to understand biological impact. Combined risk prediction with other risk features improve the prediction power and helps to create better classification in MM.

E1207

DYNAMIC IMMUNOHISTOCHEMICAL EVALUATION OF MARROW MICROENVIRONMENT MODIFICATIONS IN PATIENTS WITH SMOLDERING MYELOMA

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Background: In most cases, multiple myeloma (MM) is preceded by an asymptomatic status known as monoclonal gammopathy of unknown significance (MGUS) or smoldering multiple myeloma (SMM). The mechanisms of progression from SMM to MM are not well understood. Despite an increasing evidence of an immune system dysregulation in the setting of MM characterized by a tumor tolerating microenvironment, the immunosurveillance profile in the setting of SMM has never been investigated.
Aims: Our aim was to identify a progressive dysregulation of marrow microenvironment in sequential samples of SMM patients. Secondly, we hypothesized a difference in the microenvironment of the patients with progressed SMM *versus* those with stable SMM.
Methods: We performed extensive immunohistochemical analysis of bone marrow samples of 16 patients affected by SMM at time 0 (16 samples) and at +24 months (+/- 4 months, 16 samples). Half of these patients developed MM at 24 months (progressed SMM), the other half remained asymptomatic (stable SMM). Immunohistochemical panel comprised the following markers: microenvironment cell composition (CD138, CD4, CD8, CD3, CD45, CD56, CD68), loss of immunogenicity (PDL1, PDL2, PD1, LAG3, CTLA-4, IDO), loss of antigenicity (HLA-DR). Immunogenicity and antigenicity markers expression was described as percentage on the total of marrow plasma cells and non-plasma cells separately. A first analysis compared the samples of the whole cohort at time 0 and +24 months (32 samples, paired t-test). A linear correlation was performed to study the expression of T cell inhibitory ligands on plasmacells and their respective receptors on marrow non-plasma cells (HLA-DR with LAG3, PDL1 with PD-1). A second analysis compared, at time 0 and +24 months, the samples of stable SMM *versus* progressed SMM (16 samples each analysis, t-test).
Results: In the first analysis, we found a significant increase between time 0 and +24-month samples of CD4+ (11% *vs* 17%, p<0.01), CD8+ (15% *vs* 18%, p<0.01), CD4/CD8 ratio (0.75 *vs* 0.94, p<0.01), PDL1 on plasmacells (1% *vs* 12%, p=0.03), HLA-DR on plasmacells (7% *vs* 10%, p=0.04), HLA-DR on non-plasmacells (29% *vs* 39%, p=0.01), LAG3 on non-plasmacells (4% *vs* 10%, p=0.04). Interestingly, we found a significant correlation between LAG3 and its ligand HLA-DR (r=0.47, p=<0.01). In the second analysis we compared stable SMM *versus* progressed SMM at time 0 and at +24 months. At time 0, we found only an increased CD68-KP1 expression in favor of stable SMM group (28% *vs* 23%, p=0.01). At time +24 months, no differences were observed but an increased plasma cell marrow infiltration in the progressed SMM group (50% *vs* 26%, p<0.01).
Summary/Conclusions: First, we observed an increase in inflamed microenvironment markers (increase in CD4+ and CD8+ cell count in favor of CD4+ population and HLA-DR expression on plasmacells and non-plasmacells) during the course of SMM. Secondly, expression of T cell inhibition markers (PDL1, LAG3) was significantly augmented during disease progression. For the first time, we reported a comprehensive analysis of microenvironment modifications in the setting of SMM with a paired-samples analysis. Features of an immune dysregulated microenvironment were observed. In particular, increased in PDL1 and LAG3 expression could be of clinical interest due to the current availability of checkpoint inhibitors drugs targeting these molecules. The results of this study should be confirmed on prospective studies with larger number of patients.

E1208

IMMUNE CELL PROFILING IN BONE MARROW OF MYELOMA PATIENTS POST AUTOLOGUS STEM CELL TRANSPLANT SHOWS PRESENCE OF CYTOTOXIC CD4 AND CD8 CELLS, WITH PROMINENT LAG-3 EXPRESSION AND OTHER CHECKPOINT MARKERS

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Background: Multiple myeloma (MM) is a plasma cell malignancy that remains incurable, despite therapeutic advances. Immunotherapies have recently shown much promise in this and other cancers, and are under intense investigation. Autologous stem cell transplantation (ASCT) is standard of care in young fit newly diagnosed patients. In the post-ASCT setting, the minimal disease burden and re-constituting immune system may be a favourable context for immunotherapies, including cellular therapy and checkpoint blockade.
Aims: We aimed to characterise the immune landscape in the bone marrow (BM) of MM patients post- ASCT, to identify candidate immune checkpoint proteins for therapeutic targeting.
Methods: BM aspirates were obtained from patients with MM at 3months post ASCT (n=28), and 6-12months post ASCT (n=41) at University College Hospital. Control BM aspirates were collected from healthy volunteers undergoing BM harvesting with the Anthony Nolan. Immunofluorescence surface staining was performed using antibodies to CD3, CD4, CD8, LAG-3, PD-1, HLA-DR, ICOS and the intracellular markers GzmB and Foxp-3. All p-values indicate differences from normal donors unless otherwise stated.
Results: In the post-ASCT BM, absolute numbers of CD8 cells exceeded CD4 cells as early as 3months post-ASCT, suggesting the BM compartment is rapidly filled with CD8 cells. Although absolute numbers of CD4 effectors (CD4+Foxp3-) were either similar (3 months) to or lower (6-12months, p<0.05) than healthy donors, there was a higher proportion of cytotoxic (GzmB+) CD4 cells (3months median 30.4%, range 0.2-89.7%, p<0.01 *vs* control median 2.6%, range 0.2-33.4% and 6-12months, median17.6%, range 0.4-100%, p<0.05). CD4 effectors also expressed activation markers: Inducible co-stimulator (ICOS, 3months median 20.2%, range 2.9-80.6% p<0.05 *vs* control median 8.6%, range 2-22% and 6-12months median 33%, range 1.8-80.7%, p<0.01) and HLA-DR (p<0.05), and high levels of lymphocyte-activated gene-3 (LAG-3, 6-12months median 29.6%, range 12.2-62.1%, p<0.05 *vs* control median 17.9%, range 8.2-32.4%) Significant numbers of CD4 effectors co-expressed LAG-3 and GzmB (p<0.05), as well as LAG-3 and ICOS (p<0.05). Absolute numbers of CD8 effectors (CD8+Foxp3-) in post ASCT patients were not different from healthy donors. Similar to CD4 effectors, a significantly higher proportion of CD8 effectors displayed cytotoxic phenotype (GzmB+, 3months median 88%, range 15.2-98.3%, p <0.0001 *vs* control median 30.3%, range 5.8-71.4% and 6-12months median 81.5%, range 19.4-100%, p <0.0001) as well as increased levels of HLA-DR (p<0.05) post ASCT. CD8 effectors also displayed high co-expression of LAG-3 and GzmB (p<0.05), but overall levels of LAG-3 were not significantly higher than controls. Both CD4 and CD8 effectors expressed PD-1, however, levels were not significantly higher compared to healthy donors. Patients whose BM contained higher levels of activated (and cytotoxic) CD4 effectors also had similar phenotype of CD8 effectors. We correlated immune checkpoint protein expression with disease response in patients at 12months post ASCT. Patients with residual disease (PR) had higher levels of cytotoxic (Foxp-3-GzmB+) CD4 and CD8 effectors (p<0.05) with co-expression of LAG-3 on cytotoxic CD4 effectors, but no differences in PD-1 expression were seen.
Summary/Conclusions: The BM of MM patients following ASCT contains activated CD4 and CD8 effectors, but high co-expression of LAG-3 suggests that these cells may be functionally suppressed. Patients with larger amounts of residual disease have higher numbers of cytotoxic CD4 and CD8 cells, and the co-expression of the checkpoint protein LAG-3 may provide a rationale for blockade of this pathway.

E1209

INHIBITION OF EXTRACELLULAR VESICLE SECRETION INDUCES APOPTOSIS OF BONE MARROW STROMAL CELLS: TOWARDS SOIL-TARGETED THERAPY IN MULTIPLE MYELOMA

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Background: Bone marrow stromal cells (BMSCs) interact with multiple myeloma (MM) cells in the bone marrow, and also create a permissive microenvironment for MM cell growth and survival. Recent evidence indicated that MM cell-BMSC communication via extracellular vesicles (EVs) plays an important role in the MM microenvironment.
Aims: In this study, we investigated the biological property of EVs and miRNAs in EVs derived from BMSCs, aiming to establish the emerging strategies to target MM microenvironment to prevent tumor growth and spread.
Methods: Bone marrow samples were obtained from MM patients (age 56 to 82, n=20) and monoclonal gammopathy of undetermined significance (MGUS) patients (age 44 to 82, n=13) in accordance with the Declaration of Helsinki and using protocols approved by the research Ethics Committee of Tokyo Medical University (IRB No. 2648), and BMSCs derived form MM patients (MM-BMSCs) andmgUS patients (MGUS-BMSCs) were isolated by the classical adhesion method. EVs were isolated from conditioned medium of BMSCs using a Exoquick-TC (SBI). The size of EVs was confirmed using a NanoSight LM10 (Malvren). The RNA from cells and EVs was profiled for 381 miRNAs using a TaqMan low-density array (ABI). For functional analysis of candidate miRNAs, the miRNA mimics (Ambion) were transfected into BMSCs using HiPerFect (Qiagen). Cell viability of miRNA-overexpressed BMSCs were determined using WST-8 (Dojindo), and Apoptosis rates were determined using Caspase-Glo assays (Promega). To assess the effect of the inhibit on EV secretion, BMSCs were treated with 10 μM GW4869 (nSMase2 inhibitor, Sigma) for 48h.
Results: MM-BMSCs andmgUS-BMSCs had a fibroblast-like morphology in culture, and were homogeneously $CD73^+$, $CD90^+$, $CD105^+$, $CD34^-$, and $CD45^-$. MM-BMSCs had a higher expression of α-smooth muscle actin (α-SMA) thanmgUS–BMSCs. The nanoparticle size distribution of EVs derived from BMSCs was approximately 50 nm. We found high expression of miR-10a in the EVs derived MM-BMSCs, while the expression of intracellular miR-10a was low in MM-BMSCs. We therefore hypothesized that low expression of cellular miR-10a might be important for survival of MM-BMSCs; As a result, miR-10a was packaged into EVs, and they were released to the extracellular space. To test the hypothesis, miR-10a mimic was transfected into MM-BMSCs andmgUS-BMSCs. Of note is that overexpression of miR-10a inhibited cell proliferation and induced apoptosis of MM-BMSCs, while the cell proliferation and apoptosis ofmgUS-BMSCs were not affected by the overexpression of miR-10a. We also found that inhibition of EV release with GW4869 promote the accumulation of intracellular miR-10a in MM-BMSCs, and EV-release inhibitor also can inhibited cell proliferation and induced apoptosis of MM-BMSCs.
Summary/Conclusions: Our results provide the possibility that the inhibition of EV secretion induced apoptosis of MM-BMSCs that can support MM cell growth and survival in BM microenvironment.

E1210

SINGLE-NUCLEOTIDE POLYMORPHISM IN THE PBK GENE IS CLOSELY ASSOCIATED WITH MYELOMA CELL PROLIFERATION

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Background: Elevated expression of *PDZ binding kinase (PBK)*, which encodes a serine/threonine kinase, has been reported to be associated with a poor prognosis in a variety of cancers. The public gene expression profiling data also showed that higher expression of *PBK* was related with a poor prognosis in myeloma. However, the molecular mechanisms of *PBK* expression have never been investigated in myeloma.
Aims: The aim of this work was to elucidate *PBK* gene functions associated with myeloma cell growth *in vitro* and *in vivo*.
Methods: Eight human myeloma cell lines including ANBL-6, 8226, OPM2, and KMS-11 were used in this study. The expression levels of mRNA and protein of *PBK* were detected by real-time RT-PCR and western blotting, respectively. The genome sequence of the whole *PBK* gene was determined using the dye terminator method. Knockout of *PBK* was performed using CRISPR-Cas 9 system. A single guide RNA sequence for *PBK* was in exon 3 and *PBK* expression was completely disrupted (Fig. 1). Transfection of the plasmid expressing *PBK* to cells was performed using with the Amaxa Nucleofector system. Cell viability and proliferation were examined by the MTT and colony formation assay. The KMS-11 cells were subcutaneously injected to mice and tumor volumes were observed every 3 to 4 days.
Results: High expression of mRNA and protein of PBK was observed in 8/8 myeloma cell lines. Genome sequencing revealed the rs3779620 polymorphism in the *PBK* exon 5, in which the A to G transition results in the N107S substitution. A/A, A/G and G/G were found in 88, 0 and 12%, respectively. Of note, *PBK* inhibition by CRISPR-mediated knockout enhanced cell proliferation in ANBL-6, 8226, and OPM2 cells, all of which carry $PBK^{A/A}$. Surprisingly, in the KMS-11 cells carrying $PBK^{G/G}$, *PBK* inhibition by CRISPR-mediated knockout suppressed cell growth *in vitro* and in xenograft mice (Fig. 2). Moreover, exogenous expression of $PBK^{G/G}$ augmented cell proliferation in the PBK-deficient OPM2 cells, which carry $PBK^{A/A}$ originally. Furthermore, Thr 9 phosphorylation on PBK was increased in cells expressing $PBK^{G/G}$ compared with those cells expressing $PBK^{A/A}$.

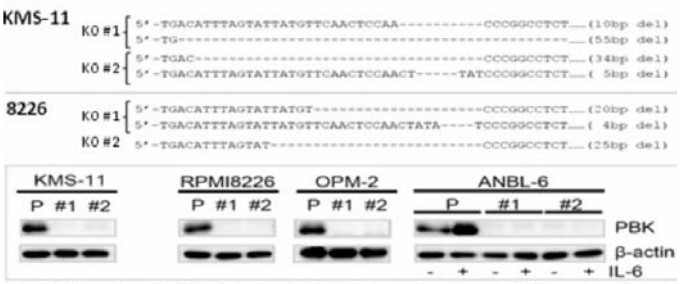

Fig. 1. Knockout of *PBK* using CRISPR-Cas 9 system. *PBK* expression was completely disrupted. 8226, ANBL-6, and OPM2 carry $PBK^{A/A}$ and KMS-11 $PBK^{G/G}$. P; positive control. #1 and #2; clone numbers.

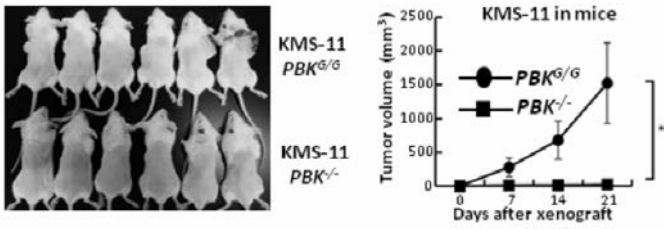

Fig. 2. $PBK^{G/G}$ inhibition by CRISPR-mediated knockout suppressed cell growth in xenograft mice. $PBK^{-/-}$; PBK-deficient cells.

Figure 1.

Summary/Conclusions: Our findings indicate that expression of $PBK^{G/G}$ was associated with myeloma cell proliferation, while $PBK^{A/A}$ was likely linked to tumor suppression. Increased phosphorylation of Thr 9 on PBK might contribute to proliferation in cells with $PBK^{G/G}$. These results provide a novel insight into the mechanisms underlying myeloma cell growth and *PBK* rs3779620 genotype is a potential stratification and therapeutic target for plasma cell dyscrasias.

E1211

THE HISTONE METHYLTRANSFERASES G9A/GLP REPRESENT NEW PROMISING TARGETS FOR THE TREATMENT OF MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) is a clonal plasma cell malignancy which mainly resides in the bone marrow. In cancer cells, the epigenetic landscape is known to be highly disturbed. In line, numerous epigenetic aberrations have been described in MM, resulting in deregulated gene expression, disease progression and drug resistance. Targeting deregulated epigenetic modifiers therefore represents an interesting therapeutic approach. G9a (EHMT2) and GLP (EHMT1) are 2 histone methyltransferases which catalyze mono- and dimethylation of histone 3 lysine 9 (H3K9). Importantly, G9a is overexpressed in several cancers, correlating with a poor prognosis.
Aims: Currently, data about the expression and role of G9a/GLP in MM is lacking. The aim of this study is therefore to investigate the functional role of G9a/GLP in MM pathogenesis.
Methods: The prognostic value of G9a/GLP in terms of overall survival was determined in the UAMS-TT2 cohort of newly diagnosed MM patients (n=345, GSE4581) using Maxstat analysis. In addition, we used a panel of 10 human cell lines, 3 murine cell lines and 5 primary patient samples to evaluate the effect of the small molecule inhibitors UNC0638 and BIX01294 on MM cell viability, cell cycle progression and apoptosis. We also assessed the *in vitro* anti-MM activity of BIX01294 in combination with bortezomib or ABT-199. The *in vivo* anti-MM activity of therapeutic BIX01294 treatment was tested using the murine 5TGM1 model. Difference in overall survival between groups was assayed with a log-rank test and survival curves plotted using the Kaplan-Meier method.

Results: Here we report that high expression levels of both G9a and GLP are associated with a worse disease outcome in newly diagnosed MM patients. Moreover, gene set enrichment analysis of patients with high G9a/GLP expression levels displayed a significant enrichment of genes involved in pathways associated with MM disease progression, including the RAS pathway, NF-κB canonical pathway, IRF4 multiple myeloma program and mRNA splicing. Next, we demonstrated that the specific G9a/GLP inhibitors BIX01294 and UNC0638 significantly and potently reduced MM cell viability *in vitro*. Moreover, both inhibitors also induce cell cycle arrest and apoptosis. When comparing between both inhibitors, BIX01294 was found to be the most potent in induce apoptosis. Mechanistic studies for BIX01294 furthermore indicated that BIX01294 treatment resulted in autophagic programmed cell death as evidenced by a strong increase in the formation of LC3B puncta and an increase in LC3II and beclin-1 protein levels. In addition, we found that BIX01294 sensitizes MM cells to the proteasome inhibitor bortezomib and the Bcl-2 inhibitor ABT199. Lastly, therapeutic treatment of 5TGM1 inoculated mice with BIX01294 resulted in a clear delay in tumor progression, as evidenced by a clear decrease in tumor burden and a significant increase in the overall survival of BIX01294 treated mice compared to vehicle treated mice.
Summary/Conclusions: Altogether, our results demonstrate for the first time the importance of the histone methyltransferases G9a/GLP in MM pathogenesis. Furthermore, we show that specific targeting of G9a/GLP induces MM cell apoptosis, enhances MM sensitivity to ABT- 199 and bortezomib and significantly delays tumor progression in the murine 5TGM1 model. Thus, G9a/GLP targeting represents a promising strategy to improve treatment of MM.

E1212

CYTOTOXIC LYMPHOCYTES IN NEWLY DIAGNOSED MYELOMA HAVE REVERSIBLE FUNCTIONAL AND PHENOTYPIC ABNORMALITIES THAT MAY OFFER THERAPUTIC OPPORTUNITIES

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Background: A bi-directional interaction exists between malignant cells and those of the immune microenvironement. This dynamic relationship results in gradual loss of clonal control associated with loss of cytotoxic lymphocyte (CTL) response. Mechanisms of immune escape are varied and include the induction of immune checkpoint pathways, notably the PD1-PDL1 axis. Multiple myeloma is a disease characterised by a pre malignant phase which can evolve into periods of asymptomatic and symptomatic disease. One possible mechanism for disease progression is progressive loss of immunological control. The malignant plasma cell has multiple potentially immune modifying effects including the expression of PDL1 and induction of a pro-tumour micro-environment. The role of CTLs is less well understood.
Aims: To undertake deep immune profiling of the CTL landscape in myeloma in order to establish whether features of immune dysregulation are present and to identify potential therapeutic opportunities.
Methods: Cryopreserved bone marrow from 18 patients with newly diagnosed and untreated myeloma and 9 controls were assessed using a 36 parameter mass cytometry panel. The panel was designed to assess 9 immune checkpoint regulators, 5 cytokines, and markers of proliferation and degranulation across multiple lymphocyte subsets. Samples were stimulated with CD3 and CD28 to assess functional capacity. Dimensionality reducing clustering algorithmic analysis was used alongside traditional data analysis techniques to identify functional subpopulations characterised by expression of multiple markers.
Results: The cytokine profile in newly diagnosed myeloma is shifted towards a pro-tumour microenvironment with particularly marked elevation of TGFb throughout resting CTLs (36.4% v. 66.2%, p=<0.0001). IFNg production is reduced in the resting myeloma effector population (0.33% v. 0.18%, p=0.0099). Stimulation restores the cytokine profile to match that of controls. Myeloma CTLs retain the capacity to proliferate and produce the constituents for cytotoxic granule formation, however elevated PD1 expression alongside other markers of exhaustion suggests a transition towards an exhausted phenotype is occurring. Strongly PD1 expressing populations in myeloma are larger (26% v.43%, p=0.05) and have increased rates of co-expression of CTLA4 (32%v. 64%, p=0.0015), PDL1 (26% v. 47%, p=0.0198) and TIM3 (34% v. 56%, p=0.0241). Populations of CTLS from myeloma up-regulate expression of the TCR co-stimulatory receptors DNAM1 (74% total CD8), NKG2D (45% total CD8) and OX40 (33% total CD8) following stimulation.
Summary/Conclusions: Clear differences can be identified in the functional and phenotypic features of CTLS in myeloma compared to those of controls.The partial nature of these defects and the fact that reversibility can be demonstrated suggests that these cells have not yet reached the stage of irreversible exhaustion. Taken together this data suggests that targeting immune checkpoint regulators at an early disease stage, in order to optimise immunological function and reverse partial defects, is a viable therapeutic strategy to explore. PD1, PDL1, CTLA4 and TIM are all potential immune checkpoint targets. In addition the expression of NKG2D, DNAM1 and OX40 raises the possibility of activating TCR co-stimulation in order to up-regulate antigen specific CTL activity. Combining immunological blockade with other immune optimising agents may enhance the benefit, leading to greater malignant cell clearance.

E1213

P53-RESTORING SMALL MOLECULE CP-31398 INDUCES APOPTOSIS VIA INDUCTION OF REACTIVE OXIDATIVE SPECIES IN HUMAN MULTIPLE MYELOMA

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Background: Reactive oxygen species (ROS) are normal byproducts of a wide variety of cellular processes. ROS have dual functional roles in cancer cell pathophysiology. At low to moderate levels, ROS act as signaling transducers to activate cell proliferation, migration, invasion, and angiogenesis. In contrast, high levels of ROS induce cell death. In multiple myeloma (MM), ROS overproduction is the trigger for apoptosis induced by several anticancer compounds, including proteasome inhibitors. However, no drugs that mainly affect oxidative stress are currently used for treatment of MM in the clinic. In MM, p53 status is an independent prognostic marker, since patients harboring p53 abnormalities are highly resistant to standard therapies, and the incidence of p53 mutations and deletions increases during disease progression. Therefore, restoration of p53 is an attractive strategy for treating advanced relapsed and refractory MM (RRMM) patients. CP-31398 (CP) is a small molecule that activates wild-type p53 or restores tumor-associated p53 mutants to wild type p53 function in multiple human cancer cell lines; this leads to cell cycle arrest and/or apoptosis. The growth of rhabdomyosarcoma cell lines can be inhibited by p53-dependent induction of ROS, but it is not clear whether CP-induced cytotoxicity proceeds via a similar pathway.
Aims: Our study was aimed at evaluating the anti-myeloma activity of CP.
Methods: MM cell lines (MM1S, RPMI8226, U266, KMS5, OPM2, Delta47, KMS11) and three primary MM samples were treated for 48 h with CP. Subsequently, the inhibitory effect of CP on MM cell line growth was assessed using a WST-1 assay. In order to elucidate the cytotoxic mechanism of CP, immunoblotting and flow cytometry analysis were performed. Measurements of cytosolic and mitochondrial ROS were performed with CellROX Deep Red or MitoSOX Red. For quantification of ROS, cells were analyzed by flow cytometry and fluorescence microscopy. The therapeutic potential of CP was evaluated by its ability to suppress tumor growth *in vivo* using the subcutaneous RPMI8226 murine xenograft model for human MM.
Results: In this study, we have demonstrated that the p53-activating small molecule CP-31398 effectively inhibited the growth of MM cell lines and primary MM isolates from patients with IC_{50} values in the range of 2.51–11.2 μM. CP also suppressed the growth of MM xenografts in mice. Mechanistically, CP was found to induce intrinsic apoptosis in MM cells via increasing mitochondrial and cytosolic ROS production. Interesting, CP-induced apoptosis occurs regardless of the cells' p53 status, suggesting that CP has additional mechanisms of action. In addition, we found that CP acted synergetic with the protease inhibitor carfilzomib (CFZ) in MM cells, providing a framework for further studies of CP alone and in combination with CFZ to improve the prognosis for MM patients.
Summary/Conclusions: Our findings indicate that CP could be an attractive candidate for treatment of MM even in patients with p53 abnormalities; this would satisfy an unmet clinical need, as such patients currently have a poor prognosis.

E1214

TUMOR MICROENVIRONMET TRANSFORMATION FROMmgUS TO MYELOMA IS ASSOCIATED WITH PRO-TUMORAL ACTIVATION OF MESENCHYMAL STROMAL CELLS (MSC)

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Background: A well-recognized feature of MM is the intimate relationship between plasma cells (PC) and bone marrow microenvironment, characterized by a modified extracellular matrix, enhanced angiogenesis and presence of cells with immune suppressive activity, including tumor-associated macrophages and myeloid-derived suppressor cells (MDSC). Recently, we demonstrated that MM-MSC are able to convert normal immature myeloid cells in MDSC contributing to immune-escape mechanisms.
Aims: We hypothesize that MSC derived from Smoldering myeloma (SMM) and MM are in an activated status that promotes tumor growth and and tumor microenvironment trasformation.
Methods: Human peripheral blood mononucleated cells (PBMC) isolated from healthy subjects (HC) were cultured with healthy controls (HC-),mgUS-, SMM- or MM-MSC. After 6 days,. neutrophils (N) were isolated using anti-CD66b magnetic microbeads and were tested *in vitro* for their ability to induce angiogenesis and suppress T cell proliferation.

Results: Only N educated by SMM- and MM-MSC (both from patients at diagnosis, relapsed and refractory) significantly up-regulated Arg1, NOS2 and TNFα and exhibited suppressive effect with a reduction of T cell proliferation ($p<0.001$). By co-culturing educated-N with Human Brain Microvascular Endothelial Cells (HBMEC), we observed increased both tube length and number of branch points only in conditions where HBMEC were incubated with MM-MSC or MMref-MSC educated-N ($p<0.05$). Adding Bortezomib, Lenalidomide or Pomalidomide during co-culture of PBMC with MM-MSC, isolated N showed a significant reduction of pro-angiogenic activity but did not lose immunosuppressive ability. To examine if PC play a role in MSC "activation", before performing co-cultures with PBMC, we pre-treated HS-5 or HC-MSC with MM cell lines. PC pre-treatment drives healthy MSC to activate N in immunosuppressive and pro-angiogenic cells. Implanting of mixtures of fluorescently labeled MM cells and healthy- or MM-MSC into zebrafish, animals coinjected with PC and MM-MSC showed enhanced tumor colonization and growth compared with those injected with PC and healthy MSC.
Summary/Conclusions: Tumor microenvironmet transformation frommgUS to MM is associated with progressive activation of MSC that have a pro-tumoral activity. Indeed SMM- and MM-MSC polarize N in immunosuppressive and pro-angiogenic N (N2) in vitro. In addition, MM-MSC facilitate MM growth *in vivo* confirming their central role in tumor progression.

E1215

LONG TERM CR MULTIPLE MYELOMA PATIENTS STUDIED WITH NEXT GENERATION FLOW SHOW PREDOMINANTLY CURED *VSmg*US-LIKE MINIMAL RESIDUAL DISEASE PATTERNS: A STUDY OF THE GTMM-TUSCAN GROUP FOR MULTIPLE MYELOMA

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Background: CR is a prerequisite for long term responses, progression free survivals, and ultimately overall survivals and cure. In the era of novel agents, many MM patients can achieve stringent CR (sCR), *i.e.* disease disappearance at serological, immunoistochemical level plus negativity of free light chains (FLC). On the other hand most of these patients still will relapse and minimal residual disease (MRD) detection will play a crucial role in the very next future. Recently, two 8 colours tubes panel developed by the EuroFlow Consortium can detect MRD with an increased sensitivity and can be applied as standardized method to study multiple myeloma (MM) patients.
Aims: While many studies have looked at MRD status sequentially and soon after autologous or allogeneic stem cell transplantation with flow or molecular techniques, little is known about long term remission patients (>5-10 years) and in particular if more sensitive techniques such as NGF or NGS can still detect minimal disease in those patients. Aim of the study was to analyse patients with MM in >VGPR with next generation flow at>2 and>5 years of lasting remission.
Methods: Clinical assessment definition of CR status included serum and urine immunofixation, free light chain determination, imaging study with CT-PET, bone marrow aspirate, bone core biopsy. 56 MM patients (M/F= 30/26), were studied with NGF at two GTMM centers between February 2016 and February 2017. 28/56 (50%) patients were in sCR at the moment of the study at a median of 40 months after therapy (range 3-140). 28/56 (50%) patients were in VGPR at study analysis according to new IMWG response criteria. N= 12, 25 and 44 patients had a remission disease >5 years, >2 years, and <5 years, respectively. Two tube assay incorporated 8 antibodies each: CD38, CD56 β2-Microglobulin, CD19, κ Anti-Kappa Anti-Lambda CD45 CD138, and CD38, CD28, CD27, CD19, CD117, CD81, CD45 and CD138 (OneFlow™ *PCST* and PCD, BD Biosciences) and were utilized to detect MRD level with a lyse-wash-and-stain sample preparation protocol by flow cytometry (FACScanto II, BD, Biosciences). Accurate identification of BM plasma cells (PCs) and discrimination between phenotypically aberrant (aPC) and normal PC (nPC) were carried out after acquisition and analysis of >2 x 10^6 cells (Diva 8, BD Biosciences).
Results: MRD+ status was detected in 23/56 (41%) of the patients. 4/12 (2 33%) were MRD positive at >5 years remission (2 sCR, 2 VGPR) (median 96 months range 72 – 186 months); 20/44 (45%) were positive at<5 years of remission (3 CR; 17 VGPR)(median 9,5 range 3 – 46 months). 9/25 (36%) were MRD + after >2 years of remission (2 sCR, 7 VGPR) (median 46 months range 24 – 186 months). As expected being in sCR was correlated with a low MRD+ status 5/28 (18%) (2 patients after >5 years, 3 patients after <5 years). Interestingly looking at long lasting remission, *i.e.* >5 years, the 4/14 patients that resulted MRD+ displayed anmgUS like –plasmacell immunophenotype (prevalence of normal plasmacells *vs* aberrant monoclonal) with a PCn/PCtot ratio of 48%, 95%, 35%, 30%. CT/PET was positive in 22/56 patients. All patients in sCR were CT/PET negative.
Summary/Conclusions: In conclusion NGF showed that MM patients with long remission status can be considered disease free/cured with a high sensitivity method. MM patients that display anmgUS–like phenotype after achieving a CR can have long lasting remissions meaning disease control. Patients in sustained CR after 2 years can have high percentage of MRD negativity. Larger studies are warranted to identify patients who need treatment consolidation or continuous treatment based on MRD+ status *vs* others who could stay treatment free with social and economical benefits.

E1216

THE NOTCH PATHWAY IN THE INTERPLAY BETWEEN MYELOMA CELLS AND ENDOTHELIUM IN THE BONE MARROW NICHE

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Background: Angiogenesis is a hallmark of tumors, and it is a peculiar characteristic in bone marrow (BM) of multiple myeloma (MM) patients. MM is a still incurable disease that strongly depends on interactions with BM microenvironment. Endothelium of MM patients displays malignant behavior as compared to a healthy counterpart (1). MM displays a dysregulation of the Notch pathway due to Jagged ligands and Notch receptors overexpression. This condition brings to the generation of homotypic and heterotypic interaction loops that sustain MM cells. Moreover, Notch pathway represents a bridge in the dialogue with BM resident cells, including osteoclast and BM stromal cells (BMSCs), although its role in the crosstalk of MM and endothelium is still to be clarified.
Aims: The aim of this study is to investigate Notch role in MM crosstalk with endothelium exploiting 2D assays and 3D organoid systems to mimic tumor microenvironment (TME).
Methods: The Notch ligands, Jagged1 and 2, were silenced in the MM cell line RPMI8226 (RPMI8226$^{shJAG1/2}$) using an inducible lentiviral vector carrying two short hairpin RNAs targeting Jagged1 and 2. To mimic the endothelial compartment the human pulmonary arterial endothelial cells (HPAECs) were used and for the stromal compartment, the GFP$^+$HS5 cell line. Matrigel and wound healing assays were set up to investigate Notch role in modulating respectively the angiogenic potential of MM cells co-cultured with HPAECs and HPAEC motility in response to MM-derived soluble factors. To develop a TME-like system, a decellularized extracellular matrix (dECM) was used as a physiologic scaffold for organoid generation. dECM was produced by treating murine fibroblast NIH3T3 with ascorbic acid and was loaded with cells for organoids generation. We evaluated apoptosis of MM cells in single culture and co-culture with BMSCs or HPAECs by flow cytometry.
Results: Matrigel assay of HPAEC co-cultured with MM cells showed that direct contact increased angiogenic potential of HPAEC to form a grid of tubes; this effect is significantly reduced when HPAECs are co-cultured with RPMI8226$^{shJAG1/2}$ cells, indicating a key role of Notch signaling in endothelial stimulation. Wound healing assay demonstrated that Notch signaling affects HPAEC motility, since it is reduced when Jagged ligands are silenced. Concerning the 3D-organoid generation, our results indicate that the handcrafted dECM was a suitable scaffold. Moreover, apoptosis assays indicated that MM cells displayed an increased survival when cultured in the presence of BMSCs, that consistently with their recognized protective role; no significant difference in MM cell apoptosis was observed in the presence of endothelial cells. On the contrary, we have observed that endothelial cells were protected by MM cells suggesting that MM cells improve angiogenesis by preventing endothelial cells apoptosis.
Summary/Conclusions: These results indicate a novel role for Notch pathway in MM-EC crosstalk suggesting that the Notch pathway activation in MM cells can increase their proangiogenic potential. 3D-organoid mimics BM microenvironment and may be used as a novel tool to recapitulate the interactions of BM and tumor cells beyond the animal models.

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E1217

MIR-101-3P REGULATES BONE MARROW STROMA-INDUCED DRUG RESISTANCE IN MULTIPLE MYELOMA CELLS BY TARGETING SURVIVIN AND MODULATING CELL-CELL ADHESION

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Background: In multiple myeloma (MM), bone marrow stromal cells (BMSCs) protect MM cells against cell death by direct or indirect interaction. This phenomenon can partly explain *de novo* or acquired drug resistance in MM. Findings of relevant studies indicate activation of some oncogenic or survival pathways including PI3K/mTOR, Ras/MAPK, NFkB and Wnt. However; the potential regulatory mechanisms and druggable targets have not been clearly elucidated.

Aims: To understrand the role of stomal induced drug resistance and to identify new therapeutic target in myeloma
Methods: GFP-tagged human myeloma cell lines, 8226, U266 and MM.1s, were co-cultured with MM patient-derived BMSCs or HS.5 cells with or without BTZ for 24 h. MM cells in monocultures were used as controls. Co-cultures were then applied to magnetic cell separation to isolate MM cells for downstream analyses including western blotting and mRNA or miRNA qPCR arrays. Furthermore, percent apoptosis of gated GFP^+ cells was determined using FACS. In other experiments, MM cells were exposed to BMSCs pre-treated with Brefeldin-A (BFA) or separated with a transwell (TW) insert. For functional analysis, miR-101-3p was overexpressed using lentiviral transduction and survivin gene was silenced using siRNA. MM cells were then seeded on BMSCs in presence or absence of BTZ. GFP fluorescence-based adhesion, cytotoxicity and annexin-V/PI apoptosis were applied.
Results: qPCR arrays showed that BMSCs up- or down-regulated several mRNAs and miRNAs in MM cells. Survivin (BIRC5) was confirmed to be consistently upregulated in 3 MM cell lines at both mRNA and protein levels. In contrast, miR-101-3p was confirmed to be significantly downregulated by stroma in MM cells. Moreover, suppression of miR-101-3p or upregulation of survivin was reversed partially when BMSCs were pre-treated with BFA but highly significantly when they were separated from MM cells with a TW insert. The same rescue pattern was observed in apoptosis FACS analysis indicating that direct cell-cell adhesion was more effective in BMSC-induced modulations in MM cells. Next we identified that survivin was a direct target of miR-101-3p, overexpression of miR-101-3p suppressed survivin mRNA/protein. As indicator of involvement in stroma-mediated drug resistance, survivin and miRNA-101-3p were not affected by BTZ in MM cell-BMSCs co-cultures compared to monocultures. Furthermore, miR-101-3p overexpression or silencing of survivin increased BTZ-induced apoptosis in MM cells in the absence or presence of BMSCs significantly overcoming stroma-mediated drug resistance. To test whether miRNA-101-3p could also regulate adhesion of MM cells to BMSC, we found that miRNA-101-3p significantly reduced adhesion of MM cells to HS.5 and primary MM BMSCs compared to scrambled control. This finding suggests that miRNA-101-3p regulates cell adhesion-mediated drug resistance (CAMDR) by modulation of MM-BMSC adhesion.
Summary/Conclusions: Our results identify a mechanism whereby BMSCs induce drug resistance in MM cells by upregulating survivin and downregulating miRNA-101-3p which directly targets survivin. Overexpression of miRNA-101-3p or silencing of survivin sensitizes MM cells to BTZ significantly overcoming stroma-induced drug resistance. These findings disclose a role of survivin-miRNA-101-3p axis in regulation of BMSCs-induced BTZ resistance in MM cells, thus provide a rationale to further investigate the anti-myeloma activity of miR-101-3p in combination with BTZ as a potential therapeutic strategy in MM.

E1218

ARQ-197, A SMALL-MOLECULE INHIBITOR OF C-MET, REDUCES TUMOUR BURDEN AND PREVENTS TUMOUR-ASSOCIATED BONE DISEASE IN A MURINE MODEL OF MYELOMA

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Background: The receptor tyrosine kinase c-Met, its ligand HGF, and their signalling pathway, have all been implicated in the pathogenesis of myeloma. In myeloma patients with elevated levels of HGF their prognosis is known to be poor. Therefore, targeting these molecules or their pathway in such patients may be of great benefit. We hypothesised that ARQ-197 (Tivantinib), a small molecule c-Met inhibitor, would reduce myeloma cell growth and prevent myeloma-associated bone disease in a murine model.
Aims: 1. Assess effects of ARQ-197 on myeloma cell proliferation. 2. Assess effects of ARQ-197 treatment on tumour burden in murine models of myeloma and 3. Assess effects of ARQ-197 treatment on myeloma bone disease in murine models of myeloma.
Methods: *In vitro* we assessed the effects of ARQ-197 (0.1563 μM - 5 μM) on myeloma cell proliferation, cytotoxicity and c-Met protein expression in the JJN3 human cell line. *In vivo* we intravenously injected NOD/SCID-γ mice with 10^6 JJN3 cells and 1 week later treated mice with either ARQ-197 (200mg/kg/day, 5 times per week by oral gavage) or vehicle for 2 weeks.
Results: *In vitro* exposure of JJN3 cells to ARQ-197 (0.625 μM - 5 μM) resulted in a significant inhibition of cell proliferation ($p<0.0001$) and an induction of cell death ($p<0.001$), probably caused by significantly [SL1] reduced levels of phosphorylated c-Met. *In vivo* ARQ-197 treatment of JJN3 tumour-bearing mice resulted in a significant reduction in tumour burden ($p<0.001$), where tumour infiltration of the bone marrow was reduced by approximately 43% (96±4.9% vehicle *vs* 55±20% ARQ-197 treatment). ARQ-197 treatment also significantly prevented the formation of myeloma-induced bone lesions ($P<0.001$) and the loss of trabecular bone ($p<0.01$) compared to vehicle treated JJN3-tumour bearing mice. Dynamic histomorphometry showed ARQ-197 treatment prevented significant decreases in the mineralising bone surface ($p<0.001$), the mineral apposition rate ($p<0.01$), the bone formation rate ($p<0.01$), and prevented complete loss of osteoblasts on the cortico-endosteal bone surface compared to the vehicle group.
Summary/Conclusions: In summary, these results suggest that ARQ-197 could be a promising therapeutic in myeloma patients who express high levels of HGF, leading to both a reduction in tumour burden and an inhibition of myeloma-induced bone disease.

E1219

Abstract withdrawn.

E1220

THE GENETIC LANDSCAPE OF THE MURINE 5T MODELS FOR MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) is a plasma cell malignancy which remains incurable in most cases. This is mainly attributed to the large genetic and clonal heterogeneity between patients and within individual patients. The mutational landscape of MM patients has led to the discovery of several potential driver mutations and copy number alterations reflecting this genetic heterogeneity. Genetic lesions affecting RAS/MAPK and NF-KB pathways, apoptosis and cell cycle signaling are the most commonly found in patients. Moreover, mutations disturbing the DNA damage response are linked with poor prognosis.
Aims: The use of suitable murine MM models is important to gain understanding of the functional consequences of the genetic heterogeneity observed in patients. However, to date, the genetic landscape of murine MM models have not been analyzed. Our aim of this study is to analyze the genetic landscape of the 5T murine models for MM.
Methods: In this study, we used the 5T2, 5T33vv and 5TGM1 murine models for MM. As control samples, we used C57Bl/KaLwRij and C57Bl/6J germline DNA. We analyzed the copy-number alterations and the mutational landscape using shallow whole genome sequencing and whole exome sequencing.
Results: Among the tested models, the 5T2 model displayed the most copy number alterations. Over the entire genome, 11% and 17% showed copy number alterations for the 5T33vv and 5TGM1 of which 6% is shared reflecting their clonal relationship. Overall, the copy-number alterations affects genes involved in RAS/MAPK, PI3K/AKT1 and JAK/STAT signaling, DNA damage response, cell cycle and epigenetic regulation. Exome sequencing revealed the presence of 417, 407 and 314 non-synonymous mutations and 8, 14 and 24 indels in the 5T33vv, 5TGM1 and 5T2 models, respectively. Moreover, a statistically significant overlap of mutated genes between the 5T33vv and 5T2 models and multiple myeloma patients from two large cohorts published by Lohr *et al.* and Walker *et al.* was observed ($p<1E-8$). Similar to MM patients, we identified damaging mutations in Trp53, Rb1, Pik3ca, Fat3, Kdm6a and Nf1.
Summary/Conclusions: In summary, our results show that the disturbed genetic landscape of the 5T models shows heterogeneity and a partial overlap with multiple myeloma patients. The genetic defects affect pathways known to be involved in multiple myeloma cell survival. The 5T models thus represent reliable models to study the characterized genetic defects.

E1221

CHARACTERIZING THE CONTRIBUTION OF BONE MARROW STROMA-DERIVED IL-6 TO MYELOMA GROWTH AND RESISTANCE

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Background: The bone marrow niche is a specialized microenvironment, which allows for the survival, growth and differentiation of hematopoietic stem and progenitor cells. This niche also provides the optimal growth conditions for hematological malignancies, such as multiple myeloma (MM). A complex interaction between cytokines, adhesion molecules, cell receptors and their ligands provides the MM plasma cells with survival signals and contribute to therapy resistance.
Aims: To unravel the role of the bone marrow mesenchymal stem/stromal cells (BMMSCs) in MM cell growth, progression and drug resistance.
Methods: Hypothesizing that the interaction between MM cells and the BMMSCs is bidirectional, we have compared BMMSCs from healthy individuals, mgUS, and MM patients and used our "humanized" bone marrow-like model to characterize the molecular impact of MM cells on BMMSCs. Finally, we have validated targets by generating HS-5 knock-out lines using CRISPR/Cas9 targeting.
Results: Analyzing the BMMSCs of healthy individuals,mgUS, and MM patients, as well as BMMSCs impacted by MM in our humanized bone marrow-like model, allowed us to confirm established disease biomarkers (*e.g.* IL-

6, HGF, IGF and GDF15) and identify novel mediators of MM disease progression and bone disease. To further elucidate the role of IL-6 in BMMSC-induced growth of MM plasma cells and drug resistance, we have established HS5 stromal cell lines that upon CRISPR/Cas9 targeting have reduced or no expression of IL-6. RNA sequencing analysis of these cells revealed IL-6 to be a master regulator of cytokine production (e.g. IL-1B, CXCL8, CSF2 and CSF3). Disruption of the IL-6 gene did not result in a reduced growth rate of the IL-6 deficient stromal cells as compared to wildtype. Using the compartment specific bioluminescent imaging co-culture system, where luciferase gene-marked MM cells are co-cultured with non-marked stromal cells, we have documented a contribution of the stromal cells to both growth and drug resistance to known chemotherapeutics (*e.g.* bortezomib, doxorubicin) of MM cells. Using this same co-culture system we compared wildtype and IL-6 deficient stroma. Although disruption of IL-6 in the stromal cells resulted in a reduced proliferation of MM cells and stromal cell mediated drug resistance, it did not entirely reverse these stroma-mediated effects.

Summary/Conclusions: Taken together these data suggest that although IL-6 is one of the most deregulated genes in MM-derived BMMSCs, it certainly is not the sole contributor to BMMSC-induced MM cell growth and drug resistance.

E1222

THE PAN-PIM KINASE INHIBITOR, PIM447, POTENTLY SYNERGIZES WITH POMALIDOMIDE PLUS DEXAMETHASONE IN PRECLINICAL IN VITRO AND IN VIVO MODELS OF MULTIPLE MYELOMA

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Background: PIM kinases are a family of serine/threonine kinases recently proposed as therapeutic targets in multiple myeloma. Recent work from our group has shown the dual antimyeloma and bone-protective effects of the pan-PIM kinase inhibitor, PIM447, and its *in vitro* synergism with current standards of care. Since myeloma remains an incurable disease, the preclinical evaluation of new drug combinations is of utmost importance, in order to support the development of future clinical trials. In this scenario, effective all-oral combinations are particularly attractive.

Aims: The aim of the present work has been the evaluation of the efficacy and mechanism of action of the all-oral triple combination PIM447 + pomalidomide + dexamethasone in preclinical *in vitro* and *in vivo* models of multiple myeloma.

Methods: *in vitro* cytotoxicity of PIM447, pomalidomide and dexamethasone alone or in double and triple combinations was evaluated on myeloma cell lines. The combination index (CI) was calculated with Calcusyn software based on results from MTT assay. Effects on apoptosis and cell cycle were evaluated by flow cytometry. Glucose uptake was analyzed by incubation with 2-NBDG. The mechanism of action was explored by analysis of different protein levels by western blot. Finally, a plasmacytoma model in CB17-SCID mice was employed for *in vivo* studies.

Results: Triple combination PIM447 + pomalidomide + dexamethasone showed a strong synergism (CI<0.3) in MM1S and RPMI-8226 cell lines. The clear reduction of cell viability promoted by this combination was due to induction of apoptosis and cell cycle arrest at G_0-G_1 phase. Accordingly, cleavage of caspase 3 and PARP, as well as reduction of cyclin D2 was observed by western blot. In addition, triple combination inhibited mTORC1 as shown by decreased levels of p4EBP1 and pS6RP. Interestingly, treatment with PIM447 + pomalidomide + dexamethasone remarkably reduced the levels of the glucose metabolism-associated enzime hexokinase II and also reduced glucose uptake by cells. Finally, the efficacy of this combination was confimed in a plasmacytoma model in CB17-SCID mice, where it clearly reduced tumor growth as compared to single and double treatments.

Summary/Conclusions: Our preclinical data suggest that myeloma patients could benefit from treatment with the triple combination PIM447 + pomalidomide + dexamethasone and would support future clinical trials with this combination.

E1223

EXPRESSION OF CD38 AND ECTOENZYMES OF THE ADENOSINERGIC PATHWAYS IN MYELOMA BONE NICHE: A RATIONAL BASIS FOR THE USE OF DARATUMUMAB TO TARGET OSTEOCLAST FORMATION IN MULTIPLE MYELOMA

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Background: Bone disease is the hallmark of multiple myeloma (MM). It is known that MM cells express CD38 and that a recently developed human anti-CD38 monoclonal antibody, Daratumumab (DARA), induces and mediates MM cell killing. However, the expression of CD38 and other functionally related ectoenzymes in the bone niche of MM patients and the potential effects of DARA on bone cells are still unknown.

Aims: The aim of this study was to define the expression profile of CD38, CD31, CD39, CD73 and CD203a on MM and bone niche cells. Then, the effect of DARA on bone cells was evaluated by *in vitro* osteoclastogenesis.

Methods: In order to evaluate the expression profile of CD38 and the ectoenzymes, we firstly performed immunohistochemical analysis on bone biopsies in a cohort of 37 patients with MM and 14 with monoclonal gammopathy of uncertain significance (MGUS). The same antigens were analyzed by flow cytometry on primary MM cells, mesenchymal stromal cells (MSC), osteoblasts (OB), monocytes and osteoclasts (OC). Then, we tested DARA effects, in the presence or absence of *All-trans* retinoic acid, compared with human IgG isotype control, on OC differentiation from either $CD138^-$ cell fraction or purified MM bone marrow (BM) $CD14^+$ cells. We also investigated the effect of microvesicles isolated from a MM cell line treated with DARA or the human IgG isotype control, on OC differentiation.

Results: MM cells showed a high expression of CD38 and were positive for CD31, CD39, CD73 and CD203a at variable levels. However, we did not find any significant difference in the expression of CD38 and related ectoenzymes between MM andmgUS patients. CD38 was expressed by monocytes and early OC progenitors but not by OB, mature OC and MSC, that were positive for CD73 and CD203a. Indeed, CD38 was lost during OC differentiation. Consistently, we found that DARA reacts with CD38 expressed by monocytes and its binding inhibits early *in vitro* osteoclastogenesis from total mononuclear cells. *All-trans* retinoic acid treatment increased the inhibitory effect of DARA on OC formation.

Summary/Conclusions: Our data suggest that DARA inhibits osteoclastogenesis, targeting monocytes and early progenitors. These observations provide a rationale for the use of an anti-CD38 antibody-based approach as treatment for MM-induced osteoclastogenesis.

E1224

TRIM33 IS A POTENTIAL TUMOR SUPPRESSOR IN MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) continues to be an incurable plasma cell neoplasm, regardless of recent therapeutic advances. The success of proteasome inhibitors in MM validates the ubiquitin proteasome system (UPS) as a therapeutic target. Using a UPS-specific microarray (PIQOR) we identified aberrant expression of an E3 ligase TRIM33 (tri-partite motif containing protein 33) in MM. TRIM33 has previously been identified as a tumor suppressor in chronic myelomonocytic leukaemia and hepatocellular carcinoma.

Aims: The aim of this study was to examine TRIM33 expression and to investigate its role as a potential tumor suppressor in MM.

Methods: Western blotting and qPCR were used to analyse TRIM33 expression at basal level and following knockdown in four MM cell lines representing a range of MM translocations; JJN3 t(14;16), U266 t(11;14), KMS-18 t(4;14), OPM-2 t(4;14). TRIM33 knockdown was performed using shRNA pLKO lentiviral plasmids. CellTiter-Glo® was used to determine cell viability following knockdown and drug treatments. Partek ®Genomics Suite was used to analyse publically available datasets to look at TRIM33 expression and correlation with survival in subsets of newly diagnosed MM; GSE19784 (N=320) and GSE2658 (N=551). qPCR was used to validate the changes in expression of the TRIM33 gene signature.

Results: Compared to normal bone marrow, lower expression of TRIM33 was observed at both gene and protein level (p=0.03) in the t(4;14) cell lines, KMS-18 and OPM-2. Conversely, expression was found to be high in the non t(4;14) cell lines, JJN3 (p=0.001) and U266 (p=0.015). Knockdown of TRIM33 expression did not alter cell viability in the t(4;14) cell lines. However, cell viability was significantly increased in JJN3 (P<0.04) and U266 (P<0.05). Analysis of a publically available dataset, GSE19784, showed lower levels of TRIM33 present in patients with a t(4;14) compared to other MM subtypes, particularly t(6;14) (p≤0.004) and hyperdiploid cluster (p≤0.03). Low TRIM33 expression has also been associated with poor overall survival (GSE2658; p=0.00034). Forty-seven genes associated with TRIM33 expression in non t(4;14) MM were identified; these genes were analysed using QUADrATiC tools connectivity mapping to identify FDA approved agents predicted to enhance the TRIM33 gene signature. One of the top enhancers identified was the tyrosine kinase inhibitor (TKI) Imatinib. The OPM-2 cell line showed greatly increased sensitivity to Imatinib compared to other cell lines (IC50: 0.8 μM *vs* 20 μM). Similar effects were observed with second generation TKIs, Dasatinib (IC50: 0.003 μM *vs* >1μM) and Nilotinib (IC50: 0.08 μM *vs* >1μM). An increase in genes associated with TRIM33 expression in non t(4;14) subtypes was also observed following Imatinib treatment.

Summary/Conclusions: We have shown that TRIM33 exhibits lower expression in t(4;14) cell lines, compared to non t(4;14) cell lines and that knockdown of TRIM33 increased the viability of non t(4;14) cell lines. This suggests that TRIM33 may act as a tumor suppressor in MM and that expression is dysregulated in a subset of MM. Connectivity mapping identified Imatinib as an

enhancer of the TRIM33 signature that potently decreased the viability of the OPM-2 cell line. This study suggests that enhancing the TRIM33 gene signature could potentiate the tumor suppressive effect of TRIM33 and identify novel therapies for this subset of MM.

E1225

LONG NON-CODING RNAS EXPRESSION HETEROGENEITY AND FUNCTIONAL INVOLVEMENT IN MULTIPLE MYELOMA

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Background: Increasing amount of evidence indicates that deregulation of long non-coding RNAs (lncRNAs) is a common feature of cancer and therefore, its investigation may uncover new molecular oncogenic mechanisms. In multiple myeloma (MM), altered expression of small number of lncRNAs has been associated with decreased disease-free and overall survival, suggesting that these elements may play a more important role in MM than previously anticipated. Nevertheless, an extensive high-throughput analysis that characterizes the deregulation of lncRNAs in MM has not yet been performed.
Aims: We aim to characterize the lncRNA transcriptome of MM and its heterogeneity, and determine whether altered lncRNAs have a functional involvement in this disease.
Methods: Paired-end strand-specific RNA sequencing (ssRNA-seq) was performed in 38 purified plasma cell (PC) samples from MM patients, as well as in 5 tonsil PCs (TPCs) and in 3 bone marrow PCs (BMPCs) of healthy donors as controls. We also performed ssRNA-seq of populations from B cell differentiation (Naïve, Germinal Center, Memory and PC). To study the heterogeneity of lncRNAs expression we performed sample level enrichment analysis (SLEA), in which each individual lncRNA was compared to BMPCs. To determine the epigenetic regulation of lncRNAs we used whole-genome bisulfite sequencing and CHIP-seq. shRNA-mediated knockdown using 2 different shRNAs and MTS (cell proliferation) and annexin V (cell death) assays were utilized to study the functional effect of lncRNA overexpression.
Results: We identified 40.552 novel lncRNAs in MM samples that were present in at least 3 of the 38 patients. Principal component analysis demonstrated that TPCs and BMPCS cluster separately, suggesting that, in spite of being the same cell type, their transcriptomes are very different. We observed that the expression of lncRNAs was more heterogeneous than that of coding genes. More importantly, SLEA showed 11.067 lncRNAs that were overexpressed and 5.601 underexpressed in >40% of patients. Thus, the number of deregulated genes analyzed by SLEA was much larger than the 70 lncRNAs that appeared as deregulated when all MM were compared to BMPCs, demonstrating the relevance of studying the heterogeneity in this disease. To determine the functional role of heterogeneously altered lncRNAs in the biology of MM cells we focused on the study of *LINC-SMILO* (*Specific Myeloma INtergenic LOng noncoding RNA*), a lncRNA that it is overexpressed in ~ 40% of MM patients and not at different stages of B-cell differentiation. DNA methylation analysis demonstrated that CpGs located upstream of *LINC-SMILO* showed a significant hypomethylation inmgUS, that was even more pronounced in MM samples. We also have observed a gain of active chromatin modifications in the promoter region of *LINC-SMILO* in MM patient samples. These data suggest that epigenetic mechanisms, namely DNA hypomethylation and the gain of active histone modifications, may be the cause of *LINC-SMILO* overexpression in MM. Knockdown of *LINC-SMILO* in 3 different cell lines (MM.1S, MM.1R and KMS-11) resulted in reduced proliferation and induction of apoptosis, indicating this lncRNA is essential for the survival of MM cells.
Summary/Conclusions: All together, these data demonstrate that alteration of lncRNAs is an important and unexplored feature of MM. Moreover, overexpression of *LINC-SMILO* is required for the survival of MM cells and could represent a potential therapeutic target for the treatment of this disease.

E1226

ROLE OF EPHA3 IN MULTIPLE MYELOMA: A PERSPECTIVE FOR A NOVEL TARGET THERAPY?

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Background: The tyrosine kinase Eph receptor A3 (EphA3) has recently emerged as a potential therapeutic target, since it resulted to be overexpressed in many cancers, including some hematological malignancies (Keane *et al.* 2012). Furthermore, EphA3 has been found overexpressed not only in neoplastic cells, but also in the microenvironment of different human cancers, where its targeting inhibits tumor growth by disrupting supportive stroma and vasculature (Vail *et al.* 2014).
Aims: Due to the absence of relevant information about the role of EphA3 in multiple myeloma (MM), we aimed to evaluate the expression of this molecule in primary bone marrow plasma cells (BMPCs) from MM patients and MM cell lines compared to healthy controls (HCs). In addition, using a "loss of function" approach by mRNA silencing and an anti-EphA3 monoclonal antibody (EphA3mAb), we studied *in vitro* plasma cells (PCs) viability and movement. Finally, we analysed the *in vivo* effects of EphA3mAb in a MM mouse xenograft model.
Methods: EphA3 mRNA and protein where investigated in 15 MM BMPCs, 11 MM cell lines and 10 HCs by qRT-PCR and flow cytometry. The effects of EphA3 targeting by lentiviral RNA silencing (shRNA) and anti-EphA3mAb on PC trafficking and viability were studied by adhesion assay on fibronectin and on bone marrow stromal cells (BMSCs), invasion assays and proliferation MTS assay, respectively. Gene expression profiling (GEP) was performed in shEphA3 *versus* shControl cells. Furthermore, the effects of EphA3mAb were analysed in a MM xenograft model by measuring tumor size and by assessing angiogenesis, proliferation and apoptosis rate on tumor biopsies using immunohistochemistry (anti-CD31, anti-ki67 and TUNEL assay, respectively). Statistical significance was determined by the t-test or One-way ANOVA analysis.
Results: EphA3 was found overexpressed in primary MM BMPCs and MM cell lines when compared with HCs (figure 1A-B). The EphA3 loss of function by siRNA and by EphA3mAb significantly inhibited *in vitro* the ability of MM PCs to adhere to fibronectin, to BMSCs and to invade (figure 1C-E), without affecting cell proliferation and viability (data not shown). GEP showed that knockdown of EphA3 modulated some molecules that regulate adhesion, migration and invasion processes. Importantly, the treatment with EphA3mAb *in vivo* significantly reduced tumor size and inhibited angiogenesis, as revealed by decrease of CD31+ vessels at immunohistochemistry (data not shown).

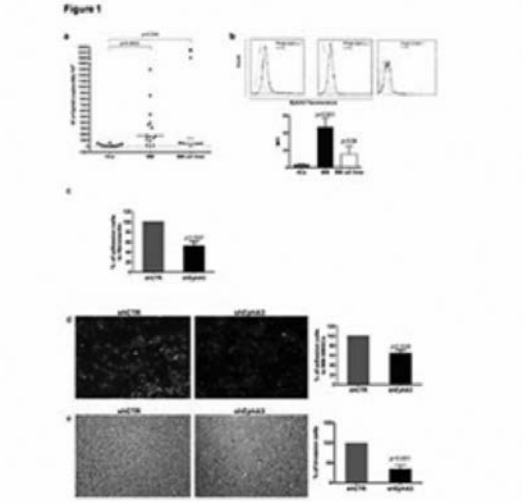

Figure 1. EphA3 expression was verified by absolute quantitative qRT-PCR (normalized to Abelson=ABL) in bone marrow plasma cells (BMPCs) from 5 healthy controls (HCs), 15 MM patients and 11 MM cell lines (◆ SKMM1, ● KMS-11, ▼ KMS-12-BM, ◇ KMS-18, * KMS-34, ⁄ KMS-28, ▲ MM1S, ■ JJN3, ⁷ U266, ⁻⁺ RPMI-8226, □ OPM-2; β-actin = loading control; (lines inside scatters represent the median values)] (a) and by cytometry analysis in PCs from a representative MM patient, HC and MM cell line (b). Mean fluorescence intensity (MFI) as mean ± SD of 3 HCs, 5 MM BMPCs and 4 MM cell lines. shEphA3 and shCTR cells were tested in adhesion to fibronectin (FN; c) and to MM bone marrow stromal cells (BMSCs; d) and Matrigel® invasion test (e). Data are presented as percentage of the respective controls (mean ± SD of triplicates from 3 independent experiments) for viability, proliferation, adhesion to FN and invasion assays. For the adhesion to BMSCs data are presented as percentage of the respective controls and are shown as means ± SD of n=3 experiments on 3 BMSCs by analysing five random fields for each experiment.

Summary/Conclusions: Our findings suggest that EphA3 is a novel regulator of MM PC trafficking, in part via effects on adhesion and invasion; its targeting using EphA3mAb inhibits tumor growth, possibly by reducing angiogenesis, though other possible mechanisms of tumor death cannot be excluded. These data, together with the favourable clinical properties of a humanized EphA3mAb reported in a phase I trial on acute myeloid leukemia and myelodysplastic syndrome (Swords *et al.* 2016), support EphA3 targeting as a new potential therapeutic opportunity for MM that would warrant to be further investigated.

E1227

PROGNOSTIC SIGNIFICANCE OF AMP1Q21 IN MULTIPLE MYELOMA

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Background: Multiple Myeloma (MM) is a genetically heterogeneous and complex disease with widely diverging survival times from months to years. Amplification of locus 1q21 (amp1q21) is among the most commonly reported genetic abnormalities in MM, but its prognostic value remains unclarified.
Aims: To define the frequency of amp1q21 in MM and its correlation with other chromosomal abnormalities, clinical course and prognosis.
Methods: In 134 patients (pts) with newly diagnosed MM from December, 2009 to March, 2016, 67 male and 67 female, median age 57 years (30-81), we performed FISH with locus-specific and centromere DNA probes (XL 1p32/1q21, XL IGH plus, XL t(11;14), XL t(4;14), XL t(14;16), XL t(14;20), XL t(6;14), XL cMYC BA, XL 5p15/9q22/15q22, XL P53 (MetaSystems), D13S25 (Cytocell). Induction therapy with bortezomib-based courses was initiated for 131 pts, 3 pts with smoldering MM remained under observation. Response was evaluated according to the IMWG criteria for 127 pts, because 4 pts died in induction. 48 pts were underwent ASCT. The median follow-up of group was 19.3 months (3,2 – 77,4). Progression was diagnosed in 69 pts, in 12 of them FISH-analysis was performed also in disease progression.
Results: Chromosomal aberrations were revealed in 133 of 134 (99%) pts. TIgH/14q32 were detected in 42.5% (57/134), hyperdiploidy in 57.5% (77/134), hypoploidy in 2.4% (3/134) pts. In 11.2% (15/134) a concurrent tIgH/14q32 and a trisomy were found. The IgH translocations t(11;14), t(4;14), t(14;16), t(14;20), t(6;14) were observed at a frequency of 16.4%, 12.7%, 3.7%, 2.2%, 0.7% respectively, chromosomal partner is not found in 6.7%. Del(13q) was detected in 40.3% (54/134), del(17p) in 12.7% (17/134), tcMYC/8q24 in 17.2% (23/134). Amp1q21 was detected in 39.6% (53/134). We identified 3 copies of 1q21 in 32 (60.4%) and>3 copies 1q21 (4-7) in 21 (39.6%) pts. Cases with amp1q21 had a high incidence of del(13q) (OR=2.71 (1.32-5.55); p=0.006) and t(4;14) (OR=4.49 (1.47-13.51); p=0.005), as well as higher LDH levels (OR=2.27 (1.09-4.72); p=0.027). From 12 pts investigated in progression amp1q21 was found in 9 pts (75%): in 2 cases amp1q21 was not found at diagnosis and was revealed in disease progression only; in 7 cases - amp1q21 was detected at diagnosis and in progression, and its copy number did not change. The difference in response after induction between pts with or without amp1q21 was not statistically significant: CR – 11.8% *versus* 14.5%; VGPR –39.2% *versus* 27.6%; PR –37.2% *versus* 27.6%; therapy resistance 11.8% *versus* 30,3% (p=0.07). Pts with amp1q21 had significantly worse 5-year overall survival (OS) (43.5% *vs* 79.4%; p=0.07). According to copy number of 1q21 the 5-year OS pts carrying 3 or >3 copies of 1q21 were 67.3% and 20.9% (p=0.0016) (Figure 1). On multivariate analysis>3 copies of amp1q21 (HR=4.29, p=0.0094), tcMYC/8q24 (HR=6.51, p=0.0082), del(17p) (HR=3.46, p=0.007) were found to be an independent adverse predictors of shorter OS.

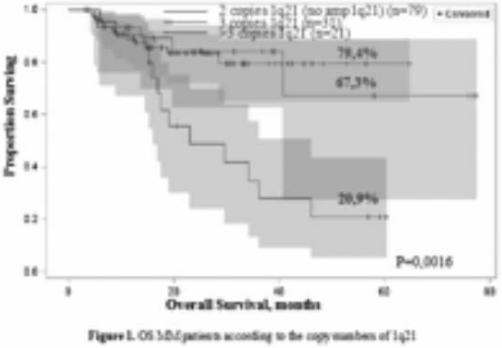

Figure 1.

Summary/Conclusions: Our results show that amp1q21 has a significant impact on OS MM pts in cases of more than 3 copies of locus only. In cases of 3 copies of 1q21 OS pts is comparable with OS in group without amp1q21. Amp1q21 can appear in the course of MM, therefore FISH-analysis of locus 1q21 should be performed at diagnosis, as well as in disease progression.

E1228

ADAPTIVE IMMUNE RESPONSE IN PLASMA CELL DYSCRASIAS: IMMUNE PROFILING AND DETERMINATION OF CIRCULATING B CELL LEVELS AS A SURROGATE ASSAY FOR BONE MARROW TESTING

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Background: Immune paresis is commonly identified in patients with plasma cell dyscrasias (PCD). Often, in newly presenting multiple myeloma (MM), it is associated with intractable infections for which the patient first seeks medical help. Furthermore, recent evidence suggests the importance of assessing levels of bone marrow (BM) derived B cells for risk stratification of MM patients as reduced levels of B-cells in the BM have been associated with poorer outcomes and reduced progression free survival[1]. This cellular measure of adaptive immune function (ie: B cell enumeration) is, however, seldom analysed in the peripheral blood (PB) of patients with PCD.
Aims: This study was designed to examine measures of the adaptive immune response in PCD patients, by measuring relative and absolute numbers of T, T cell subset, B, NK and NKT cells at different stages of PCD, and to determine if the PB B-cell component can act as a surrogate marker for B cell enumeration in the BM.
Methods: PB and BM lymphocyte subset analysis was performed on samples obtained from a range of PCD patients (n=70) using directly conjugated monoclonal antibodies (MAB) and multicolour flow cytometry, carried out on a FACSAria III cell sorter (BD, Oxford, UK). Serum protein electrophoresis was performed to identify and quantify paraprotein, and uninvolved Ig levels were quantified immunoturbidometrically. sFLC were estimated using the Freelite assay on the SPAplus instrument (Binding Site, Birmingham, UK).
Results: Data is presented on 102 PB samples obtained from 70 PCD patients at different stages of disease, including monoclonal gammopathy of undetermined significance (MGUS), smoldering myeloma (SMM), and MM at diagnosis (MMD), throughout treatment (MMT) and at relapse (MMR). Quantification of circulating lymphocyte subsets showed reduced, absolute, numbers of B cells (56/102), T cells (19/102), T_H cells (32/102), CTLs (17/102), NK cells (32/102) and NKT cells (72/102). Furthermore, these reduced B cell levels were more frequently seen in the MMD and MMT groups (50% of samples) compared with the other PCD groups (10-25% of samples). Lymphocyte subset analysis was also performed on paired PB and BM samples from 14 patients with MM and a significant, positive, correlation was seen between relative numbers of B cells in both PB and BM ($p<0.0001$, $r^2=0.94$). No clearcut correlations were found between reductions in uninvolved Ig or sFLC levels, and numbers of cells involved in the adaptive immune response.
Summary/Conclusions: The results presented here are further evidence of immune paresis in PCD with specific effects seen at the cellular level. The highest frequency of reduction was in B lymphocytes and NKT cells, in keeping with reduced levels of circulating uninvolved Ig levels, followed by T cells, particularly T_H cells which have a crucial role in B cell Ig production. Relative B cell levels in BM were significantly correlated with B cell levels in PB and we suggest that monitoring of B cell levels in the PB of PCD patients may serve as a surrogate assay for enumeration of B cells in BM.

References

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E1229

NOVEL MONOCLONAL ANTIBODY THERAPY TARGETING CD26 IN MULTIPLE MYELOMA

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Background: Bone disease is a hallmark of multiple myeloma (MM) and targeting osteoclasts (OCs) to alleviate bone destruction is a component of the standard care for MM. CD26 is a 110-kDa cell surface glycoprotein with DPPIV enzyme activity and has well-defined roles in T cell activation and several tumor developments, including malignant lymphoma. However, little is known about the role of CD26 in regulating bone remodeling.
Aims: In this study, we examine the CD26 expression in human normal OCs and OCs of MM patients. We explore the function of CD26 in osteoclastgenesis (OCG) and investigate the effects of humanized anti-CD26 monoclonal antibody (CD26mAb) on human OCG. We further define the molecular targets of CD26 signaling cascade in OCG and explore the therapeutic potential of CD26mAb for treating MM.

Methods: Human BM-MNCs derived from normal human subjects or MM patients were cultured with M-CSF plus sRANKL with or without CD26mAb for OC formation for TRAP staining and functional assay. To assess the mechanisms of action of CD26mAb on OCG, RANK signaling proteins were examined by immunoblotting.
Results: CD26 is expressed on normal human OCs and is intensely expressed on activated OCs in MM. M-CSF and sRANKL induced human OC differentiation, in association with CD26 expression on monocyte-macrophage lineage cells. CD26 expression was accompanied by increased phosphorylation of MKK3/6 and p38MAPK, which is crucial for early human OC differentiation with its downstream activation of microphthalmia-associated transcription factor (mi/Mitf), which plays an important role in OC functions. CD26mAb decreased the number of multinucleated OCs (>3 nuclei) by TRAP/CD26 staining and down-regulated the secretion of TRAP-5b and type 1 collagen. It decreased the size of OCs and the number of nuclei per OC, with significantly defective bone resorption activity. It was revealed that in the presence of CD26mAb, when it bound to CD26 on OC precursor cells, only MKK3/6-p38MAPK phosphorylation pathway was specifically, rapidly inactivated and subsequently, its downstream mi/Mitf phosphorylation was persistently inhibited. Thus, OC maturation with its bone resorption was impaired by suppressing the expression of TRAP and OC fusion proteins. In contrast, MKK3/6-p38MAPK-mi/Mitf was not phosphorylated at all in mature OCs after RANKL stimulation, regardless of the absence or presence of CD26mAb. These results suggest that CD26mAb blocked RANKL induced p38MAPK phosphorylation in OC precursor cells, but not in OCs. The activation of other MAPKs including ERK and SAPK/JNK, or NFκB was rapidly induced in response to RANKL both in OC precursor cells and OCs, regardless of the absence or presence of CD26mAb. Moreover, CD26mAb did not directly affect mature OC functions. Next, although CD26mAb did not demonstrate direct inhibition of proliferation of MM cells, to further investigate the role of CD26 in MM cells in the BM, co-cultures of 11 MM cell lines with CD26-stained OCs were performed. We examined the expression of CD26 in MM cells. Although CD26 expression was only slightly detected in any of MM cell lines in mono-culture, CD26 expression level was upregulated in all MM cell lines, co-cultured with OCs by flow cytometry and immunohistochemistry. CD26 protein level in these cell lines was also increased by immunoblotting or ELISA. To further explore the CD26 expression in the BM of MM patients, we performed immunohistochemical staining on decalcified biopsy specimens. CD26/CD138 positive plasma cells were detected around CD26 positive OCs and certain endothelial vascular cells in several cases. Anti-myeloma efficacy of CD26mAb on MM cells, co-cultured with OCs was also revealed.
Summary/Conclusions: Our data imply that the blockade of CD26 signaling with CD26mAb impairs the development of human functional OCs. Targeting CD26 in both OCs and MM cells with CD26mAb may be a promising novel therapeutic strategy in MM-associated bone disease and MM progression.

E1230

KYNURENINE INHIBITS T-CELLS THROUGH THE ARYL HYDROCARBON RECEPTOR AT IDO-POSITIVE TUMOR MICROENVIRONMENT

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Background: IDO is an intracellular enzyme proceed the essential amino acid tryptophan metabolism. Accumulation of tryptophan derivatives such as kynurenine blocks antigen-specific T-cell proliferation and induces T-cell death. A recent study indicated that kynurenine was an endogenous ligand of aryl hydrocarbon receptor (AhR), which is a ligand-dependent transcription factor well known to generate biological responses to dioxins. We previously showed that both IDO expression by tumor cells and high serum kynurenine levels correlate with poor prognosis in non-Hodgkin lymphoma patients. We also revealed that tumor IDO activity can inhibit CD19-specific CAR T-cell therapy through the action of tryptophan metabolites (*Blood*. 2015; 125(25): 3905-3916).
Aims: Therefore, we now focus on the AhR in T-cells to escape from IDO-positive tumor microenvironment.
Methods: We collected PBMCs from healthy blood donors, and bone marrow (BM) samples from multiple myeloma (MM) patients at first diagnosis. The cells were activated with anti-CD3 and anti-CD28 Abs. We measured the expression of AhR in activated T-cells using by flow cytometry. We then studied the effect of kynurenine on the proliferation and apoptosis of T-cells. To evaluate the effect of inhibiting AhR in T-cells, we used AhR inhibitor (CH-223191). Next, to assess the functional effects of tryptophan metabolites on the T-cells, we cocultured T-cells with GFP-transduced wild-type Raji cells or IDO-transduced Raji cells. After 72 hours, cells were stained with CD8 antibody to distinguish tumor (GFP+ CD8–) and T-cells (GFP- CD8+).
Results: We found that the range of AhR expression in activated T-cells were from 4.5% to 38%. We found that kynurenine significantly inhibited the proliferation of T-cells with high expression of AhR. The percentage inhibition with kynurenine 25μM was 33%. While T-cells with low expression of AhR were not inhibited with even 50μM of kynurenine. We found that kynurenine was also associated with increased the apoptosis of T-cells with high AhR, as assessed by Annexin-V staining (percent Annexin-V positive and 7-AAD negative cells in 0 and 25 μM kynurenine was 11.8% and 36%, respectively). We found that CH-223191 could blocked the inhibitory effects of kynurenine on T-cells. We found that the percentage of T-cells with high AhR in culture with IDO negative and positive Raji cells was 46.7% and 61.4%, respectively, while the percentage of T-cells with low AhR was 41.6% and 39.4%. These data showed that only T-cells with high AhR were inhibited at IDO-positive tumor microenvironment. Next, we could generated the activated T-cells from 10 of 12 BM samples from MM patients. We found that there was a positive correlation between the expression of AhR and the proportion of plasma cells in BM (r=0.76, P=0.04) and clinical stage of MM. The mean expression of AhR in T-cells of stage2 and stage3 were 7.3% (3.3-11.6) and 19.5% (11.3-26.6), respectively.
Summary/Conclusions: Kynurenine produced by IDO, induce inhibitory signal in T-cells through the AhR. Anti-PD-1 and anti-CTLA-4 therapies, which block directly the inhibitory signal in T-cells, have been getting some clinical benefits against such as melanoma and Hodgkin lymphoma. Therefore, the AhR in T cells might be a target for IDO-positive hematological malignancies.

E1231

THE ANTI-MYELOMA ACTIVITY OF PERK KINASE INHIBITOR IN TARGETING MORE THAN 50 UPR-RELATED GENES INVOLVED IN THE PROLIFERATION OF MM CELLS

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Background: Due to the immunoglobulin production, multiple myeloma (MM) plasma cells are dependent on the unfolded protein response process (UPR), which controls protein production and ensures its proper translation and folding. A study by Michallet et al (2011) showed that knockdown of one of the three well-known arms of the UPR, PERK (protein kinase R (PKR)-like ER kinase) in MM cells resulted in autophagic cell death. This outcome indicated the important role of PERK activation for the metabolic shift of plasma cell to myeloma cell but also its ability to impede the apoptotic effect. In this study we used a small ATP competitive molecular inhibitor, GSK2606414, that acts by targeting PERK enzyme activity in its inactive DFG conformation at the ATP-binding region, while displaying ≥385 fold selectivity over c-kit, Aurora B, BRK and many other kinases.
Aims: In this study we aimed to use a small ATP competitive molecular inhibitor, GSK2606414, that acts by targeting PERK enzyme activity in its inactive DFG conformation at the ATP-binding region, while displaying ≥385 fold selectivity over c-kit, Aurora B, BRK and many other kinases.
Methods: We initially screened 25 CD138+ MM patients and 6 human myeloma cell lines (HMCLs) for PERK mRNA expression. Our results showed that PERK mRNA is highly expressed in almost all patients (5-10 fold higher than the mean PERK expression of HMCLs).
Results: To test the effect of GSK2606414 on the proliferation of MM cells, 4 HMCLs were treated with different doses of GSK2606414 at two time points (24 and 48 hours). Treatment of cells with 3-30μM GSK2606414 resulted in a dose-dependent inhibition of cell proliferation in all HMCLs ranging for 20-95% reduction of proliferative activity, thus, indicating the dependency of these cells on PERK activity. Treatment with GSK2606414 at 20μM in H929 and L363 HMCLs for 24 hours resulted 25% and 15% increase in apoptotic cells by Annexin-PI staining respectively compared to the untreated cells. However, the most important finding was a significant synergistic effect of GSK2606414 with bortezomib in these cells. Specifically when H929 and L363 cells were treated with 5nM bortezomib in combination with 20μM GSK2606414, synergistic effect was seen where apoptotic cells reached 99% and 77% respectively, compared to bortezomib-treated cells (87% and 42% respectively). In addition, the effect of GSK2606414 in combination with bortezomib in the proliferation of H929 and L363 cells was examined. As seen in the apoptosis assay, pretreatment with GSK2606414 for 2 hours prior to bortezomib treatment resulted in 40% and 30% reduced cell proliferation in H929 and L363 respectively compared to bortezomib only treated cells. Under ER stress conditions, the activation of ATF6 and PERK/eIF2α leads to the induction of ATF4 translation and results in the upregulation of CHOP. To determine the gene target effects of GSK2606414, ATF4 and CHOP mRNA expression levels were determined in H929 cell line after 24 hour of treatment. Treatment with GSK2606414 alone did not alter the expression levels of CHOP but reduced more than 50% the expression levels of ATF4. When combined with bortezomib CHOP and ATF4 levels were reduced 20% and 60% respectively while treatment with bortezomib alone increased the levels of CHOP and ATF4 by 50-100%. Changes in RNA expression of 84 UPR-related genes were analyzed in H929 cells. Specifically H929 cells were pre-treated with GSK2606414 and then subjected to ER stress conditions by treatment with tunicamycin (TM). After 24 hours of treatment, 50 genes were found to be transcriptionally regulated by 5-fold in response to TM-induce UPR activation. Thirty of these genes (ERN1, ERN2,XBP1, DDIT3, CEBPB, PPP1R15A, etc.) were downregulsted by >5 fold, whereas 10 of these genes (HERPUD1, EIF2AK3, CREB3L3, HSPA2, HSPA1B, etc.) were upregulated similarly.

Summary/Conclusions: In conclusion, given the on-target pharmacological effects of PERK inhibitor on MM, development of PERK inhibitors may offer a therapeutic advantage that would affect MM pathogenesis and treatment.

E1232

ENVIRONMENTAL CONTROL OF PLASMA CELL FITNESS IN MULTIPLE MYELOMA: MALIGNANT CO-OPTATION OF ARGININE AS NOVEL IMMUNO-METABOLIC CHECKPOINT

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Background: The bone marrow (BM) environment plays a crucial role in the incurable plasma cell (PC) malignancy multiple myeloma (MM). Our previous work showed that autophagy is essential to sustain long-lived PC fitness, regulating Blimp-1, immunoglobulin (Ig) production and ATP availability (Pengo, 2013) and that MM is addicted to the autophagy receptor SQSTM1/p62 (Milan, 2015). Among putative pro-tumoral players are immunosuppressive BM-derived high-density neutrophils (HDNs), but their role in MM is unknown.
Aims: We hypothesized that in MM HDNs sustain PC fitness sustaining p62 through environmental arginine deprivation.
Methods: We integrated diverse unbiased and hypothesis-driven approaches: (1) gene expression profiles (GEP) of patient-derived circulating HDNs (60 MM, 30mgUS, 30 healthy controls), (2) metabolomic profiling by UHPLC/GC-MS of *ad hoc*-collected BM and peripheral plasma (16 MM, 17 smoldering MM, 30mgUS, 29 controls), and (3) functional and expression *in vitro* studies on human MM cell lines. We validated our observations in primary MM cells using bioinformatic analysis of transcript expression levels detected by RNA sequencing (RNAseq) available from the open-access, public clinical and molecular database, the CoMMpass Researcher Gateway (RG, (https://research.themmrf.org, v IA8, n=549).
Results: *In vitro*, selective and progressive arginine deprivation (range 1000-0 μM) in four MM cell lines (MM.1S, U266, OPM2 and RPMI8826) activated the GCN2/CHOP axis, resulting in increased p62 and Blimp-1 expression, increased ATP availability and immunoglobulin production. Conversely, stable lentiviral p62 silencing significantly reduced Blimp-1 and ATP, and led to complete extinction of MM cell lines within 10 days of culture. Bioinformatic analysis of MMRF-Encompass trial data showed a positive correlation between p62 and Blimp-1 expression (r^2=0.92, p<0.0001). Both high Blimp-1 and p62 transcripts predicted shorter progression-free survival at 24 months (4.56 months *versus* 11.6 months, p<0.0001).
Summary/Conclusions: Taken together, our findings disclose a novel environmental circuit co-opted by MM evolution, whereby immunosuppressive HDNs sustain PC cell fitness through arginine depletion to increase p62 and Blimp-1 via the GCN2/CHOP pathway.

E1233

ESTIMATED GLOMERULAR FILTRATION (EGFR) CALCULATED BY CKD-EPI EQUATION COMBINED WITH THE INTERNATIONAL STAGING SYSTEM PROVIDES A POWERFUL PROGNOSTIC MODEL FOR EARLY MORTALITY IN MYELOMA PATIENTS

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Background: During the last decades, the introduction of autologous transplantation and novel agents has improved early mortality rates (EM, henceforth defined as death within one year after diagnosis) in Multiple Myeloma (MM). However, the incidence of EM remains high. Data relating to prognostic factors for EM in MM are limited.
Aims: The aim of this study was to explore for possible prognostic factors for EM, which could be a useful tool for planning treatment strategy in MM.
Methods: We have studied the medical records of 479 patients with MM (M/F: 258/221, median age: 68 years, range 29-88, IgG: 269, IgA: 123, light chain: 72, non-secretory: 15), diagnosed and treated in our Department between January 2001 and January 2016; 86 patients (18%) had EM. Comparisons of patients' characteristics between the EM group and the rest of the patients, were performed with χ^2, one-way ANOVA and Mann Whitney *U* test. Prognostic factors for EM and overall survival (OS) were studied by using logistic regression and cox regression analysis, respectively; OS was plotted by Kaplan-Meier; p<0.05 was considered as statistically significant.
Results: Patients with EM were more often men with a higher median age; hemoglobin, platelets and albumin were lower whereas β2 microglobulin, lactate dehydrogenase (LDH) and calcium were higher in the EM group compared to the rest of MM patients (p<0.05). The percentage of patients with abnormal estimated Glomerular Filtration (eGFR) calculated by chronic kidney disease epidemiology collaboration (CKD-EPI) creatinine equation (<40ml/min/1.73m^2) was higher in EM group compared with the rest of the patients (69% *vs* 17%, *p*<0.001). In accordance with the International Staging System (ISS), advanced MM stage (*i.e.* ISS3) was observed more often in the EM group compared to the rest (65% *vs* 31%, *p*<0.001). High risk cytogenetics including t(4;14), t(14;16) and del17p were present in 48% of patients in the EM group *vs* 21% of the others (p<0.001). The causes of EM included infection/sepsis: 54%, relapsed/refractory disease: 26%, other causes: 6%. Univariate logistic regression analysis demonstrated that ISS, revised ISS (R-ISS), abnormal LDH, hemoglobin <10g/dl, high risk cytogenetics, and CKD-EPI <40ml/min/1.73m^2 were independent prognostic factors for EM. In the multivariate analysis ISS and abnormal eGFR were the only independent prognostic factors for EM. When we incorporated ISS and eGFR in a single prognostic model (CKD-EPI/ISS) we identified 3 distinct prognostic groups: 1) low risk group including patients with ISS1 and CKD-EPI ≥40ml/min/1.73m^2, 2) high risk group including patients with ISS3 and CKD-EPI <40ml/min/1.73m^2 and 3) intermediate risk group including patients that did not fit in either low or high risk group. The incidence of EM in each group was 8.1% 39% and 15.3%, respectively (OR: 2.8, 95% CI:1.9-4.1, *p*<0.001). Multivariate cox regression analysis of prognostic factors for OS in the whole population demonstrated that CKD-EPI/ISS model was the strongest independent prognostic factor for OS (HzR: 0.38, 95% CI: 0.23-0.6, *p*<0.001).
Summary/Conclusions: Based on our data, the combination of eGFR estimated by CKD-EPI with ISS (CKD-EPI/ISS) represents a powerful independent prognostic model for EM and OS, in the era of novel agents. The markers constituting eGFR and ISS are cheap and available for most of MM patients, therefore the CKD-EPI/ISS prognostic model may have a wide application. Nevertheless, the establishment of CKD-EPI/ISS model requires further validation.

E1234

ACTIVATED AND EXPANDED NATURAL KILLER CELLS FROM MULTIPLE MYELOMA PATIENTS DESTROY TUMOR DRUG RESISTANT CELLS AND CLONOGENIC TUMOR CELLS

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Background: Multiple myeloma (MM) remains an incurable disease. Novel therapeutic strategies targeting drug resistant cells (DRC) and clonogenic tumor cells (CTC) are needed. Our group has conducted a phase I clinical trial with activated and expanded autologous NK cells (NKAEs) in patients with refractory MM with a relevant clinical effect. Likewise, it has been possible to discriminate DRCs in MM by side population (SP) detection.
Aims: The aim of this study was to characterize DRC and to check the activity of NKAEs against these DRCs and CTCs while preserving the hematopoietic progenitor cell.
Methods: Flow cytometry of the side population was performed by Dye Cycle Violet efflux detection to characterize DRC of MM cell lines and bone marrow samples from MM patients. The side population was purified by sorting and characterized by RNAseq. NK cells from MM patients' peripheral blood were obtained and cocultured with the genetically modified K562-mb15-41BBL cells in order to obtain NKAE cells. The activity of NKAE cells against SP was evaluated by time lapse microscopy and the activity against CTCs was evaluated by methylcellulose assay. I*n vitro* safety against CD34+ progenitors was evaluated by time-resolved fluorescence cytotoxicity with europium-TDA and cultures of methylcellulose of healthy bone marrow CD34 + progenitors.
Results: SP cells from both cell lines and samples from different stages of MM showed overexpression of stemness markers. Patient NKAE cells were shown to have much higher adhesion, migration and detection capacity of MM tumor cells than NK cells from the same patient. After SP purification by sorting, NKAE cells were able to detect and destroy the SP cells without significant differences. NKAE cells were also able to destroy CTCs from different MM cell lines. Cytotoxicity studies revealed that NKAE cells did not destroy mononuclear cells from healthy bone marrow, even at maximum ratios of 32:1 the mean cytotoxicity was 1.85% (range 0 - 4.47%). Experiments on CD34$^+$ hematopoietic progenitor cell cultures also showed that NKAE cells do not destroy CD34$^+$ clones from healthy bone marrow, confirming the safety of NKAEs.
Summary/Conclusions: SP cells have molecular characteristics of the tumor stem cell compartment in MM. Likewise, NKAE cells from MM patients could

destroy drug resistant MM cells and clonogenic tumor cells with high efficiency, preserving CD34+ hematopoietic cells, and thus constitute an effective and safe therapy against MM.

E1235

UNMASKING THE RETROTRANSPOSON-ORCHESTRATED PRODUCTION OF SOLUBLE RANKL IN MULTIPLE MYELOMA CELLS

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Background: Growing evidence suggest that production of soluble receptor activator of nuclear factor-kappa B ligand (sRANKL) directly by myeloma cells is causally related to generalized bone loss in multiple myeloma (MM). Notably, sRANKL may be produced either by proteolytic cleavage of membrane-bound RANKL or by alternative splicing of *TNFSF11* gene (*TNFSF11* variant 2, sRANKL mRNA). Recent analysis argues against proteolytic processing of the membrane-bound form being the main mechanism of sRANKL production by myeloma cells. Accumulative data indicate that sRANKL mRNA presents a restricted transcriptional pattern, namely is expressed predominantly in malignant cell types. Accordingly, sRANKL mRNA (over)expression in primary MM cells and human MM cell lines has been validated in three independent studies. Furthermore it was recently demonstrated that sRANKL mRNA proximal promoter and first exon are of retroviral origin, residing within a large genomic cluster of transposable elements (TEs).
Aims: To unmask the TE-shaped transcriptional and epigenetic apparatus impelling the expression of sRANKL mRNA in a cell type- and cell context-specific manner.
Methods: RepeatMasker software was used to reveal the presence of integrated TEs in the genomic segment comprising *TNFSF11*. *TNFSF11* RNA-Seq data, generated by the GTEx project across 51 normal human tissues, were analyzed via GTEx Portal. *TNFSF11* RNA-seq data from 4 bone marrow samples and 8 white blood cells samples, generated from the PRJEB4337 and PRJNA182351 BioProjects, were analyzed via the NCBI portal. *TNFSF11* transcription factor (TF) ChIP-seq data were downloaded from the UCSC Genome Browser Database. Data on *TNFSF11* proximal promoter methylation status in 63 cell lines were downloaded from the HAIB Methyl450 ENCODE track.
Results: RNA-Seq data from 51 normal human tissues show that sRANKL mRNA is expressed exclusively in testis, which is in accordance with the retroviral origin of the transcript. Data analysis from the PRJEB4337 and PRJNA182351 BioProjects further validates the null expression of sRANKL mRNA in normal human bone marrow and white blood cells. Methylation status of sRANKL mRNA promoter in 5 lymphoblastoid cell lines (LCLs) signifies that the retroviral promoter remains heavily methylated in these cell types. *TNFSF11* TF ChIP-seq data show that 5 of 161 TFs can bind to the TE-derived sRANKL mRNA promoter region. Four of the five TFs (EBF1, PAX5, IKZF1, and PU.1) bind to this genomic segment exclusively in LCLs, signifying a cell-type specific transcriptional regulation. Notably, all 4 TFs are known to play a major role in normal and/or malignant lymphopoiesis. Furthermore, IKZF1 and PU.1 represent direct targets of immunomodulatory drugs (IMiDs) for down-regulation.
Summary/Conclusions: Transcription of sRANKL mRNA is driven by a retroviral promoter which remains heavily methylated, thereby inactive, in normal lymphocytes. Epigenetic derepression of this promoter during the course of myelomatogenesis resulting in the (over)expression of sRANKL mRNA by myeloma cells represents a plausible scenario. Should the IKZF1 and the PU.1 TFs act as enhancers of sRANKL mRNA expression, directly contributing to upregulation of sRANKL production in MM, is a tantalizing hypothesis that warrants further investigation especially because this type of transcriptional boost could be intercepted following treatment with IMiDs. That Lenalidomide treatment downregulates the amount of sRANKL in the serum of patients with MM through inhibiting PU.1 expression (Breitkreutz *et al*., Leukemia 2008) is in accordance with the above and further raises the interest on the mechanisms promoting the anti-osteoclastogenic properties of IMiDs.

E1236

THE RATIO OF PATHOLOGICAL PLASMOCYTES, ASSESSED BY 8-COLOR FLOW CYTOMETRY, PREDICTS OF RISK OF EVOLUTION IN MONOCLONAL GAMMOPATHY OF UNKNOWN SIGNIFICANCE AND SMOULDERING MULTIPLE MYELOMA

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Background:mgUS and SM are defined by the presence of a monoclonal immunoglobulin and bone marrow infiltration by plasmocytes, with no associated symptoms of Multiple Myeloma (MM). The risk of development of symptomatic MM justifies identification of the factors associated with an increased risk of evolution. Flow cytometric quantification of the ratio of bone marrow pathological/total plasmocytes (PP/PT) has been reported to be predictive in this context (Pérez-Persona E. *et al.* Blood 2007; 110: 2586–2592).
Aims: We undertook to test this in a single center study.
Methods: All patients undergoing bone marrow evaluation following identification of a monoclonal peak (at diagnosis or during follow-up) during a 7.5year period with a diagnosis ofmgUS (n=154) ou SMM (n=56) and at least 6 months follow-up were analysed by 8-color FC (including 11 antibodies) from fresh whole bone marrow. PP/PT ratios ≥95% were considered high risk. Disease evolution was indicated by a necessity to treat.
Results: The 210_PP/PT ratios were on average 77% (10-100). Amongst the 154mgUS patients, 24 had a ratio >95%, of which 8 (33%) evolved, compared to 9/130 (7%) with a ratio below 95%. Only 2 of these 8mgUS demonstrated other high risk factors (a non-IgG monoclonal peak or a peak at >15g/L). Amongst SMM patients, 22/30 (73%) patients with a high ratio evolved, of which 9 (41%) had a non-IgG peak, compared to 10/26 (38%) evolution in SMM with low PP/PT ratios. The risk of evolution to active MM was significantly higher in patients with a PP/PT ≥ 95%, both in themgUS (p<0.0001) and in the overallmgUS + SMM group (p<0.0001). The delay to treatment start was significantly longer in the PP/PT <95% group, in both themgUS (p<0.0001) and overall (p=0,0004) groups. There was a discordance between PP/PT ratio and disease evolution in 11% (17/154)mgUS patients and 23% (48/210) of the overall group but no other FC markers associated with an increased risk of evolution could be identified.
Summary/Conclusions: We confirm the clinical value of a simple, rapid, two-tubes FC quantification of the proportion of pathological plasma cells in the evaluation of the risk and kinetics of disease evolution inmgUS and SMM. It's use allows identification of patients which require more frequent follow-up.

E1237

ADENOSINE IN THE MYELOMA BONE MARROW NICHE: IMMUNE CHECKPOINT AND KEY PLAYER IN DISEASE PROGRESSION

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Background: The tumor microenvironment is rich in extracellular mono- and di-nucleotides (ATP, NAD) which are metabolized by cell surface ectoenzymes to produce adenosine (Ado), a nucleoside involved in the control of inflammation and immune responses. Multiple myeloma (MM), a plasma cell malignancy that develops within the bone marrow (BM) niche, overexpresses CD38, a molecule with complex functions. As a nucleotide-metabolizing ectoenzyme, CD38 catalyzes the initial disassembly of NAD (to cADPR and ADPR), which is followed by adenosinergic activity, provided that CD38 is operating in the presence of other ectoenzymes (CD203a and CD73).
Aims: To demonstrate that adenosinergic pathways contribute to customize homeostasis in MM.
Methods: Evaluation of the expression of adenosinergic ectoenzymes was assessed by immunohistochemical and flow cytometric analysis on cell lines, and primary myeloma cells and BM biopsies from patients with MM or with asymptomatic monoclonal gammopathies (MGUS and SMM). Having investigated the cell antigenic pattern, the adenosinergic functionality of BM cell populations and of microvesicles (MVs) isolated thereof was analyzed by HPLC to evaluate Ado production.
Results: Using BM plasma aspirates, we showed that NAD within the MM niche is able to activate a discontinuous (*i.e.*, multicellular) pathway for Ado production that relies upon CD38, CD203a and CD73 (or TRAP) ectonucleotidases, depending on the environmental pH. Ado production was likely due to interactions taking place between myeloma and other cells lining the BM niche. The results of a pilot study revealed a parallel between Ado levels and disease progression, and patients with symptomatic MM usually have higher levels of Ado. This is reflected statistically in the International Staging System (ISS) for MM. Preliminary studies showed that MVs deriving from MM patients are peculiar in that i) they are enriched for CD38, CD203a and CD73 ectoenzymes and ii) are able to generate Ado. These MVs may adhere or fuse with neighboring cells, egress the BM niche and eventually reach the bloodstream. Thus, one aspect of the dynamics of available anti-CD38 antibodies for MM therapy is the role of polarization and release of the MVs after treatment. *In vitro* studies showed that MV/IgG antibody complexes can be captured by Fc receptor-expressing cells.
Summary/Conclusions: 1) We hypothesize that MM cells deploy the adenosinergic metabolic strategy to silence immune effectors during the progression of MM from indolent monoclonal gammopathy to symptomatic overt disease; 2) While these observations may only be correlative and a reflection of the tumor burden, Ado levels in the BM plasma may prove to be a useful marker of myeloma progression; 3) The therapeutic IgG on the surface of adenosinergic MVs may acts as a link determining the final destination of MVs.

E1238

TREATMENT OPTIMIZATION FOR MULTIPLE MYELOMA: SCHEDULE-DEPENDENT SYNERGISTIC CYTOTOXICITY OF POMALIDOMIDE AND CARFILZOMIB ON AN IN VITRO AND EX-VIVO MODEL

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Background: In recent years significant progress has been made in the understanding of Multiple Myeloma (MM) biology. These advances have translated into the development of new drugs and a different approach to treatment, which has ultimately translated into an unprecedented rate of complete remissions. Immunomodulatory drugs (IMiDs) and proteasome inhibitors (PIs) form the backbone of modern MM treatment,but new and more targeted treatments are under development and are being tested in the context of clinical trials.Pomalidomide (POM) is a third-generation IMiD with immunomodulatory, antiangiogenic, and direct anti-MM activities, and greater *in vivo* potency than its sister Lenalidomide. Carfilzomib (CAR) is a second generation irreversible PI that is structurally and mechanistically distinct from Bortezomib. Preclinical study suggested that the timing and dosing schedules of IMiDs in combination with PIs treatment is critical, proposing a first evidence that established treatment regiments need to be carefully re-evaluated to maximize the anti-tumor effects.
Aims: In this study we tried to optimize the anti-MM therapy using the new class of agents of IMiDs and new generation PIs, by evaluating a possible synergistic effect between POM and CAR.
Methods: For the purpose of this study we used five *bona fide* MM cell lines (MM1.S, OPM-2, NCI-H929, KMS12.BM and U266), a human bone marrow stromal (BMS) cell line (HS-5 cells) and primary samples from newly diagnosed MM patients. Apoptosis analysis was done up to 48h after administration of the first drug. For each drug, three different concentrations were used: low dose, intermediate dose and high dose. Since the BM microenvironment is a complex and active system, with potential contributions of both physical adhesion and soluble factors, we used three experimental conditions to differentiate these interactions: 1) MM cells cultured in complete medium, 2) MM cells suspended in medium conditioned in the prior presence of BMSCs, or 3) MM cells co-cultured with BMSCs in a transwell system.
Results: Using the median effect method of Chou Talalay, we evaluated the combination indices for simultaneous and sequential treatment schedules, and we found that the schedule of administration is important to maximize the synergistic effects. Indeed, schedule-dependent synergistic cytotoxicity was demonstrated for the combination of IMiDs and PIs and a maximal apoptosis consistently observed in IMiDs pre-exposure schedule. The superiority of this schedule was maintained throughout BM microenvironment models. Our data overall suggest that the administration of IMiDs before PIs can improve efficacy. Clinical trials are needed to investigate the most effective schedule, which could be to start the administration of IMiDs a day before PIs to increase cells killing.
Summary/Conclusions: Schedule-dependent synergistic cytotoxicity was demonstrated for the combination of CAR and POM and a maximal apoptosis consistently observed in POM pre-exposure schedule. The superiority of this schedule was maintained throughout BM microenvironment models using low dosage of both drugs. Whilst the clinical efficacy of CAR and POM combinations has been demonstrated, the synergistic cytotoxicity may be further exploited by using optimized schedule. Utilizing such a schedule with IMiDs pre-treatment may improve the depth and duration of response of MM patients both as upfront therapy and in the relapsed/refractory setting.

Myeloma and other monoclonal gammopathies - Clinical

E1239

ASSESSMENT OF THE IMPACT OF POST-AUTOLOGOUS STEM CELL TRANSPLANT MAINTENANCE THERAPY ON SURVIVAL OUTCOMES IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA IN THE COMMUNITY-BASED CONNECT MM REGISTRY

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Background: Randomized phase 3 clinical trials have shown that maintenance therapy after autologous stem cell transplant (ASCT) can extend time to progression, progression-free survival (PFS) and overall survival (OS) for patients (pts) with newly diagnosed multiple myeloma (NDMM) (Sonneveld, *J Clin Oncol,* 2012; McCarthy, *N Engl J Med,* 2012; Attal, *N Engl J Med,* 2012; Palumbo, *N Engl J Med,* 2014; Attal, ASCO, 2016). Connect MM is a largely community-based, US prospective observational cohort study designed to characterize diagnosis, treatment patterns and outcomes in pts with NDMM in clinical practice.
Aims: The Connect MM registry was used to assess impact of maintenance therapy on survival outcomes in pts with NDMM receiving ASCT.
Methods: Adult pts with NDMM were eligible to enroll in the registry within 60 days of diagnosis. Pts were enrolled in 2 sequential cohorts and were treated at the clinician's discretion as per standard of care. Cohort 1 pts receiving induction and ASCT were included in the analysis and characterized into 4 maintenance regimen subgroups: no maintenance, lenalidomide (LEN)-based maintenance, bortezomib (BORT)-based maintenance, and LEN+BORT maintenance. Duration was from 100 days post-ASCT (no maintenance group) or start of maintenance until progressive disease, death, discontinuation, or data cutoff of January 7, 2016. End points were PFS, second PFS, OS, and safety. An exploratory analysis of the impact of baseline characteristics on survival outcomes was performed.

Table 1.

	LEN Maintenance (n = 213)	No Maintenance (n = 165)	Hazard Ratio (95% CI)
Median PFS, months	50.3	30.8	0.62 (0.46, 0.82) *P* = .0009
3-yr PFS	56%	42%	
Median OS, months	not reached	not reached	0.54 (0.36, 0.83) *P* = .0050
3-yr OS	85%	70%	

Results: A total of 1493 pts were enrolled in Cohort 1 from Sep 2009 to Dec 2011; 1450 were treated, 81% (n=1173) in a community setting. Of those, 432 (29%) met analysis criteria. Median follow-up was 39.3 months. Median age was 60 y (range, 24-78); 60% were men; and 86% were white. A total of 165 pts did not receive maintenance. Of 267 pts receiving maintenance, 213 (80%) received LEN-based maintenance; 30 (11%) received BORT-based maintenance; and 16 (6%) received LEN+BORT maintenance. Of the maintenance groups, only data from LEN maintenance is presented; small sample sizes in the other maintenance groups limited interpretation. The median treatment duration was 35.2 months for pts who received LEN maintenance and 26.1 months for those who did not receive maintenance. Median PFS was significantly longer for pts who received LEN maintenance *vs* no maintenance (50.3 months *vs* 30.8 months; hazard ratio [HR]=0.62 [95% CI: 0.46, 0.82); *P*=.0009; Table). OS was also significantly improved for pts who received LEN maintenance *vs* no maintenance (HR=0.54 [95% CI: 0.36, 0.83]; *P*=.0050). Second PFS (PFS for second-line treatment) was similar for both LEN and no maintenance groups. Exploratory analyses showed generally similar PFS and OS improvements across subgroups (age, ECOG status, International Staging System stage, risk group, and induction regimen). No new safety signals were observed.
Summary/Conclusions: In this observational study, post-ASCT LEN maintenance therapy significantly improved PFS and OS compared to no maintenance. These improvements appeared to be independent of induction regimen. Preliminary analysis of second PFS suggests no adverse impact of maintenance treatment on the efficacy of second-line therapy. These data, from a largely community-based setting, support results from randomized phase 3 trials.

E1240

DARATUMUMAB-BASED COMBINATION THERAPIES IN HEAVILY-PRE-TREATED PATIENTS WITH RELAPSED AND/OR REFRACTORY MULTIPLE MYELOMA

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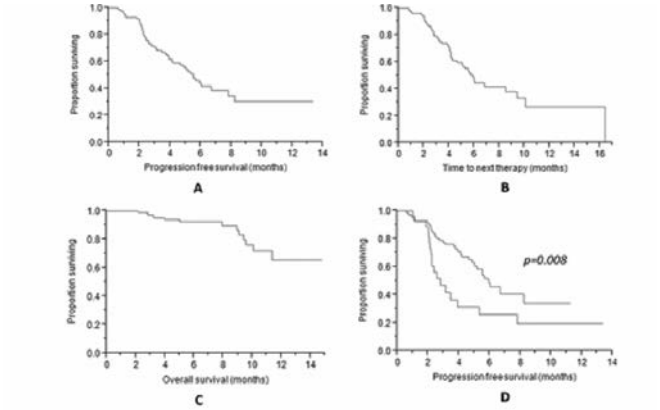
Background: Daratumumab-based Combination Therapies (DCT) with bortezomib (V)/ lenalidomide (R)/ pomalidomide (P) and dexamethasone (d) have shown exceptional activity in relapsed and/or refractory multiple myeloma (RRMM) in trials. Experience outside of trials since the approval of Daratumumab (D) in 2015 is limited.
Aims: We aimed to review the outcomes of patients who received DCT at our institution.
Methods: Records of RRMM patients seen at Mayo Clinic, MN from December 2015 –December 2016 were reviewed. Patients who received ≥ 1cycle of DCT were included. Time-to-event analyses were done from date of starting DCT using Kaplan Meier method. Common terminology criteria for adverse events v4.0 were used to grade toxicities.
Results: Of 130 patients, 59% were males and median age at DCT initiation was 67 (43-93) years, ECOG performance score was ≥2 in 29%. Patients were classified as mSMART high (22%), intermediate (22%) or standard (56%) risk. Median time from diagnosis to initiation of DCT was 51.3 (5-156) months, and median number of prior therapies was 4 (1-14). Eighteen (14%) of patients were refractory to prior daratumumab monotherapy. Fifty-three (41%), 34 (26%) and 25 (19%) received DPd, DRd and DVd respectively. Eighteen (14%) patients received 'other' DCT. Median time to first response (≥ PR) was 3.1 months (95% CI 2.1-4.6). Overall response rate was 46%, [complete remission-2%, very good partial remission-18%, partial remission-26%]. Minimal response was seen in 17%, with clinical benefit rate of 62%. Median estimated follow up from initiation of DCT was 5.5 months (CI 4.2-6.1). The median duration of response was 6.1 months [CI 5.1- not reached (NR)]. Median progression free survival (PFS) was 5.5 months (CI 4.1-7.8) (figure A) and median time to next therapy (TTNT) was 5.9 months (CI 4.6-9.4) (figure B). Median PFS for DPd, DRd, DVd and other DCTs were 4.6 (CI 2.7-NR), 7.8 (CI 5-NR), 3.9 (CI 2.1-NR) and 3.9 (CI 2.8-8.2) months, respectively (p=0.3). Median overall survival (OS) from starting DCT was NR (CI 11.4-NR) (figure C). Median PFS for quadruple refractory (n=28) MM was 2.8 months (CI 2.2-5.3) *vs* 5.9 months (CI 4.9-NR) for the rest (p=0.008) (figure D). Grade 3 or higher hematological toxicities were seen in 42% of patients. Other toxicities included infections (37%), fatigue (31%), infusion reactions (16%) and diarrhea (10%).



Panel showing Kaplan Meier curves for (A) progression free survival, (B) time to next therapy and (C) overall survival for the entire cohort of relapsed/refractory multiple myeloma (RRMM) patients who received daratumumab-based combination therapies and (D) the difference in progression free survival between quadruple refractory (blue curve) RRMM patients and the rest (red curve).

Figure 1.

Summary/Conclusions: DCT are effective in RRMM, but the PFS remains short, particularly in quadruple refractory patients, reflecting the challenges encountered in managing heavily-pretreated, and often less fit patients, in routine practice.

E1241

IMPACT OF METFORMIN USE IN THE OUTCOMES OF MULTIPLE MYELOMA PATIENTS POST STEM CELL TRANSPLANT

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Background: Multiple myeloma (MM), a monoclonal plasma cell disorder, is one of the most common hematologic malignancies in the US. In preclinical studies, metformin demonstrated plasma cells cytotoxicity. However there is lack of studies translating the effect of metformin into the clinical setting.
Aims: Assess the clinical effect of metformin in patients with MM.
Methods: All MM patients who underwent stem cell transplant (SCT) at the Mayo Clinic Rochester from 2007 to 2012 were reviewed. Patients were grouped based on metformin use. Initial diagnosis at our institution and ≥12 months of follow up were required. Kaplan-Meier method and Cox regression were used for time-to-event and multivariate analysis.
Results: Out of 687 patients, 78 (11.4%) patients were using metformin at the time of MM diagnosis. Baseline characteristics in the metformin and no-metformin groups were similar. Median metformin dose was 2000mg daily and median duration of metformin use from MM diagnosis was 22 months. Patients on the Metformin group achieved higher rates of complete response after SCT (41% *vs* 29% p<0.02). Median progression-free survival (PFS) after SCT was longer in the Metformin group, 31.3 months (95% CI: 10.4-52.2) *vs* 16.6 months in the no-metformin group (95%CI: 14.5-18.7) p<0.04. There was a trend toward longer overall survival in the Metformin group, but it was not statistically significant (170 *vs* 106 months, p<0.10). In a multivariate analysis of metformin use, age, sex, international staging system (ISS), LDH and cytogenetics/FISH, the former was an independent predictor of PFS after SCT (OR: 0.38, 95%CI: 0.20-0.68, p<0.001).

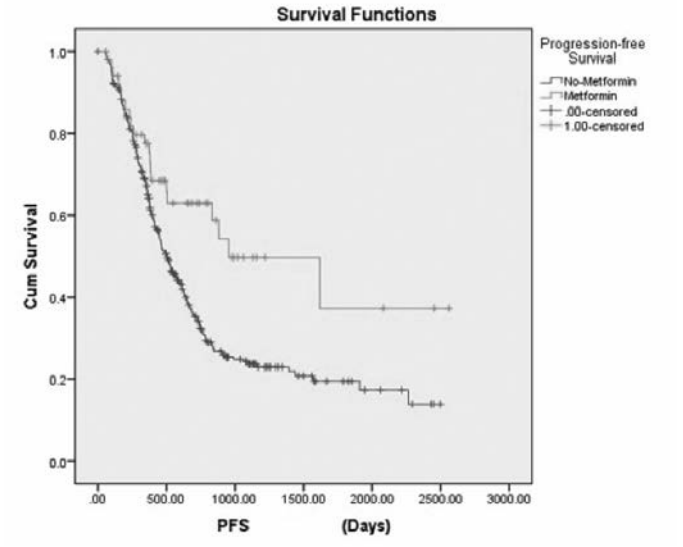

Figure 1.

Summary/Conclusions: Metformin use was associated with a better progression-free survival and higher complete response rates after SCT in our MM cohort. A trend toward better overall survival was also noted in the Metformin group. Larger studies are needed to enhance our understanding of the clinic effect of metformin on MM.

E1242

COMPARING WHOLE BODY MRI WITH PET-CT IMAGING AT DIAGNOSIS OF MYELOMA

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Background: Imaging in the diagnosis of myeloma is a rapidly developing field. First line imaging has traditionally been a skeletal survey with plain films, however new guidelines recommend whole body imaging to aid the diagnosis of plasma cell disorders. The International Myeloma Working Group recommend low-dose whole body computerised tomography (LDWBCT), PET-CT or whole body magnetic resonance imaging (WBMRI) as initial imaging modalities.
Aims: To compare WBMRI with PET-CT as initial imaging modalities at diagnosis of myeloma or plasmacytoma.
Methods: Both WBMRI and PET-CT were performed at diagnosis of myeloma or a plasmacytoma in 33 patients presenting to King's College Hospital, London. The scans were reviewed independently by two Consultants in Radiology

and Nuclear Medicine, looking for focal bone lesions, bone marrow pattern and incidental findings. Details of the patients' demographics, myeloma diagnosis and treatment were collected from the medical records.
Results: Of the 33 patients, 24 were male. The median age was 64 years(range=43-86 years). One patient had a solitary plasmacytoma, the other 32 had myeloma (21 IgG, 3 IgA, 2 non-secretory, 4 light chain disease, 2 biclonal myeloma). Most had ISS stage I disease with a median paraprotein at diagnosis of 17 (range 0-52.6). 21 patients had a bone marrow plasma cell burden of 10-60%, 10 patients >60% and 2 were unknown. Sixteen patients were diagnosed with smouldering myeloma and a 'watch and wait' policy was adopted. Eleven patients were treated with chemotherapy, 4 were entered into a clinical trial, one was offered palliative care and one was referred to our centre for autograft. WBMRI identified a focal lesion of disease in 30% of patients compared with 36% by PET-CT. This was not a statistically significant difference (p=0.18). In addition there was no statistically significant difference between PET-CT & MRI in detecting <3 or >3 lesions (p=0.705 and p=0.083 respectively). The apparent diffusion coefficient (ADC) at vertebrae L5 (using diffusion weighted MR imaging) was measured. This showed a strong correlation with the degree of bone marrow infiltration by plasma cells (r=0.64). An ADC of <600mm^2/s had a negative predictive value of 93% for a bone marrow plasma cell infiltrate of >60%. There was also a significant difference (p=0.012) in the ADC between those with smoldering myeloma and those with symptomatic disease. It was noted that 9 scans resulted in incidental findings including pneumonia, adrenal lesions and one case of colorectal cancer.
Summary/Conclusions: We have shown no difference in PET-CT and WBMRI in detecting a myeloma defining focal bone lesion, or providing prognostic estimate of burden of disease. Using MRI, a measure of the ADC at vertebrae L5 has been shown to be a semi-quantitative parameter that correlates with bone marrow plasma cell infiltration and distinguished between those with smouldering and symptomatic disease. In addition it is noted that whole body imaging has led to incidental findings of further pathology, including an unrelated malignancy, which may lead to useful clinical information or to further investigations and imaging which may not be needed.

E1243

PERSISTENCE OF MINIMAL RESIDUAL DISEASE BY MULTIPARAMETER FLOW CYTOMETRY CAN HINDER RECOVERY OF ORGAN DAMAGE IN PATIENTS WITH AL AMYLOIDOSIS

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Background: In multiple myeloma, Minimal Residual Disease (MRD) demonstrated by multiparameter flow cytometry (MFC) identifies subjects with significantly shorter survival among those who attain complete response (CR). The role of MRD in AL amyloidosis has not been assessed so far.
Aims: In the present study, we assessed the MRD by MFC in patients with AL amyloidosis who attained CR.
Methods: CR was defined as per current criteria (negative serum and urine immunofixation and normal free light chain ratio). For flow cytometry studies bone marrow samples were processed following the Euro Flow Bulk Lysis Standard Operating Protocol and stained with the EuroFlowIMF MM MRD panel. At least 5x10^6 events were measured using a FACSCanto II (USA) instrument. Data were analyzed using the Infinicyt software (Spain). Patients were identified as having residual disease if a discreet population of clonal plasma cells comprising ≥50 events was identified (10^{-5} limit of detection).
Results: Twenty-eight patients were tested (7 were found to have relapsed at the time of MRD assessment with monoclonal components detectable and MRD+) and 21 satisfied current criteria for CR. Nineteen (90%) had renal and 9 (44%) had cardiac involvement at diagnosis. More than 2 lines of therapy were required to achieve CR in 7 subjects. Median time to CR was 10 months (range: 3-82). Five patients (62%) had achieved cardiac response and 9 (50%) renal response at the time of CR. The median time from CR to MRD was 30 months (range: 6-148), this was not different in the MRD positive *vs* negative patients. Flow cytometry identified MRD in 9 patients (43%). A median of 1089 (range 256-2500) corresponding to 0.04% (range 0.02-0.3%) plasma cells with abnormal phenotype were detected in patients MRD+. No differences in organ involvement, cardiac and renal stage, type of therapy, number of treatments, and organ response at the time of CR was found between the two groups. However, a further improvement of cardiac function compared to the time of CR was observed in all 5 evaluable MRD- patients and in none of the 2 MRD+ patients (P=0.047). Compared to the time of CR, renal response was obtained in 7 MRD- subjects (84%) and in 4 (50%) MRD+ (P=0.153). Overall, further improvement of cardiac or renal function after CR was significantly associated with absence of MRD (P=0.012).
Summary/Conclusions: This proof-of-concept study indicates that 43% of patients with AL satisfying current criteria for CR have detectable MRD. MRD positivity could in part explain persistence of organ damage is patients in CR. A validation study in a larger cohort is ongoing. The possible impact of MRD should be considered in trials aiming at increasing organ response rate in patients in CR.

E1244

RATES OF PERIPHERAL NEUROPATHY (PN) IN PATIENTS WITH RELAPSED AND REFRACTORY MULTIPLE MYELOMA (RRMM) TREATED WITH CARFILZOMIB *VS* COMPARATORS IN PIVOTAL PHASE 3 TRIALS

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Background: PN is a dose□limiting toxicity for some anti myeloma agents, such as the proteasome inhibitor bortezomib (V).
Aims: Carfilzomib (K), a novel irreversible proteasome inhibitor associated with a low incidence of PN, was evaluated in two recent phase 3 studies in RRMM patients.
Methods: This analysis evaluated PN rates in ASPIRE (K [27mg/m^2] lenalidomide [R]-dexamethasone[d] [KRd] *vs* Rd in relapsed or refractory MM; Stewart 2015) and ENDEAVOR (Kd [K 56mg/m^2] *vs* Vd in RRMM; Dimopoulos 2016). We evaluated treatment emergent grade ≥2 PN, patient reported outcomes (PROs; QLQ C30 pain, FACT/GOG neurotoxicity subscales), and progression free survival (PFS) in patients with a baseline history of PN (patients with grade ≥3 PN at baseline or grade 2 PN with pain at baseline were excluded from the studies).
Results: In ASPIRE, grade ≥2 PN rate was low (8.9% [KRd] *vs* 8.0% [Rd]; Table). Pain subscale scores were similar between arms. Median PFS was longer with KRd *vs* Rd for patients with grade 2 PN at baseline. In ENDEAVOR, grade ≥2 PN rate during the study (prespecified key secondary endpoint) was significantly lower with Kd *vs* Vd (6.0% *vs* 32.0%; Table). Patients had significantly improved pain and neurotoxicity subscale scores with Kd *vs* Vd. PFS improved with Kd *vs* Vd in patients with baseline history of grade 2 PN (Table 1).

Table 1.

	ASPIRE		ENDEAVOR	
	KRd	Rd	Kd	Vd
Grade ≥2 PN during study, safety population, %	8.9	8.0	6.0	32.0
OR (95% CI)	1.132 (0.683, 1.876) p=0.6850		0.137 (0.089, 0.210) p<0.0001	
PRO, LS mean difference				
Pain: QLQ–C30 subscale, ITT (95% CI)	−1.02 (−3.77, 1.73) p=0.47		−2.35 (−4.30, −0.39) p=0.0186	
Neurotoxicity: FACT/GOG–NTx subscale, safety population (95% CI)	–		0.84 (0.40, 1.28) p=0.0002	
PFS by baseline history of PN, ITT				
Yes, n	144	137	215	244
Median (95% CI), mo	23.2 (18.0, 25.9)	17.6 (13.9, 26.0)	18.7 (13.88, NE)	9.4 (7.53, 10.39)
HR (95% CI)	0.947 (0.692, 1.296)		0.54 (0.410, 0.715)	
Grade 2, n	22	24	71	81
Median (95% CI), mo	24.2 (19.6, NE)	14.8 (7.4, NE)	18.6 (10.20, NE)	5.6 (4.47, 7.40)
HR (95% CI)	0.695 (0.321, 1.507)		0.42 (0.266, 0.677)	

Summary/Conclusions: In ENDEAVOR, the rate of PN was significantly lower with Kd then with Vd; in ASPIRE, PN rate was similar for KRd and Rd. Improved pain and neurotoxicity subscale scores with K may be attributed to better disease control and/or lower PN rates.

E1245

EARLY RELAPSE FOLLOWING AUTOLOGOUS STEM CELL TRANSPLANT IN MYELOMA IS A POOR PROGNOSTIC MARKER FOR OVERALL SURVIVAL AND IS DIFFICULT TO PREDICT AT DIAGNOSIS OR DURING INDUCTION TREATMENT

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Background: High dose chemotherapy followed by autologous stem cell transplant (ASCT) remains the gold standard treatment in myeloma for young

patients at induction. A number of factors have been shown to correlate with overall survival (OS) and progression free survival (PFS) including depth of remission prior to ASCT, Initial ISS stage and high risk cytogenetics. Emerging evidence has demonstrated that early relapse following ASCT is associated with reduced OS, and is not correlated with depth of pre-transplant response.
Aims: To characterise myeloma patients who relapsed within 12 months of ASCT, through baseline characteristics and transplant engraftment, and assess the impact of this early relapse on OS and PFS.
Methods: We performed a multicentre retrospective analysis of patients who underwent ASCT at 3 centres between 01/2009 – 02/20016 (London) and 06/2006 – 03/2013 (Cardiff). Baseline characteristics were reviewed and ASCT engraftment was assessed by; time to neutrophils 50 x 10^9/l and platelets >20 x 10^9/l. Post-transplant PFS & OS was calculated by time (months) from diagnosis to progression or death.
Results: 443 myeloma patients were identified, median age was 57 (r 31-73), 56% were male. 41% of patients were ISS stage 1, 34% stage 2, 25% stage 3. Cytogenetic data was available for 139 patients. 1st-line therapy prior to transplant was immunomodulatory drug (IMiD) based (THAL/LEN) for 318/443 patients & 72/443 were proteasome inhibitor (PI) based (BORT/CARF). In addition, 11 patients received combination PI and IMiD. Median time from start of therapy to ASCT was 10 months (r 3-109m). 67 patients progressed within 12m of ASCT (early progression). No statistical difference was found between <12m or >12m relapse for: age, gender, 1st line therapy, ISS stage, Hb, LDH, Ca or cytogenetics, confirming that this group is difficult to predict at baseline. Median OS from time of diagnosis was 103 months (95% CI 101 -137), median OS from start of ASCT was not reached, however 5-year OS was 68%. Patients with progressive disease within 12 months of ASCT, has significantly reduced median OS from diagnosis: 31 months (95% CI 21- 39) compared to non-progressive patients (median OS:103m 95% CI 89-117) p<0.0005. Median OS from ASCT was reduced in early progression median OS:18m (95% CI 14-22m) *vs* progression >12 months median OS:89 months (95%CI 79-98m) p<0.0005. 1st line therapy did not influence likelihood of PFS<12months, with no statistical difference between patients who received PIs, IMiDs or both 1st line (p=0.484). A significant difference was observed in median time to platelet engraftment between the 2 centres. Increased time for platelets to reach >20 x10^9/l was associated with reduced OS from ASCT for each centre HR 1.14 & 1.20 (p= 0.046 & 0.03) for Cardiff or London centres respectively (Cox's Method).
Summary/Conclusions: Early relapse following ASCT is a significant predictor of inferior OS in myeloma and difficult to predict from standard baseline characteristics. From our analysis; 1st line treatment prior to ASCT did not influence OS or PFS. There was an association between slow platelet engraftment following ASCT and PFS and OS. Possible explanations include: residual occult disease, toxicity of chemotherapy or patient stromal factors which facilitate disease resistance and impair normal haematopoiesis. All of these factors have been shown to drive relapse. RCTs are required to standardise bone marrow response assessment post ASCT, quantify remission status (using laboratory and imaging techniques) and definitively predict early relapse. Additionally, these studies will investigate further biological or genetic mechanisms driving early relapse to help identify novel therapeutic approaches in this extremely poor prognosis group.

E1246

PATIENT-REPORTED OUTCOMES (PROS) WITH IBRUTINIB: SUBSTUDY OF INNOVATETM FOR WALDENSTRÖM MACROGLOBULINEMIA (WM)

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Background: Anemia and fatigue are frequent indications for WM treatment. To date, patient-reported outcomes (PROs) have not been used to quantify benefits of any WM treatment. Ibrutinib (ibr), a first-in-class, once-daily inhibitor of BTK, is indicated in the EU for the treamtent of WM after ≥1 prior therapy or first-line in patients (pts) unsuitable for chemoimmunotherapy.
Aims: To prospectively collect PROs from the iNNOVATE substudy to assess patients' perspectives of the therapeutic benefit of ibr.
Methods: INNOVATE has randomized (ibr+rituximab [RTX] *vs* placebo+RTX) and substudy (RTX-refractory) components. Pts in the substudy received oral ibr 420mg daily until progressive disease (PD) or unacceptable toxicity. All pts provided informed consent. PRO assessments—FACT-An total score (TS) and FACT-An anemia subscale (AS), and EQ-5D-5L (EQ)—were performed regularly.
Results: Persistent fatigue was the main indication for treatment in 22/31 (71%) pts. Baseline PRO scores were lower for the substudy *vs* randomized pts (Table). With a median of 17 months (mo) of treatment, most pts had clinically meaningful improvement in TS (≥7 points; 77%), AS (≥6 points; 84%), and EQ utility scores (≥0.08 points; 68%). Time to clinically meaningful improvement was prompt (1 mo for TS and AS; 2 mo for EQ), corresponding with a 48% decline in median IgM (median 20 g/L) after 4 weeks. In pts with baseline anemia (hemoglobin [Hb] ≤110 g/L), sustained Hb improvement increased with depth of response. At week 65, Hb levels significantly correlated with TS (r=0.507, P=0.01) and AS (r=0.519, P=0.008), and were marginal for EQ (r=0.39, P=0.054). Although IgM levels did not significantly correlate with PRO scores, the benefit was similar in responders regardless of depth of response.

Table 1.

Table. Patient Characteristics and PROs

Characteristic	Substudy (n=31)	Randomized R/R (n=81)
Med age, y	67	70
IPSSWM*, %		
Low	23	22
Intermediate	36	42
High	42	36
ECOG PS, %		
0	42	51
1	39	41
2	19	9
Med IgM, g/L (range)	39.2 (8.66-107)	33.0 (6.2-73.3)
Med Hb, g/L (range)	103 (64 -146)	103 (66-161)
Med TS	121	153
Med AS	48	65
Med EQ	0.765	0.836

Outcome	Substudy
WM response rate†, %	
ORR	90
MRR	71
Sustained Hb improvement by response, %	
PR	64
MR	33
NR	0
Pts with clinically meaningful improvement in PRO, %	
By response, %	
TS	77
PR	77
MR	83
NR	67
AS	84
PR	86
MR	83
NR	67
EQ	68
PR	77
MR	67
NR	0

IPSSWM, International Prognostic Scoring System for WM; ECOG PS, Eastern Cooperative Oncology Group performance status; Hb, hemoglobin; TS, FACT-An total score; AS, FACT-An anemia subscale; EQ, EQ-5D-5L; ORR, overall response rate; MRR, major response rate; PR, partial response; MR, minor response; NR, no response
*Time of enrollment
†Defined as the proportion of pts ≥MR, and MRR as the proportion of pts ≥PR; PR (n=22), MR (n=6), NR (n=3)

Summary/Conclusions: Clinical response, and associated anemia improvement induced by ibr, correlated with meaningful improvements in the well-being of heavily pretreated pts with RTX-refractory WM.

E1247

INCIDENCE AND RISK FACTORS OF CARDIOVASCULAR ADVERSE EVENTS IN A LARGE POPULATION OF NEWLY-DIAGNOSED, TRANSPLANT INELIGIBLE MYELOMA PATIENTS TREATED WITH CARFILZOMIB

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Background: Cardio-vascular (CV) toxicities in patients (pts) with multiple myeloma (MM) may derive from comorbidities, MM itself and its treatment. Carfilzomib, an irreversible proteasome inhibitor, is approved as single agent or in combination with dexamethasone or lenalidomide-dexamethasone for relapsed MM.
Aims: We conducted an integrated analysis of CV adverse events (AE) in newly diagnosed, transplant-ineligible MM patients treated with Carfilzomib in 3 phase I/II studies (IST-CAR-506, IST-CAR-561, IST-CAR-601).
Methods: All pts were treated with 9, 28-day induction cycles with carfilzomib, cyclophosphamide (300mg/m^2 on days 1,8,15) and dexamethasone (40mg weekly) (CCyd), followed by carfilzomib maintenance until progression or intol-

erance. Carfilzomib was administered i.v. at the dose of 36mg/m^2 on days 1, 2, 8, 9, 15, 16 in the IST-CAR-506 trial; at 3 dose levels escalated from 45 to 70mg/m^2 on days 1, 8, 15 in the IST-CAR-561 trial and on days 1, 2, 8, 9, 15, 16 in the IST-CAR-601 trial. AEs were graded based on NCI-CTCAE v4.
Results: 148 pts with a median age of 72 years were analyzed. At enrollment, 34% of patients had at least 1 cardiovascular risk factor: 20% had peripheral vascular disease (including hypertension in 13% patients), 19% diabetes and 5% chronic pulmonary disease. After a median follow-up of 21 months, at least 1 any grade CV-AE occurred in 45% of patients; any grade hypertension was reported in 17% of patients, dyspnea in 9%, and heart failure, arrhythmia and venous thromboembolism (VTE) in 6% of patients, each. Grade 3-5 CV-AEs occurred in 15% of patients, the most common being heart failure (4%), hypertension (3%), pulmonary edema (3%) and VTE (3%). Four (3%) fatal CV-AEs occurred: 1 case of heart failure, pulmonary edema, arrhythmia and VTE, respectively. No difference in terms of CV-AEs was observed in patients treated with different doses of carfilzomib. In pts who developed at least 1 CV-AE, carfilzomib dose reduction (33%) and discontinuation (33%) were more frequent as compared to those without CV-AEs (12% and 18%, respectively; p<0.0001). A trend toward a shorter 2-year overall survival (adjusted for age) was observed among patients who experienced at least 1 CV-AE as compared with those who did not (74% *vs* 83%, HR: 0.51; p=0.066). Pts ≥75 years had a higher risk of any grade (58% *vs* 36%, p=0.02) and grade 3-5 CV-AEs (34% *vs* 15%, p=0.01); major cardiac events of any grade were more frequent in older patients (29%) than in younger ones (6%; p<0.001). Patients with at least 1 CV risk factor at enrolment had a 4-fold increased risk (odds ratio: 3.79; p<0.001) of developing a CV-AE during treatment as compared to patients with no CV risk factors: in detail, baseline hypertension (odds ratio: 4.12; p=0.012) and peripheral vascular disease (odds ratio: 3.75; p=0.002) conferred the highest risk of developing CV-AEs.

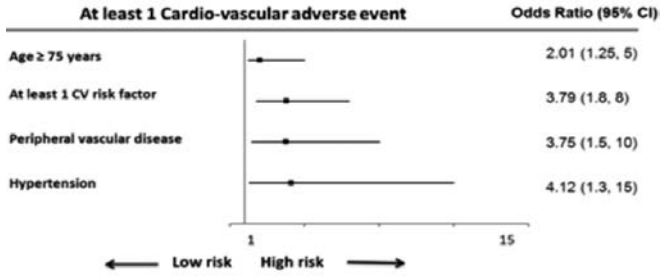

Figure 1.

Summary/Conclusions: Among newly diagnosed MM pts treated with carfilzomib, cyclophosphamide and dexamethasone, at least 1 CV-AE occurred in 45% of pts, hypertension and dyspnea were the most common. Pts ≥75 years of age and those with at least 1 pre-existing CV risk factor were at higher risk of developing a CV-AEs. The onset of CV toxicity significantly increased the rate of dose reductions and treatment discontinuation, translating into higher risk of death. CV toxicity may significantly impact on treatment compliance and survival. Therefore, to derive maximum benefit from Carfilzomib, all pts - particularly the elderly - should be carefully assessed to select the most appropriate treatment.

E1248

POMALIDOMIDE (POM) + LOW-DOSE DEXAMETHASONE (LODEX) AFTER SECOND-LINE LENALIDOMIDE (LEN)-BASED TREATMENT OF RELAPSED OR REFRACTORY MULTIPLE MYELOMA (RRMM): UPDATED PROGRESSION-FREE SURVIVAL ANALYSIS

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Background: Most recent pivotal trials of triple therapy in second- and third-line treatment excluded patients (pts) whose multiple myeloma (MM) was refractory to LEN. This is not reflective of the standard of care in first and second line where LEN is given until progressive disease (PD). To address this, the MM-014 phase 2 trial enrolled pts with RRMM and second-line LEN-based treatment failure. Cohort A enrolled pts treated with POM + LoDEX. The study was amended to include cohort B (pts treated with POM + LoDEX + daratumumab).
Aims: To present updated safety and efficacy analyses only from cohort A, in which pts received POM + LoDEX immediately after relapsing or being refractory to second-line LEN-based therapy.
Methods: Pts aged ≥18 years had documented MM, measurable disease, 2 prior lines of treatment, and PD after ≥2 cycles of second-line LEN-based treatment. Pts received 28-day cycles of POM 4mg/day on days 1-21 + LoDEX 40mg/day (20mg/day if>75 years) on days 1, 8, 15, and 22; thromboprophylaxis was mandatory. The primary endpoint was overall response rate (ORR; ≥ partial response [PR]) assessed by modified IMWG criteria. Key secondary endpoints included time to response (TTR), progression-free survival (PFS), secondary primary malignancies (SPMs), and biomarkers. All pts provided informed consent.
Results: Of 51 enrolled pts in cohort A, 39 (76.5%) discontinued treatment, mostly due to PD. Median age was 68.0 years, and 92.2% had an Eastern Cooperative Oncology Group performance status of ≤1. A total of 45 pts (88.2%) were refractory to their last treatment with LEN, and 37 (72.5%) had prior treatment with bortezomib. Median duration of prior LEN-containing therapy was 24.6 months. With a median follow-up of 13.6 months, ORR was 29.4%, with 1 (2.0%) complete response, 5 (9.8%) very good partial responses, and 9 (17.6%) PRs. Minimal response (MR) was reached in 15.7% of pts. Median TTR was 1.9 months and 66% of pts had ongoing response at 1 year. Median PFS was 13.8 months. The 2-year PFS rate was 48.6% for the intent to treat population, 69.4% for pts with ≥ MR, and 69.1% for pts with ≥ PR. In addition, pts with ≥ MR had similar treatment durations as those achieving ≥ PR (10.5 *vs* 11.5 months; Table). Common grade 3/4 adverse events (AEs) included anemia (25.5%), neutropenia (11.8%), and infections (19.6%; including pneumonia [9.8%]). No pts experienced SPMs. In the immune subset analysis, the proportions of CD3$^+$ and CD3$^+$/CD8$^+$ T cells after treatment were significantly higher *vs* baseline (72.6% *vs* 67.8% and 36.9% *vs* 32.1%, respectively; *P*<.05). Pts with response also had significantly elevated proportions of these T-cell populations, but pts with no response did not. Relative changes from baseline for CD3$^+$ and CD3$^+$/CD4$^+$T-cell populations were significantly greater in pts with response *vs* those with no response (10.4 *vs* −0.8 and 4.2 *vs* −3.5, respectively; *P*<.05).

Table 1.

Response Type	POM Treatment Duration, months
≥ PR (n = 15)	11.5
≥ MR (n = 23)	10.5
MR (n = 8)	7.7
Stable disease (n = 21)	3.7

Summary/Conclusions: This update confirms the safety and efficacy of POM + LoDEX following second-line LEN-based treatment failure in pts with RRMM. Hematologic AE rates improved, and median PFS was longer with third-line use than previously reported with POM + LoDEX use in later treatment lines. In addition, achieving disease control of ≥ MR led to similar PFS rates as reaching ≥ PR.

E1249

"REAL WORLD" DATA ON THE EFFICACY AND SAFETY OF IXAZOMIB IN COMBINATION WITH LENALIDOMIDE AND DEXAMETHASONE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA: A STUDY OF THE GREEK MYELOMA STUDY GROUP

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Background: The all-oral combination of ixazomib, lenalidomide and dexamethasone (IRd) has been recently approved as a novel standard of care for relapsed/refractory multiple myeloma (RRMM).
Aims: The aim of this study was to evaluate the efficacy and safety of IRd in the "Real World" (RW) practice, where data are very limited.
Methods: This was a retrospective, non-interventional study, which recorded IRd treatment data from patients with RRMM who participated in the name-patient program of ixazomib in Greece. The primary endpoint was the evaluation of overall response rate (ORR), according to IMWG criteria. Secondary endpoints included: treatment duration; time to response; duration of response; percentage of patients who experienced adverse events (AEs), needed dose modification or treatment discontinuation; evaluation of PFS and TTP.
Results: Forty-one patients were included in the present study. Of those, 35 (19M/16F, median age 70.5 years (range: 63-79 years) had received at least 3 cycles of IRd on the date of data analysis and thus they were included in the present report. The median line of previous therapies was one (range: 1-5): 71.4% (25/35) patients had received one prior treatment, while 20.0% (7/35),

5.7% (2/35) and 2.9% (1/35) had received 2, 3 and 5 prior treatment lines, respectively. Overall, 82.9% (29/35) of patients had been exposed to proteasome inhibitors prior to IRd (77.1% to bortezomib and 8.6% to carfilzomib] and 48.6% (17/35) to IMiDs [31.4% (11/35) to thalidomide and 22.9% (8/35) to lenalidomide]. Autologous transplantation had been given in 42.9% (15/35) of patients. Median treatment duration was 7.1 months. Among 34 patients with available data for response, VGPR was recorded in 35.3% (12/34), PR in 32.4% (11/34), MR in 2.9% (1/34) and stable disease in 26.5% (9/34). The ORR (PR or better) was 67.6%: 70.8% among those who received IRd in the second line and 60.0% among those who received IRd beyond the second line;ORR tended to be higher as the time of exposure to ixazomib increased, with patients exposed to ixazomib for at least 6, 7 and 8 months, noting ORR rates of 70.8% (17/24), 76.5% (13/17) and 91.8% (9/11), respectively. Median time to best response was 1.2 months. Treatment interruptions due to AEs were recorded for 11.4% (4/35) of patients, while 20.0% (7/35) of patients discontinued treatment. Reasons for ixazomib discontinuation were AEs for 3 patients (an event of septic shock had fatal outcome), disease progression in 3 patients and administrative reason (patient could no longer visit the site) in one. Among the 35 patients analyzed, 17.1% (6/35) had experienced disease progression or death; the 6-month PFS rate was 90.5% and the 6-month TTP rate was 93.2%. Regarding AEs of interest, 31.4% (11/35) of patients experienced peripheral neuropathy; of those events, 54.5% (6/11) resolved, while 45.4% (5/11) had not resolved (three were grade 1, one grade 2, and one of grade 3) at the end of follow-up. In addition, 31.4% (11/35) of patients developed gastrointestinal AEs, 11.4% (4/35) experienced pneumonia, 9.4% (3/32) hypertension, 5.7% (2/35) cataract and herpes zoster, and 2.9% (1/35) deep vein thrombosis; no cardiac arrhythmia or other cardiac events were recorded, while osteonecrosis of the jaw developed in 5.7% (2/35) of the patients.

Summary/Conclusions: This study showed that the IRd regimen produces an ORR of near 68% and a clinical benefit in almost all patients with RRMM who are treated in RW practice. IRd acts rapidly and has an acceptable toxicity profile with no cardiac events.

E1250

EUROPEAN POST-APPROVAL SAFETY STUDY (EU PASS) OF RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM): SAFETY IN A LARGE COHORT OF PATIENTS TREATED WITH LENALIDOMIDE, THALIDOMIDE, AND BORTEZOMIB

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Background: EU PASS is an observational, non-interventional study designed to investigate the safety of lenalidomide (LEN) and other agents in the treatment of patients with RRMM in a real-world setting.

Aims: To assess the incidence of adverse events (AEs) of special interest (ie, neutropenia, thrombocytopenia, venous thromboembolism [VTE], neuropathy, and second primary malignancies [SPMs]).

Methods: Patients with RRMM initiating LEN treatment were enrolled at the investigator's discretion into the LEN cohort (LEN + dexamethasone, the approved combination for the treatment of RRMM); patients who received ≥ 1 prior therapy initiating a non–LEN-based treatment were enrolled into a background cohort (all other treatments, including novel agents). Thromboprophylaxis was administered per local standard practice. AEs were graded per the National Cancer Institute-Common Terminology Criteria for AEs, v3.0. SPMs were defined using MedDRA terms, SPM assessments were to be conducted up to 36 mos after treatment discontinuation (Protocol amendment 2011). All patients provided informed consent.

Results: As of March 1, 2016, 3630 patients were included in the safety population. Of these patients, 2151 (59.3%) received LEN, 1187 (32.7%) received bortezomib (BORT), 137 (3.8%) received thalidomide (THAL), and 155 (4.3%) received other therapies. The median duration on study treatment was 6.6 mos (range 0.1-79.9 mos) for patients receiving LEN, 4.1 mos (range 0-61.4 mos) for BORT, and 4.6 mos (range 0.2-36.9 mos) for THAL. At the time of analysis, 3557 patients (98.0%) had discontinued treatment. Of the 73 patients (2.0%) who were on treatment, 63 were treated with LEN, 6 with BORT, 0 with THAL, and 4 with other therapies. Baseline characteristics were balanced across cohorts. Median age was 70 yrs (range 25-95 yrs) and 54.0% of patients were male. Of 2985 patients with available ECOG scores, 2865 (96.0%) had an ECOG score ≤2. The median number of prior therapies was 1 (range 1-6); the proportion of patients with only 1 prior treatment was lower in the LEN (44.2%) *vs* BORT (70.8%) and THAL (56.2%) cohorts. Overall, 1843 patients (50.8%) had grade 3/4 AEs (Table). All grade neuropathy occurred in 9.8%, 28.2%, and 17.5%, and VTE occurred at 6.0%, 1.3%, and 0% of patients in the LEN, BORT, and THAL cohorts. The incidence of SPMs per 100 patient-years was 3.87 overall and 3.45, 5.41, 2.73, and 6.51 for LEN, BORT, THAL, and other agents, respectively. Treatment discontinuation rates due to AEs were similar in each cohort (LEN: 22.4%; BORT: 20.1%; and THAL: 21.2%). Rates of treatment-emergent AEs leading to dose reductions were also similar across cohorts, occurring in 23.9% of patients in the LEN cohort, 21.2% in BORT, and 17.5% in THAL. Data on long-term responders will be presented at this meeting.

Table 1.

Grade 3/4 AEs, %	LEN	BORT	THAL
Neutropenia	20.0	4.5	7.3
Febrile neutropenia	1.5	0.4	1.5
Thrombocytopenia	10.0	8.4	3.6

AE, adverse event; BORT, bortezomib; LEN, lenalidomide; THAL, thalidomide.

Summary/Conclusions: LEN was generally well tolerated and the safety results were similar to published data. As expected, the occurrence of neutropenia, TCP, and VTEs were higher in patients in the LEN cohort, whereas neuropathy was more frequently reported in patients in the BORT cohort. VTEs were low in all cohorts. The occurrence of SPMs was generally low and comparable between cohorts.

E1251

NEW CLINICAL PATHWAYS OF THE CENTERS OF EXCELLENCE NETWORK IN GERMANY: A NEW CONCEPT FOR STANDARDIZED CARE OF MULTIPLE MYELOMA PATIENTS

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Background: Clinical outcome of multiple myeloma (MM) patients is heterogeneous and depends on various prognostic factors and available treatments. Although tremendous progress has been made in MM, so far, there is no national or international evidence-based guideline giving recommendations for clinical practice in the treatment of MM patients. In Germany, 14 Comprehensive Cancer Centers (CCC) are funded as 'Centers of Excellence' by the German Cancer Aid (DKH). All these Centers of Excellence are required to develop and provide in-house clinical pathways for standards in cancer care. These pathways include concise diagnostic and therapeutic instructions, reflecting available evidence-based recommendations. In addition, ongoing studies (in particular phase I / II) are part of clinical pathways, so that a rapid transfer of innovation is provided and patients gain access to new therapeutic approaches. The Centers of Excellence Network working group SOP has the goal to harmonize these hospital specific in-house clinical pathways differing in format, content and level of detail of guidance.

Aims: The project's objective is to harmonize diagnosis, treatment and follow-up of all MM patients in Germany by providing concise, freely available online clinical pathways.

Methods: The clinical pathway was prepared according to a methods handbook developed by the working group including recommendations for standardized methodology and evidence processing. Intensive collaboration of clinical and methodological experts in the multi-disciplinary working-group, together with experts from both German Study Groups Multiple Myeloma and the German-speaking Multicenter Myeloma Group ensured clinically relevant and up-to-date pathway drafts. In a first step, information from all available in-house clinical pathways was used as a first harmonized draft. Experts from four leading centers in the field of myeloma research and treatment in Germany (Cologne, Freiburg, Heidelberg, Wuerzburg) agreed after intense discussion during face-to-face meetings and after including latest research results from literature searches into one pathway. The resulting pathway draft was discussed with experts from all 14 Centers of Excellence during face-to-face meetings, conference calls and two online surveys until consensus was reached. The project is funded by the DKH, No. 111493.

Results: During the development process, a number of similarities and discrepancies between centers as well as evidence gaps were identified. Intense discussion and literature searches resulted in a concise, harmonized clinical pathway, released by all 14 Centers of Excellence. This is freely available on the website ccc-netzwerk.de and provides a very decisive insight according to the current state of knowledge on the CCC-level (e.g. on the diagnostic algorithm, Fig. 1). The clinical pathway is well suited for informing patients and physicians about the most up-to-date, comprehensive medical treatment standards as well as innovative procedures. Furthermore, this project initiated the idea of developing a national evidence-based clinical practice guideline for MM in the frame of the German Guideline Program in Oncology.

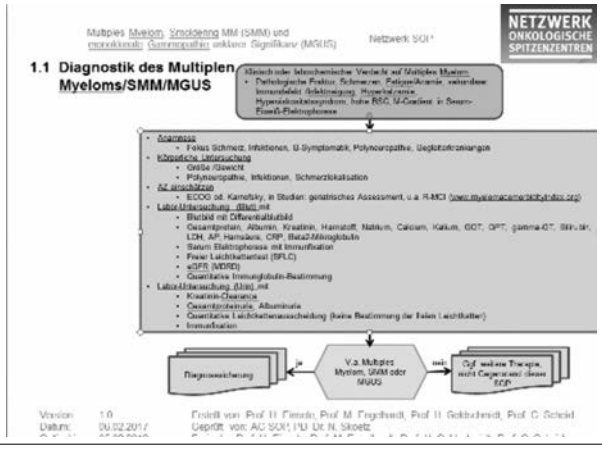

Figure 1.

Summary/Conclusions: The first clinical MM pathway developed and released by all 14 German CCCs/Centers of Excellence translates scientific evidence and expert knowledge into precise suggestions for MM patients in clinical practice. These harmonized processes might increase the quality of cancer care throughout Germany. To be up-to-date and to reflect latest research findings this clinical pathway will be updated every 18 months.

E1252

WT1 HETEROCLITIC EPITOPE IMMUNIZATION FOLLOWING AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH HIGH-RISK MULTIPLE MYELOMA (MM)

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Background: The Wilms tumor 1 (WT1) protein is a tumor associated antigen that is a target for anticancer immunotherapy. We had previously demonstrated overexpression of WT1 in multiple myeloma (MM) cells by IHC, as well as formation of a WT1 peptide fragment (RMFPNAPYL)/HLA-A*0201 complex on the engagement interface between malignant plasma cells and T-cells in HLA-A*0201$^+$ MM pts using the high-affinity fully human IgG1 mAb ESK1. We report initial results from MM pts immunized with the WT1 heteroclitic peptide mixture galinpepimut-S (GPS) after autoSCT.
Aims: To determine the safety and potential efficacy of the WT1 heteroclitic peptide immunizer GPS administered in patients with multiple myeloma following autologous stem cell transplantation.
Methods: 16 MM pts underwent autoSCT with melphalan conditioning followed by (f/b) lenalidomide maintenance starting 3 months (mos) post-SCT. 13/16 pts presented with high-risk (HR) cytogenetics [t(4;14), t(14;16), del17p, 1q21/25 gain and/or del13q]. GPS was administered with montanide s.c. starting 2 wks post-SCT and q.2 wks thereafter x 6 initial doses f/b boosters q.4 wks x 6 additional doses. GM-CSF was given on days -2 and 0 of each cycle. GPS consisted of 4 peptides: WT1-A1: Y*MFPNAPYL; 427-L (long): RSDELVRHHNMHQRNMTKL; 331-L: PGCNKRYFKLSHLQMHSRKHTG, and 122A1-L: SGQAY*MFPNAPYLPSCLES. 2 of the 4 peptides were mutated at a single residue (*) to induce stronger HLA-binding/reduce tolerance. WT1-specific immune responses (IRs) were assessed by intracellular IFN-g analyses post-challenge with PBMC's pulsed with a 'total pool' of overlapping 15mers along the entire WT1 protein; or each of the 4 WT1 peptides in GPS; or the non-mutated (native) WT1 peptides corresponding to the 2 heteroclitic sequences.
Results: 16 pts underwent auto SCT followed by WT1 heteroclitic peptide immunization; median follow-up of 18 mos (range: 5-31 mos) for survivors; median age: 61.6 y. Overall survival (OS) and progression-free survival (PFS) (95% CI) at 18 mos: 0.88 (0.73-0.99) and 0.62 (0.42-0.97) respectively. Current median PFS: 23.6 mos (15.2- not reached). No ≥G2 systemic side effects were observed, however, all pts developed local nodularity at the site of injections which resolved over 2 – 6 wks. Both CD8+ and CD4+ IRs could be detected at various levels and were induced not only against the heteroclitic peptides (within GPS), but also against the corresponding native WT1 peptide sequences as well as the 'total pool' of WT1-derived overlapping peptides.
Summary/Conclusions: Administration of the novel WT1 heteroclitic peptide immunizer GPS post-SCT demonstrates favorable safety profile along with an encouraging mPFS of currently 23.6 mos in this high-risk MM population. This 'off-the-shelf' immunotherapeutic is easy to administer and has been specifically designed to elicit responses across most common HLA Class I and II alleles. Based on these results, a larger phase II trial is being planned to optimally integrate post-transplant immunotherapeutic strategies to meaningfully delay or reduce risk of relapse in this challenging clinical setting.

E1253

ANALYSIS OF MULTIPLE MYELOMA PATIENTS WITH PROGRESSIVE DISEASE AT TIME OF FIRST AUTOLOGOUS STEM CELL TRANSPLANTATION: PREDICTORS OF PROGRESSIVE DISEASE AND FACTORS AFFECTING SURVIVAL

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Background: The impact of response depth at time of autologous stem cell transplantation (ASCT) on the progression-free and overall survival (PFS and OS, respectively) of multiple myeloma (MM) patients has been an extensively investigated area. Rosiñol et el. (2011) reported a significantly worse PFS and OS in patients with progressive disease (PD) compared to stable disease (SD) at time of ASCT; Parrish *et al.* (2015) stated a significantly shorter PFS, but not OS, in patients with PD in comparison to SD or minimal response (MR) at time of ASCT.
Aims: Our goal was to characterize MM patients with PD at time of first ASCT. We aimed to analyze the effects of PD on PFS and OS. Next, we intended to examine the influence of clinical factors (evaluated at beginning of induction therapy) as well as use of novel agents in induction therapy, response after 1st ASCT, and use of maintenance therapy in those patients to identify predictors for PFS and OS. Furthermore, we aimed to identify clinical/ therapeutic features predicting the occurrence of PD before ASCT.
Methods: Non-trial MM patients who had undergone a single or tandem ASCT at the University Hospital of Heidelberg in the years 1992-2014 were analyzed regarding their response before 1st ASCT (d -100 or, if available, response as close to the date of ASCT as possible, *i.e.* until d -4). Of the 874 ASCT-patients, 829 were eligible for a PFS- and 832 for an OS-analysis. In 51 patients, PD was present at time of ASCT. PFS and OS of those patients were compared with the survival of patients with at least SD at time of ASCT (non-PD patients). Furthermore, clinical factors at beginning of induction therapy, including age (< *vs* ≥ 65 years), ISS stage, elevated LDH, use of novel agents in induction therapy, high-risk FISH cytogenetics (at least one of the following: del17p, 1q21 gain, t(4;14)); response after ASCT, and maintenance therapy (yes *vs* no) were analyzed regarding their impact on PFS and OS of patients transplanted in PD. We also analyzed clinical factors at beginning of as well as use of novel agents in the induction therapy regarding their impact on the presence of PD before ASCT. Response was evaluated according to EBMT-criteria. PFS was calculated from date of 1st ASCT, except for prognostic impact of response assessment after 1st ASCT, where date of response assessment was used. Start of maintenance therapy was analyzed as time-dependent factor.
Results: Non-trial patients transplanted in our center between 1992 and 2014 with PD ≤100 days before ASCT had similar PFS and OS as non-PD patients. Neither the clinical parameters at induction start, response after 1st ASCT, nor maintenance therapy had a significant effect on PFS in those patients. In the univariate analysis, high-risk cytogenetics as well as elevated LDH at induction start had a significantly negative effect on OS in patients with PD before ASCT (HR= 17.12, p= 0.0017; HR=6.09, p= 0.01, respectively), compared to PD-patients with no high-risk cytogenetics or with normal LDH. Furthermore, ISS stage III was a significant predictor (OR= 3.35, p= 0.02)) of occurrence of PD before ASCT.
Summary/Conclusions: In conclusion, our analysis of 51 patients with PD at time of ASCT- among 874 ASCT-patients with MM transplanted between 1992 and 2014- shows no significant difference regarding PFS and OS between patients with PD and other response rates. It was further shown that high-risk cytogenetics as well as elevated LDH at beginning of induction therapy have a significant impact on worse OS in patients with PD at time of ASCT. In addition, ISS stage III is a significant factor for occurrence of PD before ASCT. The impact response depth at time of ASCT is not entirely clear, especially regarding the benefit of ASCT in patients with PD at time of ASCT, as reports from other centers show significantly worse PFS and OS (Rosiñol et al.) or only PFS (Parrish et al.) in patients with PD at time of ASCT.

E1254

SEVERE INFECTIONS IMPACTS OVERALL SURVIVAL IN ACTIVE MULTIPLE MYELOMA PATIENTS

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Background: Multiple myeloma (MM) represents the second most common hematological malignancy characterized by the proliferation of monoclonal plasma cells (PC) in the bone marrow. The natural history of active MM patients may be complicated in significant fraction by the occurrence of infections that can be related both to the development of therapy induced neutropenia (mainly due to high dose chemotherapy used in the setting of autologous stem cell transplantation or in salvage regimen) or to MM induced secondary immunodeficiency.
Aims: The aim of this study was to analyse the frequency, the type and the major risks factors of severe infections in our cohort of patients affected by MM and to understand the impact of these events on MM patient overall survival (OS).
Methods: A cohort of 341 patients affected by MM (104 with smouldering MM and 237 with symptomatic MM) followed from 1996 to 2016 was retrospectively studied for the presence of severe infections (si, defined by the need of hospitalization) during the natural history of the disease. Infections were classified as "not neutropenia related" or "neutropenia related" according to the Absolute Neutrophil Count > or <1,000/ml respectively. International Staging System (ISS) and Durie-Salmon (DS) were used for MM patients staging.
Results: In our cohort of patients, si were significantly associated to active MM (28,69% of symptomatic patients *vs* 3,85% of asymptomatic patients; p=0.0001, c^2=25,318). Among the 138 infective events occurred in 91 active MM patients, 38 (28%) were neutropenia related while remnant 100 not neutropenia related (72%). Furthermore, almost 44% of these events (61/138) developed during induction therapy, with 12 out of 61 (20%) being present at time of the diagnosis. Considering that majority of si was not neutropenia related and that these infective events involved most of active MM patients who developed si (68/91, 75%), our aim was to identify MM patient characteristics associated to the development of not neutropenia related si. Our results prove evidence that major features presented at the time of the diagnosis significantly associated to si were DS stage III (p=0.0004, c^2=12,14), ISS stage III (p=0.0001, c^2=21,11), age >70 years (p=0.0195, c^2=5,455), bone marrow plasma cells >60% (p=0.034, c^2=4,50), acute renal failure (p=0.0003, c^2=13,010) or MM presenting with at least three of CRAB criteria (p=0.0123, c^2=6,26). For what concern the impact of si on the natural history of the disease, patients who experienced infective event presented a reduced OS towards other patients (p<0.0001). Among infected patients no significant differences were reported referring to the number of infections (>1 or =1, p=0.11), while patients who developed exclusively neutropenia related infective events showed better OS towards patients who experienced not neutropenia related infections (p=0.0011).

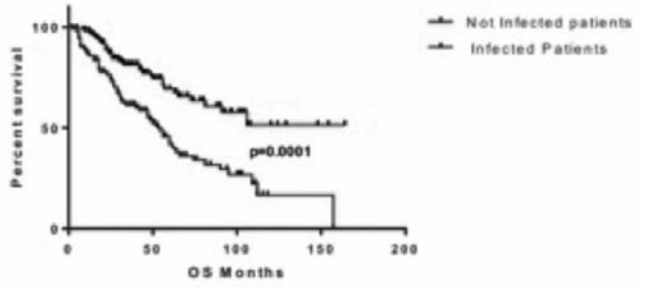

Figure 1.

Summary/Conclusions: Severe infections represent an underestimated comorbidity in MM, characterizing all phases of the disease and not only refractory/relapsed patients receiving multiple lines of therapy. Considering that severe infections impact OS mostly in the setting of not neutropenia related infections, immunoglobulin replacement therapy or antibiotic prophylaxis may possibly have a protective role in high risk old patients characterized by ISS and DS stage III, bone marrow PC >60% and aggressive disease at the time of diagnosis.

E1255

EVALUATION OF CARDIOVASCULAR EVENTS ASSOCIATED WITH DIFFERENT TREATMENT MODALITIES OF MULTIPLE MYELOMA IN THE REAL-WORLD SETTING IN THE UNITED STATES

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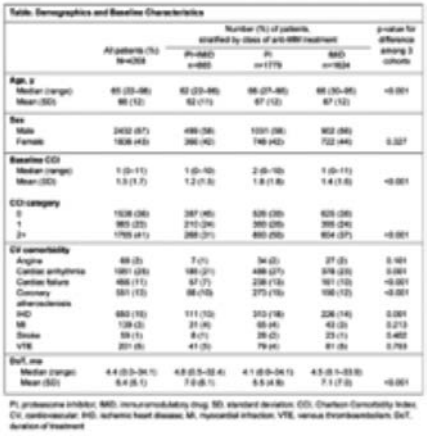
Background: Multiple myeloma (MM) is a disease of the elderly. The prevalence of cardiovascular (CV) comorbidities in the MM population is high. Past research suggests that MM is associated with a range of cardiac risks, and emerging evidence shows that both proteasome inhibitors (PIs) and immunomodulatory drugs (IMiDs) can have important CV sequelae. The improved efficacy of PI plus IMiD combination therapy (PI+IMiD) has resulted in its widespread adoption, which suggests that CV events may become a prominent concern in patients receiving PI+IMiD as contemporary treatment for MM.
Aims: To assess the risk of developing CV events in patients receiving anti-MM treatment and to test if a specific treatment modality was associated with higher risk of a CV event.
Methods: Patients with ≥1 inpatient claim or ≥2 outpatient claims with a primary diagnosis code for MM who were treated with PI and/or IMiD drugs between Jul 2012 and Sep 2014 were identified in a large US claims database. The first claim for a PI or IMiD drug in this period was defined as the index date, which was preceded by 180-d continuous eligibility with no anti-MM treatment (baseline). Patients were divided into three cohorts based on the anti-MM treatment received: PI, IMiD, PI+IMiD. CV events of interest included cardiac arrhythmia, cardiac failure, venous thromboembolism (VTE), myocardial infarction, ischemic heart disease, angina, stroke and coronary atherosclerosis, and were measured during anti-MM treatment. Kaplan–Meier methods were used to estimate the occurrence rate of a CV event, and multivariate Cox regression models were developed to identify prognostic factors of each CV event among patients treated with anti-MM therapies.
Results: 4268 patients met the eligibility criteria for inclusion in the study (57% male, median age 66 y, 41% with Charlson Comorbidity Index ≥2, mean duration of treatment 192 d; Table), 42% (n=1779) were treated with PIs, 38% (n=1624) with IMiDs and 20% (n=865) with PI+IMiDs. Patients receiving PI+IMiD were significantly younger and generally had lower prevalence of CV comorbidities than those receiving PI or IMiD (Table). Compared with patients on PI, the risk of developing VTE was 46% greater in patients on PI+IMiD (HR: 1.46; 95% CI: 1.09, 1.96). Compared with those on IMiD, the risk of developing cardiac failure and cardiac arrhythmia was 33% and 18% greater in patients on PI+IMiD (HR: 1.33; 95% CI: 1.03, 1.72; HR: 1.18; 95% CI: 1.00, 1.40). After 6 months of treatment, the rates of VTE were 8%, 10% and 11% for patients on a PI, those on an IMiD and those on PI+IMiD, respectively. The corresponding rates for cardiac failure were 18%, 11% and 11% for PI, IMiD and PI+IMiD cohorts, and 21%, 16% and 22% for cardiac arrhythmia.

Table 1.



Summary/Conclusions: PI+IMiDs may be associated with incremental occurrence of specific CV events during treatment, and may result in specific CV events earlier during therapy than PIs or IMiDs alone. These highlight a need for treatments that do not exacerbate CV risks and are appropriate for patients with pre-existing CV conditions. The lower prevalence of baseline CV comorbidities and lower mean age in patients on PI+IMiDs suggest that prevalence of a CV comorbidity and age influences treatment choice. Further analysis may be necessary to better understand the impact of baseline CV comorbidities on choice of MM treatment.

E1256

LENALIDOMIDE PLUS HIGH-DOSE *VERSUS* LOW-DOSE DEXAMETHASONE FOR THE TREATMENT OF RELAPSED/REFRACTORY MULTIPLE MYELOMA: A SYSTEMATIC REVIEW

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Background: Lenalidomide in combination with dexamethasone is approved globally for the treatment of multiple myeloma (MM). Although older pivotal registrational studies used lenalidomide combined with high-dose dexamethasone (LD), more recent studies have used lenalidomide plus low-dose dexamethasone (Ld) for relapsed/refractory MM (RRMM), as the Ld regimen demonstrated better survival with lower toxicity for the treatment of newly diagnosed MM

(NDMM; Rajkumar SV *et al. Lancet Oncol* 2010;11:29–37). Additionally, all recent Phase 3 trials establishing new triplet combinations with lenalidomide and dexamethasone in RRMM, such as ELOQUENT-2 (study of elotuzumab plus Ld in RRMM; Lonial S *et al. N Engl J Med* 2015;373:621–31), used the Ld regimen in both standard and experimental arms. Although the lenalidomide label includes Ld for NDMM, there are differences between the approved dose of dexamethasone and what is currently used in real-world clinical practice for RRMM.
Aims: We performed a historical comparison based on a systematic review of literature describing low- *vs* high-dose dexamethasone in patients with RRMM to assess effects of Ld *vs* LD on safety and efficacy outcomes.
Methods: We searched MEDLINE, EMBASE and Cochrane databases and key clinical trial registries for studies including adults with RRMM who had received ≥1 prior therapy and had a symptomatic relapse on their last treatment. Eligible studies evaluated Ld (lenalidomide: 25mg on Day 1–21 of each cycle; dexamethasone: 160mg/cycle, not pulsated) or LD (Cycles 1–4: 480mg/cycle; Cycle 5+: 160mg/cycle, pulsated). Only those trials with designs and baseline patient characteristics similar to ELOQUENT-2 were eligible to ensure comparability. Studies with a follow-up of 16–25 months were evaluated separately from studies with a follow-up of >30 months; these observation periods approximately align with those of ELOQUENT-2.
Results: From an initial bibliographic search yielding 5155 non-duplicate results and 619 registry results, 7 studies (8 publications) met the inclusion criteria (4 Ld studies, 3 LD studies). Data for overall survival and tolerability from 1153 patients in the Ld group and 353 patients in the LD group were analyzed. The median patient age was 63–68 years. Most patients were white, male and had an ECOG score of 0-1. Ld was not associated with loss of efficacy *vs* LD in terms of overall survival; after >30 months of follow-up, the hazard ratio for LD *vs* Ld was 1.04 (95% CI 0.85-1.28). Tolerability was similar for LD *vs* Ld; after 16-25 months of follow-up, LD was associated with a statistically significantly increased risk of Grade 3/4 adverse events (AEs; relative risk [RR]: 1.10 [95% CI 1.01-1.18]). However, after >30 months of follow-up, LD was not associated with an increase in overall AEs (RR: 1.02 [95% CI 1.01-1.03]), grade ≥3 AEs (RR: 1.03 [95% CI 0.95-1.12]) or serious AEs (RR: 1.08 [95% CI 0.97-1.20]); RR for AEs leading to discontinuation was 1.16 (95% CI 0.87-1.54).
Summary/Conclusions: Overall survival and safety are not significantly affected by different dosing of dexamethasone in combination with lenalidomide; thus, the use of Ld seems to be reasonable in this patient population. Further studies may provide additional evidence to inform clinicians and revision of international guidelines for dexamethasone dosing in RRMM.

Study funded by Bristol-Myers Squibb.

E1257

HIGH EFFICACY AND SAFETY OF VTD AS AN INDUCTION PROTOCOL IN NEWLY DIAGNOSED MM PATIENTS ELIGIBLE FOR HDT/AUTOSCT – A REPORT OF POLISH MULTIPLE MYELOMA STUDY GROUP

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Background: Three drug bortezomib-based regimens are nowadays generally recommended standard induction therapy for transplant-eligible patients with newly diagnosed multiple myeloma (MM). The choice between different regimens depends on drug availability in particular countries, their toxicity profile and local preferences. Observations from routine practice might have though significant clinical importance.
Aims: The aim of this retrospective analysis was to evaluate the efficacy and safety of VTD regimen in newly diagnosed MM patients eligible for HDT/autoSCT in routine clinical practice.
Methods: We collected the clinical data of 169 patients qualified to HDT/autoSCT treated with VTD as an induction regimen in 14 Polish hematology/oncology centers. VTD protocol recommended by Polish Multiple Myeloma Study Group was as follows: bortezomib: 1.3mg/m^2 (days 1,4,8,11), thalidomide: 100 – 200mg a day (days 1-21), dexamethasone 20mg a day (days: 1,2, 4,5, 8,9, 11,12) or 40mg a day (days 1- 4), every 21 days. Patients were included into analysis if ≥1 cycle of VTD was administered. Adverse events (AEs) were graded according to CTCAE v4.0. The analysis involved also the impact of VTD regimen on efficiency of stem cells mobilization as well as high dose therapy/ autologous stem cell transplantation (HDT/autoSCT) procedure.
Results: In the cohort of 169 patients, median age was 59 years (range 36-70). ISS stage 1 was found in 30.8% of patients, ISS 2 and 3 in 20.7% and 45.5%, respectively. Median number of VTD cycles was 5. In 81.6% of patients bortezomib was administered subcutaneously. Thalidomide dose was 100mg a day in 85.1% of patients. Bortezomib dose was reduced in 43 patients (25.4%) with peripheral polyneuropathy as the most common reason (75%). Polyneuropathy was also the most common grade 3/4 adverse event, observed in 20 patients (11.8%) and neutropenia was the most common hematologic toxicity, though it was noted only in 5 patients (3%). Response rate ≥ PR was achieved in 95% of patients, including 5.6% of sCR, 27.1% of CR and 35.1% of VGPR. So far, stem cell mobilization was performed in 110 patients, most commonly used protocols were cyclophosphamide (42.9%) and cytosine arabinoside (36.2%). In 69.3% of patients one apheresis allowed to obtain the number of stem cells sufficient for transplantation. Median yield of CD34+ cells was 11 x 10^6/kg (max 55.7x10^6/kg) that was sufficient for two transplantations in the majority of patients. HDT/autoSCT was performed so far in 89 patients with MEL 200 protocol as conditioning regimen in 77.6% of patients. Median number of transplanted CD34+ cells was 4.4 x 10^6/kg. Median time to reach ANC count >0.5 G/L and PLT count >20 G/L was 11 days and 12 days, respectively. In the evaluation of response 100 days after HDT/autoSCT performed in 81 patients, sCR rate increased from 5.6% to 12.7% and CR from 27.1% to 36.7%.
Summary/Conclusions: VTD regimen allowed allowed to achieve ≥ PR in 95% of patients including ≥ VGPR u 64.8% of patients as compared to 73.5% ≥ PR including 36% of ≥ nCR achieved in patients treated with CTD in our previous study (Dmoszynska *et al.* Leuk Res 2010). In 96% of patients subsequently undergoing stem cell mobilization sufficient numer of CD34+ cells was obtain during first procedure. HDT/autoSCT further increased response rate after VTD induction (≥ CR up to 43.5%, ≥ VGPR up to 83.5%).

E1258

HIGH CUT OFF HEMODIALYSIS FOR RENAL RECOVERY IN PATIENTS WITH MULTIPLE MYELOMA: FIVE YEARS OF EXPERIENCE

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Background: Up to 20% of patients with multiple myeloma (MM) present acute kidney injury (AKI) as complication and half of them can even require dialysis. The toxicity of serum free light chains (sFLC) is the main cause of renal damage in MM.
Aims: Demonstrate that rapid and sustained reduction of sFLC levels by combined chemotherapy and high cut-off hemodialysis (HCO-HD) improves renal outcome.
Methods: From July 2011 to July 2016, were performed 21 treatments on 19 patients with MM and serum concentrations of FLCs above 500mg/L who had severe AKI requiring hemodialysis according to KDIGO criteria. The HCO-HD was initiated immediately after establishing hematology diagnosis and simultaneously to bortezomib-based induction chemotherapy. A dialytic protocol was instated based on the Hutchinson scheme: HCO filter of 2.1m^2 (Theralite™ by Gambro®). Initially daily sessions on 6 consecutive days; afterwards, dialysis on alternate days until getting levels of sFLC under 500mg/L or recovering a renal function to avoid dialysis. The duration of every dialysis was 6 hours with low blood flow between 250-300ml/min, and a bath flow of 500 ml/min (ultrapure water). Blood monitoring includes renal function, sFLC, calcium, phosphorus, albumin and ions.
Results: The patients were 12 men and 7 women, aged 60±4 years (37–73 years). 10 patients were diagnosed with lambda FLC MM and 9 with kappa type. A total of 244 sessions were conducted, with an average of 11.6 sessions per patient (range 3-27) In all cases reduction of serum FLCs concentration was successfully achieved. (90% of reduction). At the end of treatment with HCO-HD, the reduction of lambda and kappa FLCs concentrations was 85% and 94%, respectively. The average reduction per dialysis session was 65% for lambda and 60% for kappa. 17 out of the 21 treated cases recovered sufficient renal function to become independent of dialysis (80.9% renal recovery). Renal recovery appears to be sustained over time. There was a significant association between the percentage reduction in serum FLCs concentrations and renal recovery. Our results confirm previous findings on the effectiveness of FLCs reduction by HCO-HD. Until randomized trials yield results, our highest percentage of improve renal outcome with respect to published studies leads us to recommend combined therapy of chemotherapy and HCO-HD in patients with MM and renal failure.
Summary/Conclusions: In dialysis-dependent AKI secondary to MM, combination HCO HD with chemotherapy allows a sustained reduction of FLCs levels, representing an effective therapy in renal recovery.

E1259

IMPACT OF IMMUNOPARESIS IN PATIENTS WITH LIGHT CHAIN AMYLOIDOSIS

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Background: The presence of immunoparesis (IP) at diagnosis in several plasma cell disorders is a risk factor for progression, associated with an unfavorable outcome with reduced progression-free survival (PFS) and overall survival (OS). However, its impact in light chain (AL) amyloidosis has been evaluated only in few series, and when present it was associated with worst response and survival.
Aims: The aim of this study was to investigate the prognostic impact of IP in patients with newly diagnosed AL amyloidosis at a single institution.
Methods: We reviewed the clinical records of patients with AL amyloidosis diagnosed from January 2006 to December 2016. Sixty-nine patients (32F/37M; median age at diagnosis 62) with available immunoglobulin (Ig) measurements were the final study population. Initial baseline demographics, clinical and laboratory data, treatment and follow-up were collected. Median follow-up was 30.2 months. IP was defined as suppression of all uninvolved Ig below the lower reference value. PFS and OS were calculated from the date of diagnosis.
Results: Forty-three patients (62.3%) were transplant ineligible while 26 (37.7%) underwent an autologous stem cell transplantation (ASCT). The distribution of the monoclonal protein isotype by immunofixation at diagnosis was as follows: light chains only (46.4%), IgG (39.1%), IgA (10.2%) and IgM (4.3%). The predominant light chain isotype was lambda (79.7%). A very good partial response (VGPR) or better was achieved in 53.6% of patients. Three-year OS rate was 64.3%. IP was observed in 27.5% of the patients at diagnosis. Patients with IP had a higher bone marrow plasma cells (BMPC) infiltration (29 *vs.* 9; *P*<0.001). Also a trend towards a higher difference between involved and uninvolved free light chains was observed in the group of patients with IP (360.2 *vs.* 221.7; *P*=0.08). IP was more frequent in those who received an ASCT (57.9% *vs.* 42.1%; *P*=0.03). There were no changes in the frequency of IP comparing at diagnosis *vs.* post-ASCT settings (39.13% *vs.* 34.78%; *P*=0.4). Regarding its prognostic value, IP did not influence survival in the whole series. In the ASCT group, the presence of IP resulted in a significantly shorter PFS (median: 30.2 months *vs.* not reached [NR]; *P*=0.019; Figure 1A) and a trend in OS (62.5 months *vs.* NR; *P*=0.097). In the group of patients with stage I and II of the Mayo risk stratification system of 2012 (Mayo12), the presence of IP resulted in a shorter PFS (21.8 months *vs.* NR; *P*=0.047; Figure 1B), but not significantly different in OS. Multivariate analysis restricted to the population of patients with stage I and II Mayo12, incorporating ASCT, BMPC and IP, indicate that IP retained its independent prognostic factor for worse PFS (HR=12.06; 95% CI, 1.9-75.7; *P*=0.008).

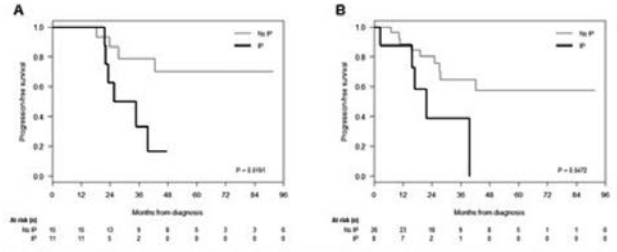

Figure 1. Progression-free survival according to the presence of immunoparesis in the group of patients who received an autologous stem cell transplantation (A), and in the group of patients with stage I and II of the Mayo risk stratification system of 2012 (B).

Figure 1.

Summary/Conclusions: The presence of IP has a negative impact on survival, especially in the sub-group of patients in early stages of the disease. The presence of IP at diagnosis could be an additional powerful discriminatory prognostic indicator in the group of patients without advanced stage of the Mayo risk stratification system of 2012.

E1260

TREATMENT PATTERNS AND DURATION OF TREATMENT IN JAPANESE MULTIPLE MYELOMA PATIENTS RECEIVING SECOND LINE THERAPY WITH NOVEL AGENTS

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Background: The introduction of novel agents, such as proteasome inhibitors (PIs) and immunomodulatory drugs (IMiDs) approved in 2006 and 2010, respectively, and/or autologous stem cell transplantation (SCT) are associated with improved overall survival of 60.6 months in Japanese multiple myeloma (MM) patients (pts) (Ozaki *et al.* Blood Cancer Journal 2015). However, the disease still remains incurable with disease relapse being inevitable after front-line therapy (FLT). Data regarding treatment patterns and duration of treatment (DOT) of Japanese pts with relapsed and refractory (RR) MM in routine clinical practice is limited.
Aims: This retrospective study aims to describe the treatment patterns and DOT of second-line therapy (SLT) with PI- and IMiD-based regimens and to assess factors that influence treatment choice and DOT of SLT in Japanese MM pts.
Methods: This is retrospective cohort study in pts with MM diagnoses with ICD-10-CM (C900) codes between April 2008 and January 2016 in Japan. This study used Japanese health insurance data provided by Medical Data Vision. MM pts receiving SLT were included. Index date was defined as the first observed claim for MM treatment and SLT was defined as switch to another drug combination>60 days or retreatment following a treatment gap of>90 days after starting FLT. Pts with salvage SCT were excluded. Observations were censored at loss to follow up, death or the end of study period. Kaplan-Meier analyses were performed to calculate DOT from the start of SLT. Welch's test was used to test for statistical significance between groups.
Results: Among 585 pts receiving SLT, mean age was 68.8 years of age (yo); 65.3% were ≥65 yo at start of SLT; 54.2% were male. Most pts received lenalidomide (L)-based SLT (35.4%), followed by bortezomib (B)-based regimens (29.4%) and other regimens not containing novel agents (35.2%); Other regimens includes thalidomide, cyclophosphamide, etoposide, melphalan, vincristine, (liposomal) doxorubicin, interferon, panobinostat, single-agent steroid; only 1.2% received B+L combination therapy. L±D and B±D were the most common (35.2% *vs* 21.7%) in SLT. Majority of Japanese pts received B-based regimen in FLT among those receiving L±D and B±D SLT (77.2% *vs* 55.1%). Pts with peripheral neuropathy (PN) and renal insufficiency (RI) prior to SLT were 30.3% and 15.6%, respectively; those with PN were more likely to receive L±D compared to B±D (35.9% *vs* 21.3%, P=0.0047), but those with RI was not independently associated with treatment choice of SLT. Median DOT of L±D was longer than B±D (13.8 *vs* 6.9 months, P=0.0001); DOT was similar for those without a front-line SCT and receiving B±D FLT in both regimens (11.9 *vs* 11.9 months). PN and RI prior to SLT and age have not shortened the DOT in SLT. Additionally, 35.4% experienced PN during SLT among pts receiving L±D and B±D in SLT but there was no statistical significant difference of DOT between pts with and without PN. Median daily dose of L was 12.0mg; there was no significant difference of DOT between pts received at least and less than 12.0mg.

Table 1.

Table 1. Patient Characteristics and DOT by SLT Regimen

Type of SLT Regimen	L ± D N=206	B ± D N=127	p-value
Median Age (yo)	67	71	0.119
Front-line SCT (%)	32.5	14.2	0.0002
PN prior to SLT (%)	35.9	21.3	0.0047
RI prior to SLT (%)	16.0	15.0	0.7960
DOT (mos)	13.8	6.9	0.0001
DOT for pts <65 (mos)	11.3	5.5	0.0030
DOT for pts ≥65 (mos)	17.0	6.9	0.0001

Summary/Conclusions: Among pts in SLT, 65% of Japanese pts obtained L- and B-based regimens. This observation is similar to the United States (Romanus *et al.* EHA 2016) and Europe (Raab *et al.* EHA 2015). Majority of pts did not receive triplet-based regimen. Pts experienced PN in FLT were more likely to initiate L-based therapy in SLT and regimen type in SLT was correlated with DOT. Future research is needed to better understand treatment changes in routine clinical practice and the impact on pts' outcomes, especially, after integration of novel agent-based triplet combinations as new standards of care in RRMM in Japan.

References

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E1261

ROLE OF HEAVY/LIGHT CHAIN RATIO IN MYELOMA PATIENTS ACHIEVING COMPLETE RESPONSE AFTER FIRST LINE THERAPY

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Background: Polyclonal antibodies against the conformational epitopes between the heavy and light chains (HLC) of immunoglobulin (Ig) have been recently introduced as diagnostic tool in multiple myeloma (MM) and other monoclonal gammopathies. They separately identify the two different light chain types of each Ig, allowing the quantification of the monoclonal component. HLC and HLC ratios may be particularly useful for monitoring the presence of monoclonal component in oligo-secretory MM or when it migrates in the β region, as frequently observed in IgA MM. The International Myeloma Working Group (IMWG) has published in 2016 new consensus criteria for assessing response and minimal residual disease (MRD) in MM, outlining the potential role of HLC assay in this setting and the need of its further investigation, particularly in patients achieving complete response (CR) after treatment.
Aims: We conducted a single center, prospective study of HLC ratio, in comparison with free light chain (FLC) ratio, for the evaluation of MRD and its prognostic role in MM patients achieving CR after first line treatments including novel agents.
Methods: Twenty-five consecutive patients were evaluated. Mean age was 63 years (range 43-82), fourteen patients were males. Ig isotype was IgG or IgA in 14 and 11 patients, respectively, with 20 patients showing kappa and 5 lambda light chains. According to International Staging System, seven patients had stage 1, ten stage 2 and eight stage 3. Fourteen patients not eligible to autologous stem cell transplantation (AuSCT) received a bortezomib-based treatment, mainly constituted by bortezomib, melphalan and prednisone combination (VMP), while eleven patients underwent AuSCT after induction therapy with bortezomib, thalidomide and dexamethasone (VTD). With a median follow-up of 52 months (range 21-92), overall survival (OS) of the entire cohort was 61 months (95% CI 52-80) and progression-free survival (PFS) was 26 months (95% CI 12-38). Ig HLC pairs (IgGk/IgGλ and IgAk/IgAλ) and FLC were analyzed on serum samples at diagnosis and at the time of immunofixation negative CR (according to 2006 IMWG criteria), using Hevylite and Freelite commercial kits, respectively, on a SPAplus analyzer (Binding Site); IgGk/IgGλ, IgAk/IgAλ and k/λ ratios were then calculated.
Results: At CR time, we found seven (28%) samples still showing abnormal HLC ratio and fourteen samples (56%) with abnormal FLC ratio. Discrepancies between the two assays occurred in 11 patients. FLC assay normalization in CR was significantly associated with better PFS (43 months, 95% CI 14-45) respect to patients with persistent abnormal FLC ratio (12 months, 95% CI 9-35, p=0,049). In contrast, normalization of HLC ratio had no impact on PFS (26 months, 95% CI 10-38, *vs* 20 months, 95% CI 10-34, p=0,51), even selecting IgA MM. Notably, in 9 patients, the negative effect of abnormal FLC ratio at CR on PFS was not mitigated by concomitant normalization of HLC ratio (19 months, 95% CI 4-35; p=0,022). Neither FLC, nor HLC affected OS. There were no differences between patients who received AuSCT and those who did not.
Summary/Conclusions: To the best of our knowledge, this is the first study to analyze HLC ratios exclusively in MM patients in CR. While our preliminary data confirm the prognostic usefulness of FLC in this setting, currently they do not support a role for HLC as putative biomarker of MRD.

E1262

REAL-WORLD RESULTS OF DARATUMUMAB MONOTHERAPY IN HEAVILY PRETREATED RELAPSED/REFRACTORY MULTIPLE MYELOMA IN POLAND: A PROSPECTIVE OBSERVATIONAL STUDY OF THE POLISH MYELOMA GROUP

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Background: Emerging resistance to modern antimyeloma agents such as proteasome inhibitors (PIs) and immunomodulatory drugs (IMiDs) remains the main clinical problem in managing multiple myeloma (MM). Daratumumab, a first-in-class anti-CD38 monoclonal antibody, has been recently approved in Europe as a monotherapy for patients (pts) with relapsed and refractory multiple myeloma (RRMM), whose prior therapy included a PI and an IMiD and whose disease had progressed during this time; based on the SIRIUS clinical trial outcome (Lonial *et al.* Lancet 2016). Nevertheless to optimize daratumumab use in clinical practice, more data on its "real life" activity and safety are still required.
Aims: This observational study of the Polish Myeloma Group (PMG) was aimed to prospectively evaluate the efficacy and toxicity of daratumumab monotherapy in RRMM pts treated within the daratumumab compassionate use named patient program in Poland (DaraCUP).
Methods: Patients were eligible for DaraCUP if they met all the following criteria a) confirmed diagnosis of RRMM, b) relapse after a minimum of 3 prior lines of therapy (including PI and IMiD) or were double refractory to PI and IMiD, c) had a ECOG performance status score 2 or lower. Data on treatment outcomes and complications were anonymously collected using electronic CRFs. The IMWG response criteria were applied.
Results: In total 30 patients were qualified to DaraCUP in Poland and all were enrolled to the PMG observational study. At the time of writing this report, 26 pts (87%) had received at least one dose of daratumumab and were included in the safety analysis, while 22 pts (73%) had received at least 2 cycles of daratumumab and were included in the preliminary efficacy analysis. Baseline pts characteristics are reported in Table 1. Pts were heavily pretreated, with a median of 4 prior lines of therapy (range, 2-10). Ten pts (38.5%) were double refractory to both PI and IMiD while 15 pts (58%) were refractory to the last line of previous therapy. Median time since initial diagnosis to start of treatment with daratumumab was 3.9 years (range, 1.4-12.2 years). At the time of analysis, the median follow-up time within the study was 5.1 months (range, 0-8 months) and median daratumumab treatment duration was 4.4 months (range, 0-8 months). Sixteen pts (61.5%) remain on treatment, while ten pts (38.5%) discontinued therapy as a result of disease progression (n=7) and adverse events (AEs) (n=3). Overall response rate (PR or better) was 31.8% including one (4.5%) CR and two (9%) VGPR (Table 1). Stable disease was reported in 11 (50%) pts. The median PFS and OS had not been reached. During the time of observation three deaths were recorded due to disease progression. Regarding daratumumab toxicity, grade 3 or 4 non-haematological toxicities occurred in 8 pts (30.7%) and included: infusion-related reactions (n=2), pneumonia (n=2), other infections (n=2), mandible inflammation (n=1), dyspnoea (n=1). The most common haematological toxicities of any grade were anaemia (n=8; 30.7%) and neutropenia (n=6; 23.1%) while thrombocytopenia occurred in 3 pts; 11.5%. Grade 3 or 4 anaemia and neutropenia were found in 3 (11.5%) and 2 (7.7%) pts, respectively. Updated results will be presented at the meeting.

Table 1.

Table 1. Patient baseline characteristics and comparison with SIRIUS trial

Parameter	PMG „real life" data n=26	SIRIUS study n=106	Parameter	PMG „real life" data n=26	SIRIUS study n=106
Age (years)			Lines of previous therapy		
Median (range)	63.4 (48-82)	63.5 (31-84)			
18 to <65	17 (65.4%)	58 (55%)	>3	16 (62%)	87 (82%)
65 to <75	5 (19.2%)	36 (34%)	median (range)	4 (2-10)	5 (2-14)
> 75	4 (15.4%)	12 (11%)			
			Previous proteasome inhibitor		
Male sex	10 (38.5%)	52 (49%)	Bortezomib	26 (100%)	105 (99%)
			Carfilzomib	0	53 (50%)
Eastern Cooperative Oncology Group score			Previous immunomodulatory drug		
0	1 (4%)	29 (27%)	Thalidomide	24 (92%)	47 (44%)
1	15 (58%)	69 (65%)	Lenalidomide	23 (88.5%)	105 (99%)
2	10 (38%)	8 (8%)	Pomalidomide	0	67 (63%)
International Staging System			Previous steroids		
I	10 (38%)	36 (35%)	Dexamethasone	26 (100%)	106 (100%)
II	7 (27%)	40 (38%)			
III	9 (35%)	40 (38%)			
High-risk cytogenetic aberrations			Previous autologous stem cell transplantation	11 (42%)	85 (80%)
t(4;14)	2 (8%)	9 (10%)			
del17p	1 (4%)	16 (17%)			
Renal function (baseline creatinine clearance)			Refractory to		
>60 ml/min	15 (58%)	60 (57%)	Last line of previous therapy	15 (58%)	103 (97%)
30 to 60 ml/min	11 (42%)	42 (40%)	Both proteasome inhibitor and immunomodulatory drug	10 (38%)	101 (95%)
<30 ml/min	0	4 (4%)			
Time since initial diagnosis (years; median, range)	3.9 (1.4-12.2)	4.8 (1.1-23.8)	Response		
			sCR	0	3 (2.8%)
			CR	1 (4.5%)	0
			VGPR	2 (9%)	10 (9.4%)
			PR	4 (18%)	19 (17%)
			SD	11 (50%)	46 (43.4%)
			PD	4 (18%)	18 (17%)

Summary/Conclusions: In this first real-world analysis we confirm that daratumumab monotherapy is able to induce response in one third of highly pretreated and double refractory RRMM patients. Regarding safety, in contrast to the SIRIUS trial where no treatment discontinuations due to AEs occured, 3/26 pts (11%) treated with daratumumab in clinical practice had their therapy interrupted due to complications.

E1263

REAL-WORLD TREATMENT PATTERNS AND PATIENTS CHARACTERISTICS IN MULTIPLE MYELOMA ACROSS EUROPE

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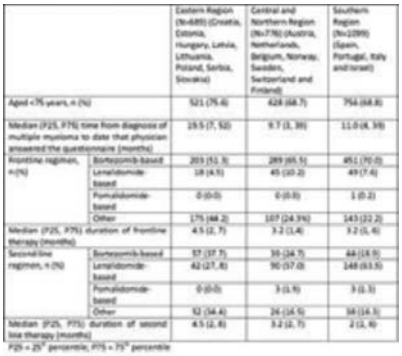
Background: Multiple myeloma (MM) is the second most common haematological malignancy after non-Hodgkin lymphomas, accounting for 13% of blood malignancies and 1% of all cancers[1]. The medical management of multiple myeloma has changed over the years and is influenced by multiple factors (e.g., evidence from clinical trials, drug approval status, level of drug reimbursement, guidelines), which vary across Europe. Information describing how patients are managed in the real world is needed.
Aims: The aim of this analysis was to investigate real-world treatment patterns and patient characteristics in MM across Europe.
Methods: Physicians in Europe were requested to answer a series of questions on patient characteristics and treatment regimens of the last eight patients that they had treated during the month prior to answering the questionnaire, according to their patients' medical charts. The questionnaire was conducted between January and June 2016. Data on 2564 patients with MM were available and are presented here. Countries were grouped into regions according to similar health care systems: Spain, Portugal, Italy and Israel (Southern Region, SR, n=1099); Austria, Netherlands, Belgium, Norway, Sweden, Switzerland and Finland (Central and Northern Region, CNR, n=776); Croatia, Estonia, Hungary, Latvia, Lithuania, Poland, Serbia, Slovakia (Eastern Region, ER, n=689). Analyses were descriptive
Results: Patient characteristics were generally similar across regions, with the majority being <75 years old (69-76%), receiving frontline therapy at study inclusion (57-58%), and being ineligible for autologous stem cell transplant (ASCT) (53-59%). The median time from MM diagnosis to the time that the physician answered the questionnaire was higher in ER (19.5 months) than other regions (9.7-11 months) (Table). The majority of frontline regimens contained bortezomib although this was lower in ER (51%) than other regions (66-70%). The median duration of frontline therapy was longer in ER (4.5 months) than other regions (3.2 months). This difference was mainly driven by ASCT eligible patients having longer duration of therapy in ER (4.5 months) than other regions (2.2 months). The number of bortezomib injections in frontline therapy, however, was higher in SR and CNR (both 24) than in ER (18). The majority of second line regimens contained lenalidomide (57-64%) in all regions except ER, where bortezomib-based regimens were most frequent (38%). The median duration of second line therapy was shorter in SR and CNR than in ER (Table). Moreover, for second line therapy, ASCT-eligible patients had shorter duration of therapy in ER and SR (3.2 months) than in CNR regions (4.5 months). The majority of later-line (3+) regimens were based on therapies that did not include bortezomib, lenalidomide or pomalidomide for all regions (57-67%) with the exception of SR where pomalidomide (29.4%), lenalidomide (12.6%) and bortezomib (14%) were the preferred options. In this analysis conducted in Europe, treatment patterns differed in terms of regimens used and duration of therapies even though patient characteristics were generally similar across regions. This may in part be related to differences in healthcare systems. Further research is required to understand how new therapies can be integrated into current treatment patterns and how these treatment patterns impact patient outcomes.

Table 1.



Summary/Conclusions: In this analysis conducted in Europe, treatment patterns differed in terms of regimens used and duration of therapies even though patient characteristics were generally similar across regions. This may in part be related to differences in healthcare systems. Further research is required to understand how new therapies can be integrated into current treatment patterns and how these treatment patterns impact patient outcomes.

E1264

FRAILTY AND MORTALITY IN ELDERLY PATIENTS WITH MULTIPLE MYELOMA

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Background: Worldwide, life expectancy continues to rise. The treatment of elderly people with cancer poses special challenges that should be better addressed. Frailty is a geriatric syndrome associated with reduced functional reserve, impairment in multiple physiological systems, and reduced ability to regain physiological homeostasis.
Aims: To evaluate the impact of the level of frailty on early death and overall survival of elderly patients with multiple myeloma.
Methods: Retrospective study of a cohort of 150 patients older than 65 years with a recent diagnosis of multiple myeloma from January 2006 to December 2012. Patients were treated with IMIDs, alkylating or bortezomib based chemotherapy based on physician preference blind to the geriatric assessment. A check list for frailty burden measurement was used based on Edmonton frailty score and included: cognitive impairment, depressive disorder, polypharmacy, urinary incontinence, functional impairment, gait disturbance or falls, low weight or weight loss and previous hospitalization. Level of frailty was scored as the sum of each area involved. Record of all the variables were obtained from a retrospective review of the centralized and computerized medical records by a hematologist and geriatrician, using predefined standardized criteria. Patients were classified as fit (0-1 frailty criteria), vulnerable (2-3 criteria) or frail (≥ 4 criteria). OS and PFS were estimated using the Kaplan Meier method using Stata13 program Group differences according to frailty were investigated using the Cox proportional hazard model accounting for ISS, age, Charlson comorbidity index and treatment.
Results: From the 150 patients evaluated, 124 patients were included in the study. The median age was 77 years (range 65-98). Thirty one percent of the patients were older than 80 years, 51% were female. The median Charlson Comorbidity index was 2 (range 0-7), 28% had renal failure and 40% of the patients presented with Myeloma ISS 3. Sixty five percent of patients met at least one frailty criteria and 31% of patients were considered frail. The most common findings were polypharmacy, gait and functional impairment. Most patients were treated with IMIDs (47%); alkylating agents (33%) or borteomib (14%) based chemotherapy. There was no difference in treatment according to frailty group (p=0.38). The median overall survival time was 75 months (95% CI 53-110), 39 months (95% CI 19-64) and 17 months (95% CI 5-37) for fit, vulnerable and frail patients respectively (log rank p 0,0002). Frailty was specially associated with early death [OR 8,2 (95% CI 1,9-34) p=0.0007]. In the multivariate analysis a higher risk of death was observed related to age [HR 1,07 (95% CI 1,02-1,12) p 0,002], number of frailty criteria [HR 1,13 (95% CI 1 – 1,3) p 0,05] and ISS [HR 2,6 (95% CI 1,8 -3,8) p 0,001]. The frailty criteria independently associated with death were incontinence polypharmacy and previous hospital admissions. Frailty was specially associated with early death [OR 8,2 (95% CI 1,9-34) p=0.0007].

Table 1.

Table 2: Univariate and Multivariate Cox Regression Analysis

Variable	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Age years	1.09 (1.05-1.3)	<0.001	1.07 (1.02-1.12)	0.002
Charlson Comorbidity index	1.2 (1.05-1.3)	0.005	1-04 (0.88-0.23)	0.61
Number of Frailty criteria	1,27 (1.14-1.43)	<0.001	1.13 (1-1.3)	0.05
ISS	2.6 (1.9 -3.9)	<0.001	2.6 (1.8 – 3.8)	0.001
Presence of Renal Failure	1.6 (0.99-2.6)	0,054		
Tipo de QT				
Alquilantes	1.38 (0.75-2.53)	0,29		
IMIDs	1.63 (0.89-2.93)	0,11		
Bortezomib	1.23 (0.45-3.37)	0,68		

Summary/Conclusions: This study shows that the prevalence of frailty syndrome is high and has a profound impact in early death. It is also independently associated with a worse prognosis. Frailty should be considered as part of the clinical assessment when treating elderly patients with myeloma.

E1265

PROGNOSIS OF AL AMYLOIDOSIS WITH KIDNEY INJURY

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Background: AL amyloidosis is a rare disease related to excessive and uncontrolled secretion of monoclonal light chains. The consequence of this proliferation is an alteration of the affected organs due to deposition of free light chains. Despite therapeutic advances in recent years based, among others, on the finding of French studies, the prognosis of this disease remains poor in particular for patients with cardiac disease. Kidney involvement is also frequently observed at diagnosis in the form of a classical nephrotic syndrome, but at present the prognosis of chronic renal failure in this context is unknown.
Aims: The study was interested in the prognosis of AL amyloidosis associated with endstage renal disease on dialysis in the era of treatment with bortezomib.
Methods: A total of 133 patients (61 from Ile-de-France region register and 72 from reference center) were analyzed.
Results: A total of 133 patients (61 from Ile-de-France region register and 72 from reference center) were analyzed. Median survival was 66.7 months compared to 70.6 months for patients without dialysis (p=0.65). Within the group

of patients on dialysis, there is no significant difference between those receiving or not bortezomib. Median survival before 2008 was 54.82 months and rose to 82.30 months for patients treated after this date (p=0.95). Age (HR: 0.2819, CI 0.1375 to 0.5782), heart disease (HR: 0.3746, CI 0.1724 to 0.8141) and serum albumin (HR: 2.500 CI: 1.077 to 5.803) were identified as prognostic factors. Transplantation is a viable treatment option for good responders.
Summary/Conclusions: Prognosis of AL amyloidosis in dialysis is heterogeneous. Prognostic scoring integrating clinical biological data could identify the patient who may benefit the most dialysis. This results need to be matched by sex and age with non-dialysis and dialysis for another cause.

E1266

REAL-WORLD DATA ON THE TREATMENT OF RELAPSED/REFRACTORY MYELOMA WITH LENALIDOMIDE AND DEXAMETHASONE IN 2ND LINE (LEGEND STUDY): THE PROGNOSTIC SIGNIFICANCE OF BIOCHEMICAL VS. CLINICAL RELAPSE

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Background: The combination of lenalidomide/dexamethasone (LenDex) is an established treatment for relapsed/refractory Multiple Myeloma (MM) patients; however, apart from clinical trials, there is limited data for the efficacy of this combination as 2nd line treatment. Furthermore, the efficacy of LenDex when administered before evident clinical manifestations, namely in the case of biochemical relapse as compared to clinical relapse, has not yet been assessed.
Aims: In the current study, we evaluated response rates and progression-free survival (PFS) in patients treated with LenDex in 2nd line and we compared survival parameters for patients treated with LenDex at biochemical relapse *vs* those treated at clinical relapse.
Methods: Medical files of 207 patients with MM diagnosed between 2000-2013 in 18 Greek centers and treated with LenDex as 2nd line treatment from January 1st 2009, up to March 1st 2014, were retrospectively studied. Overall response and PFS were evaluated for all patients. Additionally, PFS was compared in patients treated at either biochemical relapse (group A) or at clinical relapse (group B). The prognostic significance of biochemical relapse adjusted with important patients' characteristics was also evaluated. Classical methods were used for statistical analysis.
Results: Two hundred and seven patient files were recorded and analyzed (M/F: 112/95, median age: 67.2y, range 31-91y, IgG: 115, IgA: 55, Light chain: 22, non-sectretory: 2, IgD: 5, IgM: 1, unknown: 7, ISS I: 54, ISS II: 74, ISS III: 77, high risk: 13%, standard risk: 87%). First line treatment included bortezomib-based regimens (63.3%), immunomodulatory drug-based combinations (34.8%) and chemotherapy (40.1%); 25% of patients underwent autologous stem cell transplantation; 2nd line treatment with LenDex was administered at biochemical relapse in 67.5% (95% CI: 61.1% >73.9%) of patients and at clinical relapse in 32.5% (95% CI: 26.1-38.9) of patients. The overall response rate (ORR) was 73.4%; 23.7% of patients achieved very good partial response (VGPR) and 17.8% complete response (CR). The number of patients that achieved at least VGPR did not differ between the 2 groups (p>0.05). The median time to best response was 6.7 months (range 0.6- 51.9). After a median follow-up of 52.8 months, 112 (54.1%) patients are alive and 95 (45.9%) patients are deceased; 131 patients (63.3%) have relapsed (biochemical relapse: 66.4%, clinical relapse: 33.6%). Median PFS and PFS rate at 12 months was 19.2 months (95% CI: 15.6-25.2) and 67.6% respectively. The median PFS was 24 months (95% CI: 18.0-34.8) for patients in group A *vs* 13.2 months (95% CI: 8.4-19.2) for patients in group B (HR: 0.63, p=0.006). When adjusted for important prognostic patients' characteristics (ISS, age, β2 microglobulin, and LDH), biochemical relapse maintained its prognostic significance for PFS (p<0.05).
Summary/Conclusions: Our data confirm that Lendex combination as 2nd line treatment leads to high overall response rates and prolonged PFS. Additionally, we have shown for the first time in routine clinical practice that MM patients who receive 2nd line therapy with LenDex at biochemical relapse have a significantly longer median PFS compared to patients treated at clinical relapse, underlining the importance of potentially starting treatment before evident clinical manifestations at the first relapse.

E1267

FDG-PET IN MULTIPLE MYELOMA: DUAL TIME POINT FDG UPTAKE IN FOCAL LESIONS CORRELATE TO RESPONSE TO CHEMOTHERAPY

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Background: Dual Time Point (DTP) ^{18}F-FDG PET imaging has been shown to be useful in differentiating malignant from benign lesions in that increasing uptake from 1 to 3 hours is a characteristic feature of malignancy in contrast to inflammation.
Aims: The aim of this study was to evaluate the predictive role of DTP ^{18}F-FDG PET/CT imaging in assessing response to chemotherapy in multiple myeloma (MM).
Methods: 23 patients with MM (21 male, aged 53-75 years) underwent ^{18}F-FDG PET/CT in a prospective study (NCT02187731) before start of treatment and two months after high dose chemotherapy with stem cell support. All scans were performed at 60 and 180 minutes after tracer injection at Odense University Hospital and Vejle Hospital. Thirteen patients with ≥ 3 focal lesions of at least 10 mm were selected for analysis. Images were analyzed using an adaptive thresholding algorithm (ROVER software; ABX GmbH, Radeberg, Germany). Focal malignant lesions were localized in pre-treatment scans; maximum standard uptake value (SUVmax) and mean SUV (SUVmean) and partial volume corrected SUVmean (pvcSUVmean) were obtained for each lesion. Lesional response to chemotherapy was classified as complete or partial in the post-treatment scan. A complete response was defined as a complete resolution of the lesion in the post-treatment scan. Lesions with partial response were present in the post-treatment scan. All statistical analyzes were done in SPSS 24 using repeated measurements-ANOVA.
Results: Three-five focal lesions were evaluated in each patient. In the pre-treatment PET studies, the increase in SUVmean from 1 to 3 hours was significantly higher for lesions with partial response compared to those with complete response (27.7% *vs* 11.4%; P=0.050). Additionally, the increase in pvcSUVmean was more significant than the increase in SUVmean (+42.23% *vs* +12.0%; P=0.003). The increase in SUVmax of delayed scans was not significant (P =0.082).
Summary/Conclusions: These preliminary data show that a more significant increase of FDG uptake in delayed scans of DTP PET before treatment correlates with a poor response of focal malignant lesions to chemotherapy in MM. The increase in pvcSUVmean is a better index than those of SUVmean and SUVmax for this purpose.

E1268

UNDERSTANDING THE CONTRIBUTE OF THE NOTCH PATHWAY IN MULTIPLE MYELOMA BONE MARROW NICHE: A FOCUS ON EXTRACELLULAR VESICLES-MEDIATED COMMUNICATION

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Background: Multiple myeloma (MM) is an incurable cancer stemming from malignant plasma cells. MM is characterized by a strong tropism to the bone marrow (BM), where tumor cells accumulate and establish complex interactions with the normal stroma, which in turn promotes tumour survival, drug resistance and the development of bone disease. The Notch oncogenic pathway provides a key contribute to the ability of MM cells to shape the BM niche, affecting both MM cell biology and the interplay with the surrounding normal cells. Recently, extracellular vesicles (EVs) have been reported as novel mediators in creating a supportive milieu for MM. Here we investigate the role of the activated Notch signaling in EV-mediated cross-talk.
Aims: The aim of this work was to further elucidate the role played by the Notch pathway in the shaping of the BM microenvironment to provide a supportive milieu for MM cells, with a focus on the contribute of EVs to the crosstalk between MM cells and the BM stromal cells.

Methods: We established two MM cell lines stably retaining the doxycycline-inducible pTRIPZ vector containing anti-Jagged1 and Jagged2 shRNAs and a BM mesenchymal stromal cell (BMSC) line expressing shRNAs for Notch1 and Notch2. EVs were isolated by ultracentrifugation and used for functional assays and molecular analysis. qPCR was performed using SYBR Green. Apoptosis analysis was performed by flow cytometry; evaluation of protein expression was achieved by flow cytometry or western blot.
Results: We present evidences that EVs play a crucial role in the dysregulated interactions of MM cells with the BM microenvironment and that Notch regulates their release. Indeed, BMSCs knockdown for Notch1/2 results in a decrease in EVs release and reduce their ability to induce Bortezomib resistance in MM cells and to stimulate their migration. On the other side, MM-derived EVs are able to increase the production of pro-tumor factors by BMSCs (*i.e.* SDF1α), promoting their ablity to boost tumor growth; interestingly, this effect is lost when EVs are isolated from MM cells where the Notch pathway was inhibited. Finally, EVs released by co-cultures of BMSCs and MM cells where the Notch pathway is blocked display a reduced ability to increase osteoclastogenesis compared with EVs from the control culture. This is particularly relevant due to the crucial role played by bone disease in MM progression.
Summary/Conclusions: These new insights in the pathophysiology of the de-arranged BM niche represent the rationale for a Notch-directerd therapy aiming to uncouple the crosstalk of MM with the surrounding microenvironment by inhibiting Notch signaling.

E1269

THE USE OF CARFILZOMIB AND BORTEZOMIB IN ROUTINE CLINICAL PRACTICE: RESULTS FROM PREAMBLE, AN ONGOING, OBSERVATIONAL COHORT STUDY IN MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) remains largely incurable despite improvements in clinical outcomes following the approval of immunomodulatory drugs (IMiDs) and proteasome inhibitors (PIs) (Rajkumar et al 2010). Previous findings from PREAMBLE indicated shorter median duration of therapy (DoT) with PIs and IMiDs (5 and 9 mo, respectively; Palumbo et al 2016) *vs* clinical trials (Stewart et al 2014). Understanding real-world use of therapies for relapsed/refractory (RR) MM is important to determine their position in the treatment paradigm.
Aims: In this subsequent PREAMBLE analysis, treatment patterns in patients (pts) with RRMM receiving bortezomib (bort) and carfilzomib (carf) were evaluated to better understand the use of PIs in routine clinical practice.
Methods: PREAMBLE (NCT01838512) is an ongoing, observational, international cohort study exploring real-world treatment patterns and outcomes in pts with MM. Eligible pts were aged ≥18 yrs with diagnosis of RRMM, ≥1 prior therapy and initiated treatment (index therapy) with an IMiD, PI or IMiD+PI within 90 days before to 30 days after study enrollment. Treatment patterns, DoT and time to next treatment (TTNT; for pts who switched or died) were assessed. Informed consent was obtained for all pts.
Results: At data cut-off (Sept 1, 2016), data were available for 924 pts, of which 326 (35%) pts had bort-based index therapy and 86 pts (9%) received carf-based index therapy (63/72 [88%] were enrolled in North America). The most common bort-based combination was bort + dexamethasone (dex; n=99, 30%). The most common carf-based therapies were carf alone (n=40, 47%), followed by carf+dex (n=21, 24%). The most widely used bort dose per 21 days for any bort-based therapy was ≤4.0mg/m^2 (98/151; 65%). The most common carf dose per 28 days received for any carf-based therapy was ≤120mg/m^2 (28/55; 51%). Switch from carf-based index therapy occurred after a median (Q1, Q3) DoT of 3.4 (1.9, 9.5) mo (n=34); most pts switched to pomalidomide (pom)-based regimens (23/34; 68%). Switch rates increased from 17% at 3 mo to 54% at 15 mo, and then to 57% at 24 mo. Median (Q1, Q3) TTNT from index therapy was 5.6 (2.3, 9.0) mo (n=53). Median (Q1, Q3) DoT (n=113) and TTNT (n=173) for bort-based index therapy was 4.5 (2.4, 7.1) and 7.0 (3.7, 12.3) mo, respectively; most pts switched to lenalidomide (43/113; 38%) or pom (33/113; 29%). Switch rates increased from 10% at 3 mo to 57% at 33 mo. Dose reductions on carf-based therapies (6/86; 7%) were mostly determined by clinical decision (67%), whereas for bort+dex (24/99; 24%) adverse events (AEs) were the main reason (63%). Discontinuation/switching from carf-based index therapy was reported for 80% (69/86) of pts, driven mainly by disease progression (39%) and AEs (14%). Similarly, disease progression (27%) and AEs (21%) were also the main reasons for pts discontinuation/switching from bort+dex therapy (84/99; 85%). AEs were reported for 45% (39/86) of pts with carf-based index therapy, most commonly fatigue (12%) and anemia (9%); 70% (69/99) of pts receiving bort+dex had AEs, most commonly thrombocytopenia and diarrhea (each 14%).
Summary/Conclusions: Treatment duration observed for PIs in the real-world clinical practice setting was shorter than reported in clinical trials. As patient enrollment and follow-up continues for PREAMBLE, additional analyses will be conducted to evaluate the impact of these patterns on efficacy outcomes.
Study funding: BMS.

E1270

ROLE OF SERUM FREE LIGHT CHAIN *VS* BENCE JONES MEASUREMENT IN LIGHT CHAIN MULTIPLE MYELOMA (LCMM) AT DIAGNOSIS, DURING TREATMENT AND FOLLOW-UP FOR RESPONSE EVALUATION AND RELAPSE DETECTION

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Background: According to IMWG recommendations for response assessment in multiple myeloma (MM), serum free light chain (sFLC) measurement should be used to define a "stringent" complete response in symptomatic MM and, only in cases when Bence Jones protein (BJP) is deemed as not quantifiable (<200mg/24h), in light chain multiple myeloma (LCMM). However, data are available suggesting that sFLC could be a more sensitive tool than BJP for minimal residual disease assessment and an earlier indicator of progressive disease (PD). BJP measurement requires to perform both urinary electrophoresis and immunofixation, it is time-consuming for tecnicians and could be limited by poor patient compliance.
Aims: Aim of our study was to retrospectively compare sFLC and BJP results in LCMM patients (pts) at diagnosis, during treatment and follow-up.
Methods: Serum and urine samples were collected from pts affected with plasma cell dyscrasia referring to the Azienda Ospedaliero-Universitaria Careggi between 1st February 2012 and 31 December 2013. Serum and urine protein electrophoresis was performed using Capillarys II, serum and urine immunofixation using Hydrasys II (both from Sebia), sFLC were measured on Immage 800 nephelometer (Beckman Coulter) using Freelite reagents (The Binding Site).
Results: We analized samples from 387 pts having positive serum and/or positive urinary immunofixation and/or abnormal sFLC ratio. Among them, 43 symptomatic LCMM pts were identified having both sFLC and BJP measurement at baseline (at MM diagnosis or first relapse). Serum and urine lab tests results were evaluated at baseline, monthly during therapy and every 3 months during follow-up. Median duration of laboratory monitoring for the whole group was 42 months (range 3-120). Autologous stem cell transplantation was performed in 30% of pts previously treated with proteasome inhibitors (81%) and/or immunomodulating agents (40%) or chemotherapy (9%). sFLC or BJP were not available in 10% of 872 pair of samples from 43 pts. In 10% of cases (68/696 pair of samples) sFLC ratio was abnormal with increased involved FLC without any detectable BJP (FLCr+;iFLC+;BJP-); the opposite (FLCr-;iFLC-;BJP+) occurred in 1% of cases (8/696 pair of samples). Renal failure was found in 9% *vs* 13% of discrepant cases. At baseline, of the 43 LCMM pts, 6 had "measurable disease" only by sFLC due to BJP<200mg/24h and were therefore considered not evaluable for response assessment. Median time to BOR was 3 months by both sFLC and BJP (range FLC: 1-11 mesi; range BJP: 1-10 mesi). Among the remaining 37 pts evaluable for best overall response, 6/37 had complete response according to BJP but not to sFLC; interestingly 5/6 progressed after 2-8 months. Twenty-one pts progressed during follow-up: PD was detected only by sFLC in 4, only by BJP in 1. Both tests were able to detect PD in 16 pts: at the same time in 5, with sFLC-PD occurring earlier in 7 and BJP-PD occurring earlier in 4 pts.
Summary/Conclusions: Both sFLC and BJP measurement are useful in LCMM pts for disease monitoring, however, sFLC assessment appears to be more sensitive in MRD and early relapse identification. These data suggest that BJP could be substituted by sFLC assessment in LCMM. In our series only 1 case showed BJP-PD according to IMWG occurring earlier than sFLC-PD but was considered not clinically significant. On the contrary 5 pts in BJP-CR clinically progressed within few months without having reached FLC-CR. Limits of our study are a small number of pts, inhomogeneous duration of therapy and follow-up and retrospective analysis.

E1271

SUPPRESSION OF THE NON-MONOCLONAL PAIR AS NEW BIOMARKER OF POOR PROGNOSIS IN MULTIPLE MYELOMA PATIENTS AT DIAGNOSIS AND AFTER AUTOLOGOUS STEM CELL TRANSPLANT

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Background: The outcome for patients with Multiple Myeloma (MM) is highly

variable. Understanding the prognosis for a particular patient can help when selecting the intensity of treatment to be used and the frequency of reviews. The quantification of heavy/light chains pairs by the immunoassay Hevylite (HLC) allows us a precise measurement of monoclonal and non-monoclonal immunoglobulins of the same isotype.

Aims: The aim of the study is to evaluate i) the impact of the "HLC ratio" defined as monoclonal immunoglobulin over isotype matched non-monoclonal immunoglobulin (involved/uninvolved HLC ratio or i/u HLC ratio), ii) the suppression on non-monoclonal pair denominated "HLC-matched pair suppression" and III) the effect of "systemic immunoparesis" at diagnosis and at +100 days after autologous stem cell transplant (ASCT).

Methods: 85 patients (50 Male:35 Female) with a median age of 70 years (56-78) were followed (35 IgGK, 18 IgGL, 17 IgAK and 15 IgAL). The median follow-up of the patients was 19 (5-30) months. Sixteen patients (18%) presented ISS stage I, 15 (28%) with stage II and 54 (64%) with stage III disease. Thirty patients that reached ASCT were evaluated at +100 days after ASCT. Immunoglobulin heavy/light chain pairs (HLC) were assessed by Hevylite assays (The Binding Site). Clinical variables were evaluated for their impact on patient´s outcome. Overall survival (OS) and progression-free survival (PFS) were evaluated by Kaplan-Meier method and Cox regression. Statistical analysis was made with Prism 6.0.

Results: The median OS of the 85 patients was 54% and 26 patients deceased during the study due to MM. The median value of i/u HLC ratio was 80 (31.5-319.71). At diagnosis, a i/u HLC ratio>80 was significantly associated with worse OS (48 *vs* 61%, p=0,005) and shorter PFS (23% *vs* 42%, p=0,006). Severe HLC-matched pair suppression (*i.e.* more than 50% below the lower reference range) was identified in 68% of the newly diagnosed patients and was associated with significantly shorter OS (35% *vs* 81%, p=0,004) and PFS (21% *vs* 50%, p=0,013). Severe (>50%) systemic immunoparesis of non-monoclonal immunoglobulins was identified in 64% of the patients at diagnosis and was also significantly associated with shorter OS (32% *vs* 81%, p=0,030) but not with shorter PFS (26% *vs* 44%, p=0,306). The evaluation of other clinical variables on patient´s outcome are shown in table (see Table). In multivariate analysis, severe HLC-matched pair suppression and albumin were found as independent risk factors for OS whereas creatinine and i/u HLC ratio >80 were found as independent risk factors for PFS. In the post-ASCT evaluation of the patients (n=30), normalization of HLC ratio was observed in 22 patients (73%). An altered HLC ratio was significantly associated with shorter PFS after ASCT (25% *vs* 70%, HR: 3,42, 95%CI 1,12-11,97, p=0,039) and with a trend towards a worse OS (p=0,072). Severe HLC-matched pair suppression was found in 12 patients (40%) and was predictive of worse OS (0% *vs* 70%, HR: 10,63, 95%CI: 1,11-114,11, p=0,023) and shorter PFS (35% *vs* 71%, HR: 8,87, 95%CI: 1,72-45,92, p=0,002). On the other hand, the severe systemic immunoparesis observed in 17 patients (57%) was not associated with OS (p=0,644) and PFS (p=0,750).

Table 1.

Variables	Overall Survival (OS)		Progression Free Survival (PFS)	
	HR (95%CI)	p-value	HR (95%CI)	p-value
Univariate analysis				
HLC ratio >80	3,13 (1,35-7,24)	0,005	2,44 (1,26-4,71)	0,006
HLC-matched pair suppression >50%	4,94 (1,47-16,59)	0,004	2,56 (1,19-5,50)	0,013
Systemic immunoparesis >50%	2,97 (1,11-7,94)	0,030	1,42 (0,72-2,80)	0,306
Serum free light chains ratio <0,03 or >32	1,07 (0,44-1,56)	0,887	1,01 (0,45-1,82)	0,774
Calcium >10,5 mg/dL	1,02 (0,38-2,74)	0,965	1,21 (0,55-2,65)	0,642
Hemoglobin <10 g/dL	1,87 (0,70-5,01)	0,216	1,38 (0,63-3,02)	0,418
Creatinine>2 mg/dL	2,73 (1,16-6,41)	0,021	2,39 (1,17-4,88)	0,017
Lytic bone lesions	1,40 (0,42-4,73)	0,586	1,93 (0,67-5,50)	0,221
Beta-2-microglobulin >5,5 mg/L	2,013 (0,84-4,80)	0,115	1,58 (0,80-3,09)	0,187
Albumin <3,5 g/dL	3,23 (1,29-8,07)	0,012	1,83 (0,94-3,56)	0,076
Bone marrow plasma cells >10%	1,07 (0,43-2,62)	0,891	1,33 (0,62-2,82)	0,465
LDH > 250 U/mL	2,08 (0,93-4,64)	0,074	1,95 (0,99-3,79)	0,05
ISS-2 (vs. ISS-1)	1,56 (0,35-7,06)	0,561	1,80 (0,58-5,53)	0,307
ISS-3 (vs. ISS-1)	2,15 (0,64-7,27)	0,219	1,92 (0,73-5,06)	0,186
Cox multivariate analysis (backward stepwise regression)				
HLC-matched pair suppression >50%	7,03 (1,64-30,09)	0,009	-	-
Creatinine>2 mg/dL	2,29 (0,96-5,46)	0,061	3,46 (1,66-7,24)	0,001
Albumin <3.5 g/dL	3,31 (1,23-8,64)	0,017	-	-
HLC ratio >80	-	-	3,75 (1,71-8,22)	0,001

Summary/Conclusions: Severe HLC-matched pair suppression and i/u HLC>80 are associated with worse OS and shorter PFS in MM patients suggesting a potential use of these parameters as prognostic biomarkers in newly diagnosed patients. Severe HLC-matched pair suppression is an independent risk factor for OS whereas i/u HLC>80 is independently associated with shorter PFS. In patients after ASCT, severe HLC-matched pair suppression reflects the persistence of clonal cells that is not associated with severe systemic immunoparesis.

E1272

SURVIVAL STRATIFICATION OF PATIENTS WITH MULTIPLE MYELOMA (MM) AFTER FIRST RELAPSE: SENSITIVITY ANALYSES OF A NOVEL RISK STRATIFICATION ALGORITHM (RSA)

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Background: Established risk stratification tools in MM, such as the International Staging System (ISS) and the revised ISS, have improved overall survival (OS) estimates by combining the strongest known predictors of survival at diagnosis. There remains, however, a need for tools that use additional data available at relapse to improve risk stratification. We previously used real-world data from the Czech Registry of Monoclonal Gammopathies (RMG) to develop a RSA for estimating risk of death in patients with MM starting second line (2L) treatment. A multiple Cox regression model identified predictors of OS at 2L (Table); hazard ratios (HRs) for each predictor were multiplied to obtain an overall score for each patient. A K-adaptive partitioning for survival (KAPS) algorithm stratified patients into risk groups based on these scores.

Aims: To investigate how our RSA is affected by: 1) removing cytogenetic abnormalities (CAs) at diagnosis as an OS predictor, as these are not routinely measured in practice; 2) adding 2L treatment as a predictor, as 2L treatment type is likely to affect OS; 3) changing the number of stratification groups.

Methods: The analyses used data for 1418 patients aged ≥18 years who were diagnosed with symptomatic MM between May 2007 and April 2016 and who had started 2L. The Cox model was re-run for two sensitivity analyses: excluding CAs and adjusting for treatment received at 2L (adding bortezomib or lenalidomide *vs* other treatments as a predictor). The impact of different numbers of risk groups was assessed using KAPS.

Table 1.

Predictors of OS at second line (Cox regression analysis)		Original model	Sensitivity analyses	
		With CAs, without 2L treatment type HR (95% CI)	Original model without CAs HR (95% CI)	Original model, with 2L treatment HR (95% CI)
Attribute patient factors measured at 2L				
Age,* years Reference: <65 years	65–75 ≥75	1.122 (0.945–1.332) 1.425 (1.164–1.746)***	1.097 (0.925–1.302) 1.401 (1.146–1.712)*	1.116 (0.936–1.327) 1.415 (1.151–1.726)*
ECOG PS* Reference: 0	1–2 3–4	1.871 (1.384–2.530)*** 4.111 (2.808–6.017)***	1.851 (1.369–2.501)*** 3.911 (2.674–5.719)***	1.831 (1.352–2.480)*** 3.958 (2.696–5.809)***
Disease factors measured at diagnosis				
CAs* Reference: standard risk	High risk Not reported	1.616 (1.125–2.321)* 1.054 (0.709–1.444)	NA NA	1.602 (1.115–2.303)* 1.036 (0.755–1.421)
β_2-microglobulin, mg/L Reference: <3.5 mg/L	3.5–5.5 >5.5	1.154 (0.945–1.409) 1.372 (1.124–1.673)*	1.174 (0.962–1.432) 1.398 (1.147–1.704)**	1.135 (0.929–1.387) 1.361 (1.115–1.661)*
LDH, U/L Reference: ≤360 U/L	>360	1.306 (0.967–1.762)	NA	1.330 (0.985–1.796)
Disease factors measured at 2L				
β_2-microglobulin,* mg/L Reference: <3.5 mg/L	3.5–5.5 >5.5	1.072 (0.882–1.304) 1.402 (1.138–1.727)*	1.046 (0.861–1.272) 1.379 (1.121–1.697)*	1.070 (0.880–1.300) 1.422 (1.154–1.752)**
LDH level,* U/L Reference: ≤360 U/L	>360	1.985 (1.579–2.494)***	2.168 (1.753–2.680)***	2.066 (1.644–2.596)***
Thrombocyte count, 10^9/L Reference: >100 x 10^9/L	≤100	1.604 (1.281–2.010)***	1.615 (1.290–2.021)***	1.642 (1.309–2.061)***
Bone marrow plasma cell count, % Reference: <20%	20–70 >70	1.418 (1.210–1.662)*** 1.619 (1.228–2.134)**	1.409 (1.202–1.652)*** 1.754 (1.330–2.301)***	1.411 (1.203–1.656)*** 1.535 (1.149–2.051)*
Extramedullary disease Reference: not present	Present	2.291 (1.841–2.851)***	2.274 (1.829–2.828)***	2.336 (1.875–2.910)***
New bone lesions (detected by radiography) Reference: 0	>2 new lesions at diagnosis and 2L* New lesions	1.345 (1.129–1.601)** 1.081 (0.858–1.362)	1.332 (1.119–1.585)* 1.098 (0.872–1.382)	1.386 (1.161–1.655)*** 1.096 (0.868–1.385)
Treatment history				
Time to next treatment,* months Reference: >24 months	≤24	1.154 (0.951–1.401)	1.184 (0.976–1.435)	1.130 (0.930–1.372)
Refractory to previous treatment*† Reference: non-refractory + refractory to other (not new) drugs	Refractory to bortezomib Refractory to thalidomide Refractory to other new drugs	1.579 (1.240–2.012)** 1.240 (0.987–1.557) 1.411 (0.950–2.095)	1.579 (1.240–2.011)** 1.231 (0.981–1.545) 1.446 (0.975–2.145)	1.485 (1.152–1.914)* 1.291 (1.018–1.637)* 1.379 (0.926–2.052)
Grade 3/4 toxicities Reference: 0–2	3–4	1.196 (1.024–1.396)*	1.211 (1.038–1.414)*	1.185 (1.014–1.385)*
Treatment type at 2L *Reference: bortezomib only* *(Factor considered in the sensitivity analysis only)*	*Thalidomide only* *Lenalidomide only* *Bortezomib + thalidomide* *Other with new drug* *Other without new drugs*	*NA*	*NA*	*1.114 (0.878–1.413)* *1.109 (0.916–1.341)* *1.275 (0.807–2.013)* *0.538 (0.306–0.948)** *1.210 (0.893–1.639)*
AIC (model fit)		9194.3	9205.1	9194.6

*Factor was forced into the model.
†Category includes patients with accelerated osteoporosis or with >2 new lesions at diagnosis and at 2L.
‡'Non-refractory' and 'refractory to other (not new) drugs' were merged because patients in the latter group had good OS (unexpected effect) probably because they benefited from treatment with older drugs and had good treatment options available at 2L.
*p≤0.05; **p≤0.001; ***p≤0.0001.
2L, second line; AIC, Akaike's information criterion; CA, cytogenetic abnormality; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; HR, hazard ratio; LDH, lactate dehydrogenase; NA, not applicable; NR, not reported; OS, overall survival.

Results: Results are shown in the Table. The model without CAs had similar HRs and predictors to the original; however, lactate dehydrogenase level at diagnosis was not identified as a predictor. Kaplan–Meier OS analysis showed separation between groups (median OS for the lowest [group 1] to the highest [group 4] risk group: 57.2, 29.4, 14.9 and 4.9 months), but the separation was weaker than when CAs were included in the model (median OS: 57.2, 28.8, 13.4 and 4.7 months). Despite 81% of patients in the RMG having no CA data, ('missing' CA was treated as a separate level in the original model), the fit of the model (measured using Akaike's information criterion; Table) without CAs was worse than the original, reducing the accuracy of survival predictions. Adding 2L treatment as a predictor did not affect the model fit, indicating that OS predictions were not improved. KAPS analysis showed that a model with three groups for stratifying patients by risk of death was less effective than one with 4 or 5 risk groups. With group 1 as the reference, the HRs for OS were 2.4 and 8.1 for groups 2 and 3 in the three-group model (all p<0.001), 2.1, 4.2 and 11.1 in groups 2–4 in the four-group model (all p<0.001) and 1.8, 2.8, 4.9 and 10.5 for groups 2–5 in the five-group model (all p<0.001). Using five risk groups was considered less practical in a clinical setting than the four-group model, which provides a clearer difference in risk across groups.

Summary/Conclusions: These analyses indicate that our RSA incorporating data from diagnosis and relapse can identify patient groups with profoundly different survival expectations, regardless of 2L treatment type. CAs at diagnosis is a known OS predictor and, as expected, improves the strength of predictions. The practicalities of measuring CAs should be considered, but these data suggest that physicians should be encouraged to assess CAs at diagnosis; CAs at relapse may also be informative. Further validation of this model is required using other real-world and clinical trial data.

E1273

REAL-WORLD DATA ON MULTIPLE MYELOMA: A PROSPECTIVE NATIONAL REGISTRY IN URUGUAY ON 224 NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS FROM 2012-2015

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Background: The Uruguayan National Myeloma Registry is the first observational prospective Uruguayan registry designed to document clinical characteristics of newly diagnosed multiple myeloma (MM), treatment and outcomes in a real-world setting. It collects detailed data from MM patients diagnosed at all institutions from 2012, nationwide. Analysis of this non-selected data will allow us to plan strategies to improve our local approach to this disease, reducing problems derived from extrapolating information from other realities.

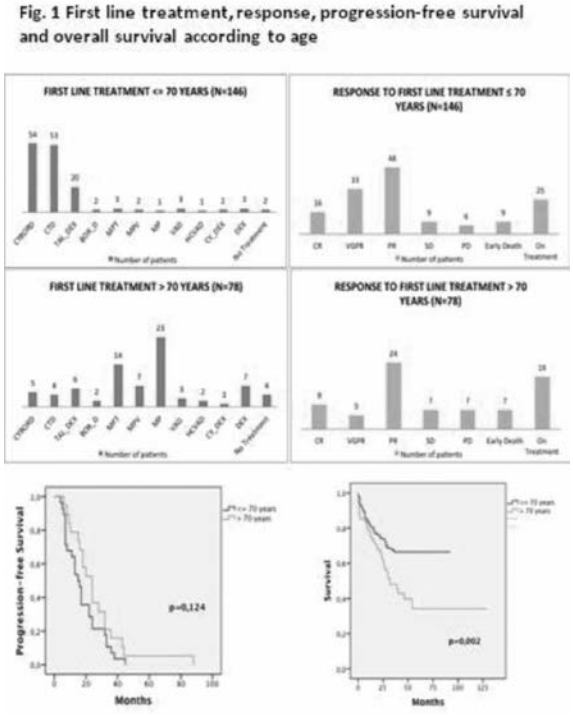

Figure 1.

Aims: To document current strategies of clinical characteristics at diagnosis, management, outcomes and treatment adverse effects of non-selected newly diagnosed MM patients in a recent period.

Methods: This registry includes all MM diagnosed from January 2012 in all institutions, nationwide. Smoldering MM are not included. We present the analysis of the first 3 years of data collection. Information was obtained from medical records at each institution. Our database includes clinical and laboratory characteristics, treatment, disease-related and treatment-related adverse events, response, progression free survival, overall survival and cause of death. Survival is obtained from the Uruguayan Ministry of Health database.

Results: With a 71% institutional coverage, 224 patients were included. Median age at diagnosis was 66 years (range 33-94 years), 54,5% were male; 10% were younger than 50 years and 34,5% older than 70 years. Distribution according Ig subtype was: IgG 50,4%, IgA 23,3%, Light chains 18,7%, non-secretor 2,2% and IgM <1%. Most patients had advanced disease: 79,6% Durie-Salmon stageIII (176/221), 48,6% ISS3 (86/177). Anemia (hemoglobin<10 g/dl) was present in 58%, osteolytic lesions in 69%, renal impairment (creatinine>2mg/dl) in 29,5% and hypercalcemia in 10%. Cytogenetics was evaluated in 150 patients; high risk features were detected in 6,3% by conventional cytogenetics and 19% by fluorescence *in situ* hybridization. First-line treatment included at least one of the new drugs (Thalidomide, Bortezomib or Lenalidomide) in 92% of patients ≤70 years and in 50% of>70 years. First-line response was available in 73%. Overall response rate (≥ PR) was 82,3%, VGPR= 23,2% and CR=15,2%; 9,8% patients achieved stable disease and 7,9% were refractory. (Fig 1.). Comorbidities and treatment-related toxicities were observed in 43,8% (47% in>70 y *vs* 41%). Most common adverse events were recurrent infections (26%), neuropathy (17%), thromboembolic events(5,4%) and grade 3-4 cytopenias(5%). Sixty out of 146 potential candidates have been transplanted as first line consolidation at the time of this analysis. After a median follow-up of 30 months, overall survival was 62,8% (median NR in ≤70 years and 32 months in>70 years) and median progression free survival (PFS) was 17 months.

Summary/Conclusions: This first national registry provides a thorough insight into the characteristics of MM patients in our country. With a high institutional coverage, we show MM characteristics at diagnosis are similar to other real-life reports.(1) MM is detected in advanced stage with a high percentage of renal impairment. Diagnosis is performed according to international recommendations. First-line treatment is defined by local policies which restrict Bortezomib to high-risk cytogenetic features and/or renal impairment and do not provide Lenalidomide. Reasons for 59% potential candidates not receiving ASCT should be addressed in future research. This analysis provides relevant real-life information to plan strategies to improve MM management and perform high quality population-based research on the field.

Reference

1. Yong K, Delforge M, Driessen C, Fink L, Flinois A, Gonzalez-McQuire S, et al. Multiple myeloma: patient outcomes in real-world practice. Br J Haematol. Oct 2016;175(2):252-64.

E1274

REPRESENTATION OF MINORITIES, THE ELDERLY AND WOMEN IN MULTIPLE MYELOMA CLINICAL TRIALS

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Background: Multiple myeloma (MM) accounts for approximately 1% of all cancers and 10% of hematologic malignancies in the United States (US). MM occurs in all races but the incidence in African Americans is two to three times higher than in non-Hispanic whites. Many clinical trials (CT) lack appropriate representation of specific patients populations, limiting the generalizability of the evidence obtained.

Aims: Determine the representation of ethnic minorities, the elderly and women in MM CT.

Methods: Enrollment data from all therapeutic trials reported as completed in clinicaltrial.gov from 2000 to 2016 were analyzed. CT including other hematologic malignancies and with recruitment outside of the US were excluded. Enrollment fraction (EF) was defined as the number of enrollees divided by the 2013 Surveillance, Epidemiology, and End Results (SEER) database MM complete prevalence. Chi-square test was used to estimate differences in categorical data.

Results: Out of 177 MM CT, 78 (44%) reported ethnicity with a total of 12,055 enrollees. Out of those 78 CT, 52 (67%) were phase II, 15 (19%) phase III and 11 (14%) phase I. Most of the results were published from 2012 to 2016 (74%). Distribution by race, gender, age and comparison with the SEER MM prevalence data are described on Table 1. Forty-six (59%) trials were sponsored by industry, 7 (9%) by NCI and 25 (32%) were investigator initiated. Participation in CT varied significantly across ethnic groups, non-Hispanic Whites (NHW) were more likely to be enrolled in CT (EF of 0.23) than African Americans (AA) (EF of 0.08, p <0.0001) and Hispanics (H) (EF of 0.05, p <0.0001). Males had

a higher recruitment rate than females (58% *vs* 42%), but this could be explained by the higher incidence of MM in this subgroup. Enrollee's median age was 62 years. Younger pts (<65 years) were more likely to be enrolled in CT than the elderly (66% *vs* 34%, p<0.0001). Industry sponsored trials were less likely to recruit AA compared with investigator initiated trials (7.6% *vs* 12%, p<0.0001).

Table 1.

	Trials Participants No. (%)	2013 MM Prevalence, %
Race/Ethnicity		
NHW	10,139 (84)	70
AA	1,034 (8.6)	20.2
H	227 (1.8)	7.2
Asian	342 (2.8)	2.5
Native American	13 (0.1)	N/A
Other	300 (2.5)	
Gender		
Male	7,055 (58)	53
Female	5,000 (42)	47
Age^		
<65	3,631 (66)	38
>65	1,908 (34)	62
Total enrollees	12,055	

^ reported by 46% of CT

Summary/Conclusions: Despite the higher incidence of MM in African Americans and the elderly, the former only represented 8.6% of the study participants and 66% of these were less than 65 years of age, perharps lacking data in the tolerability of these new agents in our aging MM population. We also observed industry studies were less likely to recruit AA patients. Future trials should take extra measures to recruit participants that adequately represent the United States MM population.

E1275

EVALUATION OF TREATMENT INDUCED NEUROPATHY IN MULTIPLE MYELOMA AND ITS INFLUENCE ON PHYSICAL AND ROLE FUNCTIONING

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Background: Peripheral neuropathy (PN) is a major dose limiting and potentially disabling adverse event of commonly therapeutic drugs used in the management of multiple myeloma (MM), including the immunomodulatory drugs (IMIDS, Thalidomide and Lenalidomide), and the proteasome inhibitor (Bortezomib).
Aims: The aims of this study were to (1) perform a psychometric evaluation of PN and (2) examine the prevalence of this complication and its influence on physical and role functioning of MM patients.
Methods: The FACT/GOG-Neurotoxicity (Ntx) subscale for assessing treatment induced PN was evaluated. The 11-item of this questionnaire was administered to patients with MM treated with IMIDS and/or Bortezomib. The subscale was evaluated in 32 patients for internal reliability, construct validity, criteria validity, and compared to NCI grading adverse events (CTCAE version 3). Spearman rank correlation was calculated to determine the impact of PN on functional, physical and role functioning of MM patients, assessed by EORTC quality of life scale (EORTC QLQ-C30). A Cronbach coefficient ≥ 0,8 is good. Spearman rank correlation is significant if p< 0.05 or r >0, 5.
Results: Cronbach'alpha coefficient for infernal consistency of FACT/GOG-Ntx subscale was 0, 92, and its correlation with the full CTCAE scale as follows: P<0, 0001. All the 11 items exhibited high correlations with the NTX subscale score (r= 0, 65- 0, 79), and the Construct validity of NTX was good. According to FACT/GOG-NTX and NCI- CTCAE, 24 (75%) patients presented PN secondary to IMID or Bortezomib. The PN was severe in 14 (43, 7%) patients, especially those who received Bortezomib associated with IMIDS (71, 4%). PN did not influence the achievement of a very good response of MM to therapy neither a complete remission (P=0.6), but patients with high scores of NTX subscale have reduced functional activities, especially physical and role functioning (P=0.0005, P=0.0001 respectively).
Summary/Conclusions: The 11-item FACT/GOG-Ntx subscale reliably and validly assesses Bortezomib/IMIDS induced PN. This complication is frequent and can alter the functional abilities of MM patients.

E1276

PROGNOSTIC SIGNIFICANCE OF T(11;14) EXPRESSION BY FISH IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA IN THE ERA OF NOVEL THERAPIES

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Background: Rearrangements of the immunoglobulin heavy chain (IGH) on chromosome 14 are identified by FISH in about 15-20% of patients (pts) with newly diagnosed multiple myeloma (MM). Historically there is variation on the significance on prognosis of these rearrangements: typically, t(4;14), t(14;16) and t(14;20) have high risk (HR), and t(11;14) have standard risk (SR). A recent study (Kaufman et al, Leukemia. 2016 30:633-9) suggests that t(11;14) may confer a worse prognosis.
Aims: To determine the prognostic significance of t(11;14) in a single-institution MM cohort.
Methods: 87 pts with t(11;14) by CD 138 selected FISH at diagnosis were identified, pts without symptomatic MM were excluded. Cox regression was used for statistical analysis. Progression free survival (PFS), and overall survivals (OS) from diagnosis and post autologous stem cell transplant (ASCT) were analyzed by Kaplan-Meier.
Results: Median age at diagnosis was 62 years, 45 pts (52%) were male, and 24 pts (27%) had ISS 3. All pts received either a proteasome inhibitor or an immunomodulatory agent, and 42 (48%) received triplet treatment as induction. Sixty-nine (79%) pts had ASCT, and overall response rate (ORR, partial response or better) post ASCT was 73%. For pts with HR FISH (defined as t(14;16), p53 del, 1q21 gain or 1p del) compared to SR FISH, the ORR post ASCT was 70% *vs* 77% (p=0.67). OS from diagnosis was 93% at 3 years, 74% at 4 years and 51% at 5 years. Seven patients (8%) developed plasma cell leukemia, and there was no association between HR and SR FISH (p=0.66). In multivariate analysis, ISS stage was an independent risk factor for mortality; pts with stage 3 had 7.3 times (CI: 1.16-36.4) and 5.7 times (CI: 1.63-20.0) the risk of mortality than pts with stage 1 and 2. Having an ASCT reduced mortality by 87% (CI: 0.04-0.41).
Summary/Conclusions: Despite the use of novel therapies the OS at 5 years of our pts with MM was not significantly improved compared to SEER data from 1992-2013 (51% *vs* 48.5%). Pts with t(11;14) who had ASCT had increased survival compared to those who did not. Our results suggest that t(11;14) may confer a worse prognosis. Further prospective studies evaluating the risk of t(11;14) are warranted.

E1277

ANALYSIS OF THE CONNECT MM REGISTRY: TREATMENT OUTCOMES AND HEALTHCARE RESOURCE UTILIZATION IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA WHO RECEIVED LENALIDOMIDE MAINTENANCE OR NO MAINTENANCE

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Background: Maintenance therapy post autologous stem cell transplant (ASCT) has been shown to improve clinical outcomes, including time to progression, progression-free survival (PFS), and overall survival (OS) in patients with newly diagnosed multiple myeloma (NDMM) (Sonneveld, *J Clin Oncol,* 2012; McCarthy, *N Engl J Med,* 2012; Attal, *N Engl J Med,* 2012; Palumbo, *N Engl J Med,* 2014; Attal, ASCO, 2016). However, the effect of continued treatment on healthcare resource utilization (HRU) is mostly unknown. Connect MM is a largely community-based, US prospective observational cohort study designed to characterize diagnosis, treatment patterns, and outcomes in patients with NDMM in clinical practice.
Aims: This analysis used the Connect MM registry to analyze the impact of maintenance treatment on clinical outcomes and HRU in a largely community setting.
Methods: Adult patients with NDMM were eligible for enrollment in the registry within 60 days of diagnosis. Patients who completed induction and single ASCT without subsequent consolidation and received lenalidomide (LEN)-only or no maintenance were included in the analysis. HRU (hospitalization rates and length of stay, surgery/procedures, concomitant medications including growth factor, bisphosphonate, and neuropathic pain medication) was assessed from 100 days post-ASCT to the end of years 1 and 2. Data cutoff was Jan 7, 2016 and the median follow-up was 39.3 months.
Results: A total of 1493 patients with NDMM were enrolled in Cohort 1 from Sep 2009 to Dec 2011; 421 patients met the analysis criteria stipulated above. Of these, 165 did not receive maintenance therapy and 256 received any type of maintenance therapy. Of those receiving maintenance, 180 (70%) were treated with LEN-only maintenance. The median age was 60 y (range, 24-78); 60% were men, and 86% were white. Baseline patient characteristics except serum

creatinine, calculated International Staging System stage, history of monoclonal gammopathy of unknown significance, presence of del(17p), and induction regimen were similar across groups. LEN-only maintenance significantly extended PFS compared to no maintenance (median 54.5 months *vs* 30.8 months; hazard ratio [HR]=0.58 [95% CI: 0.43, 0.79]; *P*=.0005; Table). OS was also significantly improved with LEN-only *vs* no maintenance (HR=0.45 [95% CI: 0.28, 0.73]; *P*=.001). HRU results are detailed in the Table. The rate of hospitalization/100 person-years (PY) was similar across groups (*P*=not significant [NS], all comparisons) at the end of years 1 and 2. The median duration of hospitalization was numerically longer for patients who received no maintenance. Procedures/surgeries and concomitant medication use were similar across both groups at the end of years 1 and 2.

Table 1.

	LEN-Only Maintenance (n = 180)	No Maintenance (n = 165)	Hazard Ratio (95% CI)
Median PFS, months	54.5	30.8	0.58 (0.43, 0.79) *P* = .0005
Median OS, months	NR	NR	0.45 (0.28, 0.73) *P* = .001
Hospitalization rate, /100 PY			Incidence Ratio (95% CI)
Year 1	28.7	32.9	0.82 (0.44, 1.52) *P* = NS
Year 2	32.0	45.2	0.65 (0.34, 1.24) *P* = NS
Median hospitalization duration, d			
Year 1	5.0	10.5	*P* = NS
Year 2	7.0	9.0	*P* = NS

Summary/Conclusions: For patients with NDMM, LEN-only maintenance significantly improved PFS and OS *vs* no maintenance with no apparent impact on HRU.

E1278

SERUM-FREE LIGHT-CHAINS (SFLC) INSTEAD OF URINE PROTEIN ELECTROPHORESIS (UPEP) FOR MONITORING LIGHT-CHAIN MULTIPLE MYELOMA (LCMM)

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Background: Response and follow-up criteria in multiple myeloma (MM) are still based on the protein electrophoretic (PEP) quantification of the monoclonal protein (MP) in serum (s) and / or urine (u). Monitoring MP by urine (u) PEP has a very low sensitivity for evaluating variations of small amounts of MP. Since 2001, serum free light-chain assays (sFLC) are available, with demonstrated clinical utility. *Dejoie et al.* have recently reported the usefulness of sFLC for evaluating response in LCMM.
Aims: In this work, we try to validate the use of sFLC assay in the context of GEM/PETHEMA clinical trials in order to evaluate the responses and its advantages in comparison to standard quantification of MP by PEP in serum (s) and —mainly— urine (u) after treatment, given the usual difficulties to collect and adequately perform urine studies in the usual clinical praxis.
Methods: We included 169 patients with Bence Jones (BJ) MM with measurable urine disease who have being treated according to GEM/PETHEMA clinical trials (GEM05menos65, GEM05MAS65, GEM2010MAS65 and GEM2012 menos65). Serum FLC assays (Freelite®, The Binding Site, Birmingham, UK) were performed on an automated nephelometer (BNII, Dade Behring / Siemens, Marburg, Germany). The electrophoretic study of the monoclonal component (CM) was performed by capillary electrophoresis (V8, Helena Biosciences Europe), and immunofixation was performed for the Ig, γ, κ and λ chains (SAS-3 and SAS-4, Helena Bioscience Europe).
Results: From a total of 169 patients with LCMM (93 Bence Jones kappa / 76 Bence Jones Lambda), 146 (86%) had FLC data at diagnosis, with 139/146 (95%) evaluable by FLCs [involved sFLC ≥100)]. In addition, 68 of the 169 patients also had detectable MP in serum and 7 of the 169 had non-evaluable MP in urine (MP <0.200 g/24h). We studied the correlation of both techniques' MP quantification results (uPEP *vs* isFLC) and we observed a low correlation (Pearson's r 0.293, p =0.003), that should be partly explained by the low profitability and subjectivity of the electrophoresis technique for quantifying paraprotein in urine. [Figure 1A]. The concordance between the classification of the response by uPEP / immunofixation (IF) and by FLCs (Kappa Index=0.425 p <0.0001) was moderate. The normalization of the sFLC ratio (r) was reached in 35/98 (36%) patients after treatment, associated to a lower risk of progression (normal *vs* abnormal sFLCr: PFS 60 *vs* 39 months, p =0.038) but without impact in overall survival in our series. We also observed that an absolute value of isFLC greater than 50mg/L after treatment was associated with an increased risk of progression, regardless of the response achieved (PFS 60 *vs* 28 months, p<0.0001). [Figure 1B].

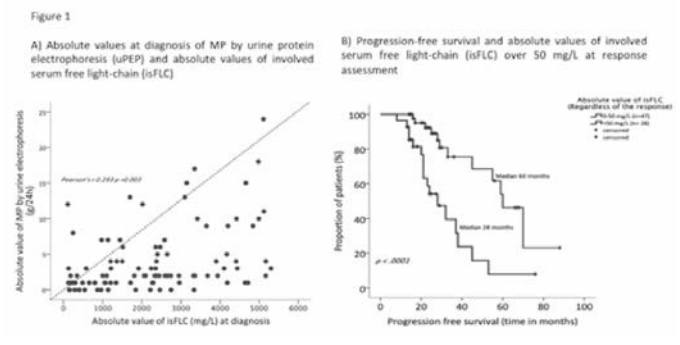

Figure 1.

Summary/Conclusions: There is an acceptable agreement between both methods for response evaluation. The sFLC assays provide a greater sensitivity than the urine protein electrophoresis for monitoring low levels of disease in certain cases with measurable disease at diagnosis (isFLC ≥100) being useful for its follow-up, and also provide prognostic value as a predictor of progression.

E1279

TOPSPIN: A NOVEL ALGORITHM TO PREDICT TREATMENT SPECIFIC SURVIVAL IN CANCER

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Background: In recent years many novel treatments have been introduced for Multiple Myeloma (MM), leading to an improved survival. However, this has also led to a situation where many different treatment combinations are used, without a clear indication which patient will benefit most from which treatment. It is increasingly recognized that genetic heterogeneity between tumors influence treatment response. Patient outcomes may be improved by selecting the right treatment for the right patient at the moment of diagnosis. This requires the discovery of predictive markers, for example gene expression signatures, that can aid in this treatment decision. Here we present TOPSPIN (Treatment Outcome Prediction using Similarity between PatIeNts), a novel algorithm to discover such markers from tumor gene expression data. We use it to identify patients more likely to benefit from bortezomib.
Aims: This algorithm aims to develop a classifier that identifies a subset of patients that will benefit more from a treatment of interest than similar patients who receive a different treatment.
Methods: TOPSPIN aims to predict whether a patient will benefit (class 1) or will not benefit (class 0) from a certain treatment of interest based on the gene expression profile of the patient. This algorithm relies on the idea that genetically similar patients who received a different treatment should have a large difference in survival, given that genetic similarity is defined in a manner that is relevant to treatment response. This principle is used to identify prototype patients: patients who received the treatment of interest and have a larger than expected survival difference with the genetically most similar patients who received another treatment. Genetic similarity is defined separately for 10 581 gene sets based on Gene Ontology (GO) annotation. These prototype patients are used to define a classifier: new samples who exhibit a similar gene expression profile as these prototypes are also expected to benefit more from the treatment of interest. Here we use TOPSPIN to predict which patients will benefit from the proteasome inhibitor bortezomib. We combine tumor gene expression data from the Total Therapy 2, Total Therapy 3 and HOVON-65/GMMG–HD4 phase III clinical trials into one dataset comprising 910 patients, split into a bortezomib arm (n=407) and a non-bortezomib arm (n=503). Progression free survival is used as outcome measure. This dataset was split in a training set (n=606) and a test set (n=304). The test set is not used at any point in the training procedure and is only used for independent validation.

Results: We successfully identify gene sets that enable us to predict which patients will benefit most from bortezomib. The top 8 performing GO sets based on Hazard Ratio (HR) were combined to achieve the final classification. In the training set 28.4% of patients are classified a class 1, resulting in an HR of 0.13 ($p=7.1*10^{-11}$) between the two treatment arms. More importantly, in an independent test set an HR of 0.47 (p=0.03) was found, as shown in Figure 1.

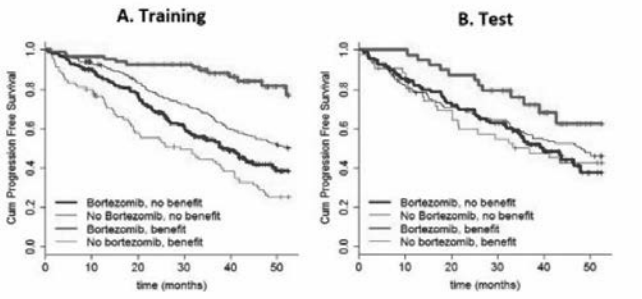

Figure 1. A. Kaplan Meier of training set classification, showing a large survival benefit for patients receiving bortezomib in class 1 (red lines) but not in class 0 (blue lines). B. Kaplan Meier of the test set classification, validating the classifier found on the training set.

Figure 1.

Summary/Conclusions: TOPSPIN is successful in predicting bortezomib specific survival in independent data. TOPSPIN can be applied to any dataset with two treatment arms and a continuous outcome measure. In a disease like MM, where many different treatment are available, selecting the right treatment is critical and TOPSPIN can aid in this decision.

E1280

AMYLOIDOSIS RESEARCH CONSORTIUM CARDIAC AMYLOIDOSIS SURVEY: RESULTS FROM PATIENTS WITH AL AMYLOIDOSIS AND THEIR CAREGIVERS

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Background: Cardiac amyloidosis is a severe disease that can lead to cardiac dysfunction and death. Amyloid light chain (AL) amyloidosis, hereditary transthyretin (hATTR) amyloidosis, and wild-type transthyretin (wtTTR) amyloidosis may result in cardiac amyloidosis. AL amyloidosis is caused by an accumulation of misfolded light chain and often involves organs other than the heart (eg, kidneys, nervous system). Initial symptoms are often nonspecific (eg, weight loss, fatigue). Consequently, a diagnosis is frequently made only after the disease has become advanced. Previous patient-directed research found that despite patients being initially referred most often to cardiologists (as opposed to hematologists and nephrologists), cardiologists diagnosed the condition much less frequently than other specialists.
Aims: To understand delays, errors, and inconsistencies in the diagnostic pathway for patients with AL cardiac amyloidosis and validate using caregiver responses.
Methods: An online survey consisting of 36 questions (for patients) and 37 questions (for caregivers) was developed by the Amyloidosis Research Consortium (ARC) and distributed to the patient mailing lists of ARC, the Amyloidosis Foundation, and Amyloidosis Support Groups in January 2017. The survey was designed for patients with all forms of cardiac amyloidosis and their caregivers; however, the present analysis is limited to AL amyloidosis.
Results: In this subanalysis, 137 patients and 115 caregivers completed the survey. Most patient respondents were >55 years of age (n=111; 81.0%); of those, 16.1% (n=22) were >70 years of age. Composition of the population was 81.8% white/Caucasian (n=112), 2.2% Asian (n=3), 4.4% African American (n=6), 2.2% Latino (n=3), 5.1% other (n=7), and 3.6% unknown (n=5). Most patients had lived with their diagnosis for >1 year (17.5% [n=24] <1 year; 23.4% [n=32] 1-2 years; 29.2% [n=40] 3-5 years; 21.2% [n=29] 6-10 years; 8.8% [n=12] >11 years). A significant percentage of patients had multiorgan involvement (54.7% [n=75] kidney; 29.9% [n=41] nerve; 14.6% [n=20] liver; 43.8% [n=60] GI; 14.6% [n=20] skin; 22.2% [n=49] other site). Before diagnosis, 43.8% (n=60) of patients were incorrectly diagnosed with one or more other conditions, predominantly by cardiologists and general practitioners (Table 1). Furthermore, more than 75% of patients visited 3 or more different physicians before diagnosis. Nearly all misdiagnosed patients (83.3%; n=50/60) reported receiving treatment for their misdiagnosed condition. Both patients and caregivers reported correct diagnoses being made most frequently by cardiologists and hematologists (Table 1). Caregivers echoed the multitude of distinct physicians visited before diagnosis (Table 1). Patients reported that biopsy of fat pad, kidney, or heart was the predominant diagnostic test performed (Table 1). Hospitalization was prevalent; 55.5% (n=76) patients reported amyloid-related cardiac hospitalization. Moreover, 31.3% (n=43) of patients reported the need for air travel for physician consultation.

Table 1.

Table 1. Patient and Caregiver Survey Responses, %, (n)*

Patients: Have you had any of the following tests/procedures?†					
Fat Pad Biopsy	Kidney Biopsy	Heart Biopsy	PYP/DPD Scan	Rectal Biopsy	Unsure
54.7 (75)	29.9% (41)	29.2% (40)	13.9% (19)	12.4% (17)	4.4% (6)

How many different doctors were seen before correct diagnosis?						
	1	2	3	4	>5	unknown
Patient	10.2% (14)	13.9% (19)	24.1% (33)	13.1% (18)	38.7 (53)	-
caregiver	11.3% (13)	17.4% (20)	21.7% (25)	13.0% (15)	19.1 (22)	17.4% (20)

What type of physician made the diagnosis?						
	Cardiologist	Hematologist	Nephrologist	Neurologist	Internist	Other/unknown
patient	18.2% (25)	35.0% (48)	17.5% (24)	1.5% (2)	4.3% (6)	23.4% (32)
caregiver	19.1% (22)	21.7% (25)	20.9% (24)	0.9% (1)	4.3% (5)	33.0% (38)

Patients: What type of doctor told you that you had something other than amyloid? †						
Cardiologist	GP	Internist	Other	Neurologist	Hematologist	Nephrologist
40.1% (55)	34.3% (47)	25.5% (35)	22.6% (31)	13.1% (18)	8.8% (12)	6.6% (9)

* not all respondents answered all questions
† respondents could check all answers that applied

Summary/Conclusions: This represents the first survey compiling both caregiver and patient experiences with AL amyloidosis. Alignment of caregiver with patient responses validates our patient-directed research. Patients with AL cardiac amyloidosis frequently receive misdiagnoses and sometimes receive incorrect treatment for the misdiagnosed condition. Disease awareness among all specialists is vital, especially among those to whom patients are initially referred due to the nature of their initial symptoms.

E1281

EFFICACY OF DARATUMUMAB-BASED REGIMENS IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA – A SYSTEMATIC LITERATURE REVIEW AND NETWORK META-ANALYSIS

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Background: Daratumumab is a new monoclonal antibody aimed to improve outcomes in relapsed or refractory multiple myeloma (RRMM), and has been investigated in combination with lenalidomide plus dexamethasone (DRd), and with bortezomib plus dexamethasone (DVd), in randomized controlled trials (RCTs), POLLUX and CASTOR, respectively. Although DRd and DVd have been compared against current standard of care (SOC), namely Rd, and Vd, it is important to assess how daratumumab plus SOC compares with other routinely used treatment regimens and investigational regimens expecting regulatory approvals.

Table 1. NMA Efficacy Results.

Network	Comparison	PFS		OS	
		HR (95% CrIs)	Prob DRd/DVd better than comparator*	HR (95% CrIs)	Prob DRd/DVd better than comparator*
IMiD-containing	DRd vs. Rd	**0.37 [0.27, 0.51]**	100.0%	**0.63 [0.42, 0.95]**	98.7%
	DRd vs. KRd	**0.54 [0.37, 0.78]**	100.0%	0.80 [0.50, 1.28]	83.1%
	DRd vs. ERd	**0.54 [0.37, 0.80]**	99.9%	0.82 [0.51, 1.30]	80.0%
	DRd vs. NRd	**0.50 [0.33, 0.74]**	100.0%	0.70 [0.42, 1.15]	91.9%
IMiD-free	DVd vs. Vd	**0.33 [0.26, 0.42]**	100.0%	**0.63 [0.42, 0.95]**	98.6%
	DVd vs. Kd	**0.62 [0.45, 0.86]**	99.8%	0.80 [0.48, 1.34]	80.5%
	DVd vs. FVd	**0.48 [0.35, 0.65]**	100.0%	0.67 [0.43, 1.05]	95.9%
	DVd vs. CVd	**0.46 [0.26-0.82]**	99.6%	0.54 [0.24, 1.23]	92.9%

**100% probability represent any value above 99.951%*

Aims: Therefore, the objective of this analysis is to compare DRd and DVd with other relevant treatment options via network meta-analysis (NMA) techniques.
Methods: A systematic literature review (SLR) based on searches of Medline, Embase, and the Cochrane Library was conducted to identify and then assess RCTs of treatments for RRMM. The specific studies of interest were those that had investigated the efficacy of other treatment options considered to be comparators to DRd or DVd. Data from trials that met the SLR's inclusion criteria and the most recent data from POLLUX and CASTOR were extracted and then included in a Bayesian NMA to allow for the indirect comparison.
Results: Data from RCTs identified by the SLR allowed formulation of two evidence networks. Network 1 included DRd and other immunomodulatory agent (IMiD)-containing regimens, and Network 2, contained DVd and other

immunomodulatory agent (IMiD) -free regimens. Analysis using a fixed-effects model found that DRd compared with other IMiD-containing regimens in Network 1, and DVd compared with other IMiD-free regimens in Network 2 prolonged PFS and OS among patients RRMM (see Table 1).
Summary/Conclusions: In the absence of prospective head-to-head trials, NMA provides potentially important information on comparative effectiveness of different treatments. This NMA suggests that the combinations of DRd and DVd are effective in improving PFS in patients with RRMM with similar trends found for OS when compared with other established and new regimens.

E1282

TRENDS IN TREATMENT PATTERNS AND SEQUENCING IN PATIENTS WITH MULTIPLE MYELOMA DIAGNOSED 2011-2016 IN THE UNITED STATES USING AN ENHANCED ELECTRONIC HEALTH RECORDS DATABASE

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Background: Over the past few years, the multiple myeloma (MM) treatment (Tx) landscape has changed considerably. Immunomodulating (IMiD®) drugs and proteasome inhibitors (PI) have emerged as mainstays of MM Tx. However, the limitations and lag time of available administrative claims databases make it difficult to assess current real-world trends in the Tx of MM.
Aims: The study aimed to describe trends in demographics, Tx patterns, and sequencing for newly diagnosed MM (ndMM) patients (Pt) in the United States (US) using an enhanced Electronic Health Records (EHR) database.
Methods: A retrospective observational study of ndMM Pts was conducted utilizing EHR from a nationally-representative database (Flatiron Health). The Flatiron MM provider network comprises over 260 clinics throughout the US. Pts with an ICD-9 (203.0x) or ICD-10 (C90.xx) diagnosis of MM between 01/01/2011–12/31/2016 were randomly selected into the study. Pts were excluded if they did not have ≥2 documented clinical visits during the study period. Diagnosis of MM was confirmed through review of unstructured chart data. Index date was defined as the Pt's date of diagnosis with MM. NdMM Pts were defined as those without a MM Tx more than 14 days prior to their first diagnosis date. Start of first-line (1L) therapy was defined as the 1st episode of an eligible systemic Tx given after or up to 14 days before the index date. Regimens were defined using the 1st eligible drug episode plus other eligible drugs given within 28 days of each other. A maximum gap of 90 days was allowed within a given line of therapy (LOT) and was considered concluded the day before the start date of the next LOT.
Results: For the 3367 ndMM Pts identified, mean(SD) age was 68.5(11) years at the time of diagnosis, 45.9% were female, 57.6% were white, 14.7% African American, and 11.1% other race. The most common immunoglobulin (Ig) classes at diagnosis were IgG (51.8%) and IgA (18.9%). Median follow-up time for ndMM Pts was 15.9 months. During the study period, 1611 received only 1 line (L), 737 were treated with 2L, 325 with 3L, 252 with 4L+; while 442 (13%) received no Tx. Mean follow-up time for these groups was 471, 730, 928, 1132, and 610 days respectively. Among treated Pts, 205 (12.7%), 208 (28.2%), 109 (33.5%), and 98 (38.1%) received at least 1 stem cell transplant (SCT), respectively. Of Pts receiving 1L therapy, 984 (33.6%) received IMiD compound +PI, 715 (24.4%) received PI-based, and 556 (19%) received IMiD compound-based therapy in 1L. The use of IMiD compound +PI in 1L increased during the study period for SCT and non-SCT Pts (NSCT) from 40.6% and 21.5% in 2011, to 66.7% and 46.8% in Pts diagnosed in 2016. In Pts who received a SCT (n=618), the most common 1L regimens were lenalidomide + bortezomib + dexamethasone (RVd; n=217, 43.9%), cyclophosphamide + bortezomib + d (CyBord; n=124, 20.1%), lenalidomide + d (Rd; n=70, 11.3%), and bortezomib + d (Vd; n=57, 9.2%). In NSCT Pts (n=2307), the most common 1L regimens were RVd (n=642, 27.8%), Vd (n=510, 22.1%), and Rd (n=412, 17.9%). Among the RVd NSCT Pts with a documented 2L (n=189), the most common 2L regimens were CyBord (13.2%), carfilzomib monotherapy (7.4%), pomalidomide + d (6.9%), and carfilzomib + d (6.9%). Documented death occurred in 785 (23.3%) Pts during the study period.
Summary/Conclusions: Over time, RVd has become the most common 1L regimen for SCT and NSCT Pts with ndMM. After a median follow up of 15.9 months, many patients remain in the initial LOT. With a longer follow up time, we will able to observe sequencing and patterns of treatment in later LOTs.

E1283

HLC PAIR SUPPRESSION AS A RISK FACTOR FOR BLOODSTREAM INFECTIONS AND EARLY DEATH IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS

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Background: Infection is a major cause of morbidity and mortality in patients with Multiple Myeloma (MM), responsible for 10-25% of deaths within 6 months of diagnosis. It is associated with suppression in primary antibody response (systemic immunoparesis (SI)), which may be aggravated following anti-myeloma therapy. Recently, the suppression of the non-monoclonal immunoglobulin pair (e.g. IgG-Lambda in IgG-Kappa MM), termed heavy+light chain (HLC) pair suppression, has been associated with poor prognosis in MM. However, the impact of HLC pair suppression as a risk factor for bloodstream infections has not been reported.
Aims: To evaluate HLC pair suppression as a risk factor for bloodstream infections and early death in MM patients.
Methods: The study retrospectively included 114 consecutive MM patients with diverse bloodstream infections, identified during investigation into unexplained fever and hospitalisation. Bloodstream infection was defined as a positive blood culture related to a febrile episode. The population consisted of 66 male:48 female patients, with a median age of 68 (56-77) years. The monoclonal immunoglobulin isotype was: 49 IgG-K, 26 IgG-L, 21 IgA-K and 18 IgA-L. In order to explore the impact of HLC pair suppression on bloodstream infections and early mortality, only events within 6 months (180 days) from diagnosis were documented. HLC pair suppression was defined as suppression in the levels of the non-monoclonal pair by >50% below the lower limit of the reference range. HLC measurements were carried out using Hevylite® immunoassays (The Binding Site) on a SPA_{PLUS} analyser. SI was defined as levels of the alternate immunoglobulins (e.g. IgA and/or IgM in an IgG patient) more than 50% below the lower reference range. Association between variables were analysed by Chi-square test and survival was estimated using Kaplan-Meier method.
Results: At diagnosis, HLC pair suppression was observed in 72 (63%) patients, and SI in 52 (45%). The incidence of bloodstream infections during the study period was 23%; and 20 patients (18%) died within 6 months from diagnosis. We found a significant association between HLC pair suppression and both the occurrence of bloodstream infections (OR: 6.10, 95% CI: 1.71-21.83; p=0.002) and early deaths (OR: 4.02, 95% IC: 1.10-14.66; p=0.03); by contrast SI had no significant association with either event (p=0.07 and p=0.3, respectively). Survival analyses demonstrated an association between bloodstream infections and shorter OS (50% *vs* 92%, HR: 7.43, 95% CI: 2.96-18.61, p<0.0001, Figure A). The risk of bloodstream infections was significantly higher among patients with HLC pair suppression *vs* those without suppression (34% *vs* 7%, respectively; HR: 5.12, 95% CI: 1.54-17.07, p=0.003, Figure B). In line with this, patients with HLC pair suppression had shorter overall survival (OS) compared to those without (76% *vs* 93%, HR: 3.47, 95% CI: 1.02-11.83, p=0.03). By contrast we found no association between SI and risk of infection (p=0.08) or survival (p=0.4).

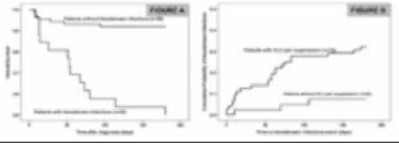

Figure 1.

Summary/Conclusions: HLC pair suppression provides information on immune status and associates with an increased risk of bloodstream infections and early deaths in newly diagnosed MM patients. Our findings highlight the importance of recognising this status at time of diagnosis, and suggest that HLC pair suppression may help guide clinical decisions about the need for adequate antimicrobial treatment during myeloma therapy.

E1284

DARATUMUMAB SIGNIFICANTLY IMPROVED PROGRESSION-FREE SURVIVAL IN COMBINATION WITH LENALIDOMIDE AND DEXAMETHASONE IN PATIENTS WITH RELAPSED MULTIPLE MYELOMA

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Background: Daratumumab is a human IgG1k monoclonal antibody which binds with high affinity to the CD38 molecule on the surface of multiple myeloma cells. It induces rapid tumor cell death through multiple immune-mediated mechanisms and showed encouraging results alone and with lenalidomide and dexamethasone in a phase 1-2 study involving patients with relapsed multiple myeloma.
Aims: The primary end point of the study was progression-free survival.
Methods: We enrolled a total of 134 patients (74 male and 60 feemale, mean age 65.4±18.2 years) with multiple myeloma who had received at least three lines of therapy to receive lenalidomide with dexamethasone (68 patients, control group A) or in combination with daratumumab (66 patients, therapy group B).

Results: At a median follow-up of 9.8 months in a protocol-specified interim analysis, 67 patients had disease progression or death were observed (in 18 of 66 patients (27.2%) in the group B *vs* 28 of 68 (41.1%) in the control group (p<0.001)). A significantly higher rate of overall response was observed in the group B than in the group A (88.7% *vs* 62.9%, p<0.001), as was a higher rate of complete response or better (39.2% *vs* 16.1%, p<0.001). The most common adverse events during the treatment was myelotoxicity (neutropenia in 68.6% of the patients in the therapy group B *vs* 42.1% of those in the control group A), anemia (in 21.5% *vs* 13.6%) and thrombocytopenia (in 13.8% *vs* 8.7%).
Summary/Conclusions: In patients with relapsed multiple myeloma, the addition of daratumumab to lenalidomide and dexamethasone appeared active and resulted in significantly improved progression-free survival. However it was associated with a higher risk of myelotoxicity.

E1285

COMPARISON BETWEEN IMMUNOFIXATION NEGATIVITY AND NORMAL FREE LIGHT CHAIN RATIO WITH MULTICOLOUR FLOW CYTOMETRY FOR RESPONSE ASSESSMENT IN PATIENTS WITH MULTIPLE MYELOMA WITH VGPR OR BETTER

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Background: Urine and serum Immunofixation electrophoresis (uIFE and sIFE, respectively) and free light chain assay (FLC) are widely accepted as standard tests for diagnosis and monitoring of multiple myeloma (MM). However, there is significant discordance between the electrophoretic method and FLC test for response assessment. Despite this discordance, previous studies did not address the differences in assessment of treatment response between the intact immunoglobulin MM (IIMM) and light chain only MM (LCMM)/oligosecretory MM (OSMM). uIFE results are poorly correlated with the serum FLC level, however, treatment response of LCMM has still been recommend to assess by 24-hour uIFE by International Myeloma Working Group guideline. However, MRD levels on uIFE negativity or normal FLC ratio (rFLC) in patients with various types of MM have not been studied.
Aims: To explore the relationship between uIFE, sIFE negativity and normal rFLC for MRD assessment in patients with IIMM and LCMM.
Methods: We initially selected 162 patients with MM (LCMM and OSMM, *n* = 41; IIMM, *n*=21) that received treatment at Kameda Medical Center, Kamogawa-shi, Japan and Kanazawa University Hospital, Kanazawa-shi, Japan between April 2008 and January 2016. Among them, 126 patients (LCMM/OSMM 40, IIMM 86), who achieve VGPR or better response, were selected on the basis of the availability of simultaneous serum and urine test, FLC data, and bone marrow MRD. To explore the relationship between uIFE and sIFE negativity and normal rFLC, MRD levels were compared by multicolour flow-cytometry (MFC) in patients with LCMM/OSMM, and IIMM that obtained VGPR or better. MRD negativity was defined as MRD $<10^{-4}$. Complete response (CR) was divided into conventional CR (cCR, CR but MRD-positive) and MRD$^-$CR (CR and MRD-negative)
Results: One hundred and fifty-four complete IFE, FLC, and MFC data set of 126 patients (LCMM/OSMM 40, IIMM 86) with ≥ VGPR were analysed. Normal FLC at VGPR, cCR and MRD$^-$CR was 65.0%, 78.4% and 78.6% in IIMM, and 0%, 21.4% and 100%, respectively, in LCMM/OSMM. The percentages of sample at MRD levels of MRD $\geq 10^{-3}$, 10^{-3} ≥MRD $>10^{-4}$ and 10^{-4}>MRD in LCMM/OSMM were 12.5%, 50.0%, and 100% for negative uIFE, and 0%, 11.5% and 100% for normal rFLC, respectively. These figures in IIMM were 23.0%, 41.6%, 81.4% for negative sIFE, and 53.8%, 75.0% and 88.8% for normal rFLC, respectively. Positive/negative predictive value (PPV/NPV) of uIFE and rFLC for MRD in LCMM/OSMM was 100%/54.8% and 100%/85.0%, respectively, while those were 90.6%/45.8% and 88.9%/32.4% in IIMM, respectively.

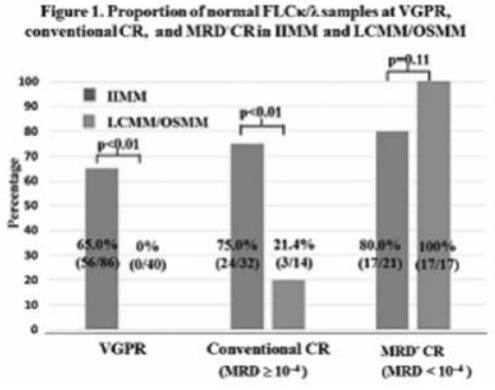

Figure 1.

Summary/Conclusions: Our observations confirmed that FLC test has greater sensitivity than uIFE for detection of the monoclonal component, and that normalization of sFLC ratio is highly predictive of MRD negativity in patients with LCMM/OSMM. The proportion of negative sIFE samples increased with depth of MRD, but the FLC response did not appear to parallel with the depth of response in IIMM. We recommend that FLC test should be incorporated into response assessment in LCMM/OSMM as an alternative to 24-h uIFE, and both negative sIFE and normal rFLC are still useful for response assessment of residual clonal PCs in IIMM.

E1286

DARATUMUMAB IS AN EFFECTIVE AND SAFE SALVAGE THERAPY IN RELAPSED/REFRACTORY PATIENTS WITH MULTIPLE MYELOMA AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: Daratumumab is a human monoclonal antibody that targets CD38, a cell surface protein that is overexpressed on multiple myeloma cells. The drug became the first monoclonal antibody as single agent therapy approved by the FDA for the treatment of multiple myeloma. The role of allo-SCT in myeloma patients (pts) remains unclear; nevertheless, the registry study of EBMT suggests an increasing rate of allografts in Europe in last years. Despite the potentially curative potential of this approach, the increased relapse rate and low PFS remain a central clinical problem.
Aims: In this single center retrospective analysis, we report on our experience on the use of daratumumab in relapsed/refractory myeloma pts after allo-SCT.
Methods: A total of 16 pts (male, n=9) with median age of 66 years (39-72) relapsing after allo-SCTs that had been performed during a period 2008-2015 at the University of Hamburg and received daratumumab as single agent salvage therapy. Before allografting 9 pts received one and 7 pts ≥2 autografts, respectively. All but one pt received at least 1 salvage therapy line prior to the allo-SCT. The allografts were performed from unrelated donors (MUD, n=9; MMUD, n=4) or matched related donors (MRD, n=3). The median number of salvage lines post-transplant and prior to first daratumumab infusion was 3 (1-4). The salvage regimens included bortezomib, lenalidomide, pomalidomide and carfilzomib. Daratumumab infusions were started at a median of 21 months (0-30) after relapse/progress.
Results: The median number of infusions was 13 (3-22). A total of 16 and 15 pts were available to safety and efficacy evaluation, respectively. The safety was assessed according to the Common Toxicity Criteria (CTC). A total of 20 adverse events were observed in 16 pts: dyspnea (CTC1, n=3; CTC2, n=1), bronchospasm (CTC2, n=2) shivering (CTC1, n=3), cough (CTC1, n=1; CTC2, n=1), musculoskeletal pain (CTC1, n=4), acute coronary syndrome (CTC3, n=1), skin rush (CTC2, n=1), pressure on eyes (n=1). Two patients developed late onset infections (pneumonia and infection of urinal tract) followed by temporarily therapy interruption. We observed a decrease of Tregs ($CD4^+CD25^{int/high}CD127^{low}$) number from a median of 5,05% at start to 0,65% at day 21 after first daratumumab infusion in four pts. There were no cases of GvHD. The adverse events appeared in all pts after the first infusion, with improved tolerance of following infusions. There were no cases, where the therapy had to be stopped due to adverse events. Within a median follow-up of 32 months (1-45) from the relapse/progression 12 of 16 pts remain alive. Two pts died due to progress of myeloma and another 2 pts died due to severe infection/sepsis. A total of 9 of 15 evaluable pts responded (60%, PR, n=7, vgPR, n=2). The responses (decrease of paraprotein and/or free light chains; reduction of extramedullary tumor in 2 pt) occurred at a median of 7 days (7-75) after the first administration of daratumumab. The median response duration is 4.5 mo (1.5-8). Six pts show ongoing responses. All responding and 2 non-responding pts (stable disease) showed clinical improvement of constitutional symptoms.
Summary/Conclusions: Daratumumab demonstrated encouraging efficacy in relapsed/refractory pts with myeloma after allo-SCT. The administrations of the drug in these heavily pre-treated pts were associated with good safety profile and development mostly non-severe adverse events mostly after the first infusion. Further studies on the use of daratumumab in post-transplant setting are warranted.

E1287

PROGNOSTIC RELEVANCE OF VEGF AND VEGFR EXPRESSION IN CD138+/CD19- AND CD138+/CD19+ PLASMA CELLS FROM PATIENTS WITH MONOCLONAL GAMMOPATHIES

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Background: Vascular endothelial growth factor (VEGF) is a potent angiogenic peptide with biologic effects that include regulation of extracellular matrix remodeling and inflammatory cytokine generation with an important role in the bone marrow microenvironment of multiple myeloma (MM). Angiogenesis is enhanced in the bone marrow of MM patients in parallel with tumor progression. Myeloma and stromal cells secrete angiogenic factors that include VEGF. Previous studies showed heterogeneity in the expression of VEGF between plasma cells (PCs) from the same MM patient. However, no clear association with expression levels, phenotypic subtypes of PCs and prognosis was demonstrated.
Aims: The present study aimed to evaluate the expression levels of VEGF and VEGF receptor (VEGFR) on phenotypic subtypes of PCs in patients with monoclonal gammopathies and to explore its role as diagnostic and prognostic biomarkers.
Methods: We include 128 patients with monoclonal gammopathies, 60 patients with newly diagnosed symptomatic MM and 68 with monoclonal gammopathy of uncertain significance (MGUS) and also from 11 non-neoplastic controls (Ctr). We evaluated the expression levels of VEGF and VEGFR by flow cytometry in the two populations of bone marrow PCs, identified by gating CD138+/CD19- (clonal PCs) and CD138+/CD19+ (non-clonal PCs). The results are presented as percentage of PCs expressing VEGF/VEGFR and as expression levels of this antiangiogenic molecules expressed in mean intensity of fluorescence (MIF). The effects of these parameters on progression-free survival (PFS) and overall survival (OS) were analyzed with Kaplan-Meier method. For statistical analysis, software IBM SPSS Statistics v22 was used. ROC curves were performed to assess the VEGF and VEGFR accuracy as diagnostic and prognostic biomarkers.
Results: In our cohort of patients, median age was 70 (39-86) years, 52% were male. We found increased expression levels of VEGF in CD138+/CD19- PCs from MM (80±7,5 MIF) compared tomgUS patients (61,7±6,2 MIF) (p=0,011), and also higher to the observed in CD138+/CD19+ PCs (39,92±1,74 MIF) in both populations of patients (p<0,001 and p=0,02, respectively). No differences were observed in the expression levels of VEGF in CD138+/CD19+ PCs from MM (39,92±1,74 MIF),mgUS patients (41,18±1,92 MIF) and controls (32,8±1,5 MIF). However, the percentage of CD138+/CD19+ cells expressing VEGF was significantly higher inmgUS (39,4±4%) and in MM patients (46,7±4,5%) compared to Ctr (13,5±0,5%)(p=0,019 and p=0,003, respectively). The differential expression of VEGFR showed thatmgUS patients with VEGFR levels higher than 23,5 MIF in CD138+/CD19- PCs have higher probability to progress to MM [AUC 0,688 (95%CI 0,592-0,784), p<0,0001, 90% sensitivity, 56% specificity, 65% PPV, 84% NPV]. In MM patients, we also found an association between increased VEGF expression levels in CD138+/CD19- PCs (³175 MIF) and inferior PFS (p=0,002) and OS (p=0,003), irrespective of first line therapy (bortezomib-based regimens for fit patients or alkylating-based treatments for unfit patients). Interestingly, we also observed an increased percentage of CD138+/CD19+ PCs (³21%) expressing VEGF in MM patients with a more favorable PFS (p=0,04) and OS (p=0,008).
Summary/Conclusions: The results of our investigation showed that CD138+/CD19- and CD138+/CD19+ PCs have differences in what concerns VEGF/VEGFR expression, not only in MM patients, but also inmgUS patients. The increased expression of VEGF in clonal PCs from MM compared tomgUS patients evidences the relevance of VEGF in myelomagenesis. We also demonstrated a negative prognostic impact of an increased VEGF expression in CD138+/CD19- PCs, highlighting the role of VEGF in the survival and maintenance of clonal PCs and as a predictor of outcome in MM progression. The association between the percentage of CD138+/CD19+ PCs and survival supports the suggestion that these cells may not be neutral players in the complex pathogenesis of MM. The results of our study should be further investigated in larger series of patients.

E1288

RACIAL DIFFERENCES OF FISH ABNORMALITIES IN MINORITIES WITH MULTIPLE MYELOMA: A SINGLE-CENTER EXPERIENCE

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Background: Racial disparities of FISH abnormalities in multiple myeloma (MM) have been well described in whites (W) but partially described in minorities (M) (Paulus et al, ASH 2016, 4432).
Aims: To explore racial-based differences of FISH abnormalities using the largest cohort of minorities to date.
Methods: CD-138 selected FISH was done on 799 consecutive patients (pts). Pts without symptomatic MM, and biopsy >6 months after diagnosis were excluded. The abnormalities evaluated included standard and intermediate risk: IGH rearrangements (IGH r), t(4;14), t(11;14), and high risk: t(14;20), t(14;16), del13q, del 17p, 1q21. Chi-square was used for statistical analysis. Due to smaller numbers, all M (Hispanic (H), Black (B), Asian (A) and Other (O)) were included into the same group for statistical analysis.
Results: 482 pts were eligible, 343 (71%) were W, 52 (10%) H, 50 (10%) B, 19 (3%) A, and 18 (3%) O. Median age was 65 years, 54% were male, and 26% ISS stage 3. There were no were no statistically significant differences in FISH abnormalities between the M. Overall W had more abnormalities in IGH r, t(4;14), t(11;14), t(14:20), 1q21 gain compared to M. Most notably W had more IGH r (39% *vs* 28%; p=0.019) and t(11;14) (20% *vs* 12%; p=0.024). There were no statistically significant differences between W and M in the high risk FISH abnormalities.

Table 1.

FISH, n (%)	W		H		B		A		O		M		p-values$
Total n=482	343		52		50		19		18		139		
IGH r	135	39%	16	30%	15	30%	4	21%	4	22%	39	28%	**<0.019***
t(4;14)	35	10%	4	7%	2	4%	2	10%	2	11%	10	7%	0.39
t(14;16)	21	6%	1	1%	5	10%	0	-	0	-	6	4%	0.43
t(11;14)	72	20%	7	13%	8	16%	2	10%	0	-	17	12%	**<0.024***
t(14;20)	6	1%	0	-	0	-	0	-	0	-	0	-	0.11
Del 13q	96	27%	16	30%	18	36%	2	10%	9	50%	45	18%	0.33
Del17p	159	45%	21	40%	22	44%	8	42%	11	61%	62	44%	0.72
1q21 gain	119	34%	12	23%	12	24%	7	36%	7	38%	38	27%	0.11

*$p-value compares W vs M. * means statistical significant (p-value<0.05)*

Summary/Conclusions: W had significant differences in FISH compared to M. W had more IGH r and t(11;14) than M, and there was no difference in high risk FISH abnormalities between W and M. This study confirms the biological racial disparities that exist in minorities with MM. Further studies with more inclusion of minorities are needed to elucidate these disparities and its effects on risk stratification and outcomes.

E1289

POMALIDOMIDE ALONE OR IN COMBINATION WITH LOW DOSE DEXAMETHASONE AS MAINTENANCE FOLLOWING INDUCTION WITH POMALIDOMIDE AND LOW DOSE DEXAMETHASONE IN RELAPSED AND REFRACTORY MYELOMA (ALLG MM14)

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Background: Whilst the addition of dexamethasone to upfront therapy with Immunomodulatory (IMiD®) agents is important to mediate rapid reduction in disease burden, preliminary findings suggest that the NK stimulatory effects of IMiD® compounds are best harnessed without the co-administration of dexamethasone, and may be especially effective in the setting of minimal disease burden (in the maintenance setting for example) when some inherent immune recovery has occurred. However this has yet to be confirmed in a prospective clinical trial.
Aims: To evaluate the effect of maintenance with POM alone (Arm 1) *versus* POM-LoDEX (Arm 2) on progression free survival (PFS), overall survival (OS), and kinetics of response (overall response rate (ORR)) in relapsed myeloma (MM) patients refractory to lenalidomide (R-LEN) demonstrating stable disease (SD) or better following salvage treatment with pomalidomide (POM) and low dose dexamethasone (LoDEX) induction.
Methods: Multicentre, open-label, randomized phase 2 study of relapsed R-LEN patients who had received>2 prior lines of therapy. POM 4mg days 1-21 (28 day cycle) was administered alone or in combination with LoDEX (40mg weekly) as maintenance following an induction with 4 cycles of POM and LoDEX. Treatment continued until toxicity or progression. Peripheral blood samples for immune studies were collected pre-induction and prior to cycles 1, 3, 6 and 10 of maintenance.
Results: 154 patients from 11 sites were enrolled on to the study (M:F 80:74), with a median age of 67 years (range 35-88). Median number of prior lines of therapy was 4.5 (2-14). All patients had failed LEN (100%), 127 (82.5%) were also refractory to bortezomib (double refractory) and 94 (61%) had received a prior autologous stem cell transplant. 72 (47%) patients achieved SD or better with POM-LoDEX induction and were randomised, 35 to POM (Arm 1) and to 37 to POM-LoDEX (Arm 2). After a median follow-up of 19 months, median PFS for all patients from study entry was 4.2m (IQR 2.1 – 8.6m). PFS for randomised patients (from time of randomisation) was 2.7m for POM (arm 1) *versus* 5.6 for POM-LoDEX (arm 2) (p=0.39). The PFS hazard rate for Arm 2 was relatively constant compared to Arm 1 which started with a hazard rate double that of Arm 2 but dropped to less than half of the rate in Arm 2 by 15 months, suggesting that with longer follow-up, there may be an emergent advantage to maintenance with POM *versus* POM-LoDEX (Figure 1.). Median OS for all patients from study entry was 13.2m (IQR 6.3-26.8m). For randomised patients, median OS (from time of randomisation) was 19m for POM (Arm 1) *versus* 13.7m for POM-LoDEX (Arm 2) (p=0.41). ORR (≥PR) for all patients was 45.5% [CR=5 (3.3%), VGPR=13 (8.4%), PR=52 (33.8%)]. Clinical benefit rate (CBR) (≥MR) was 55.2% [MR=15 (9.7%)].

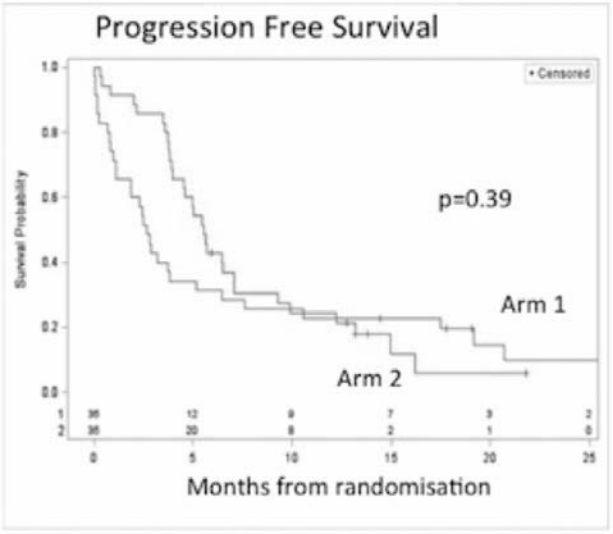

Figure 1.

Summary/Conclusions: In patients with relapsed myeloma, after initial disease control/debulking is achieved with POM - LoDEX, induction, maintenance with single agent POM may be more effective for sustaining disease control than continuation of POM-LoDEX. Correlative studies are currently underway to further investigate the immunological mechanisms behind this observation.

E1290

POMALIDOMID IS MORE EFFECTIVE IN REAL CLINICAL PRACTICE THAN IN RANDOMIZED TRIAL – AN OBSERVATIONAL STUDY OF THE CZECH MYELOMA GROUP

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Background: The combination of pomalidomide and low-dose dexamethasone (Pom-Dex) is a perspective option for patients with end-stage relapsed/refractory multiple myeloma (RRMM). We analyzed efficacy and toxicity of Pom-Dex in all patients from the Czech Republic treated from june 2013 to december 2016.
Methods: Patients were eligible if they had been diagnosed with RRMM and had failed at least two previous treatments with bortezomib and lenalidomide. They were treated with start dose of Pom (4mg/day on days 1-21, orally) plus low-dose dexamethasone (40mg/day on days 1, 8, 15, and 22, orally) until disease progression or unacceptable toxicity. We analyzed TTP and OS together with toxicity. Also, univariate Cox proportional hazards model for OS was done for standard risk factors. One hundred and twenty-two patients with median age of 67 treated with Pom-Dex were evaluated. Median follow-up was 8.7 months. Median of previous treatment lines was 4.
Results: Median TTP of Pom-Dex treatment was 7.1 months (95% CI 5.3-8.6). Median OS was 19.0 months (95% CI 13.2-25.8). The most common grade 3-4 adverse events were neutropenia in 44%, anemia in 22% and thrombocytopenie in 24% of patients. Grade 3-4 infection were observed in 10% of patients. Patients with ECOG worse than 2, B2microglobulin higher than 5, ISS stage 3, low hemoglobin, low platelet count and presenting extramedullary mass had worse OS according to univariate Cox proportional hazards model.
Summary/Conclusions: Our analyses show that Pom-Dex treatment of Czech RRMM patients is effective, well tolerated and had better results than the registration study. Performance status and tumor burden seem to be main prognostic factors according to our model. Thus, our suggestion for clinical practice is to start pomalidomide treatment as soon as possible in case of MM relapse.

E1291

UNDERSTANDING THE REAL-WORLD CLINICAL CHARACTERISTICS OF MULTIPLE MYELOMA PATIENTS IN EUROPE

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Background: Multiple myeloma is a heterogeneous disease that accounts for approximately 10% of all haematological malignancies. While European treatment guidelines exist for multiple myeloma, there is limited understanding about the characteristics of patients with multiple myeloma in Europe and how these characteristics vary by disease stage. Numerous patient and disease-related factors can have an impact on treatment choice. Data surrounding these factors would help to better characterise European patients and inform management and treatment practices in multiple myeloma.
Aims: The aim of the current study is to describe multiple myeloma patients from 5 European countries (France, Germany, Italy, Spain, and the UK) across the disease continuum.
Methods: Data were drawn from the Adelphi Real World Multiple Myeloma Disease-specific Programme (DSP), which was conducted across France, Germany, Italy, Spain, and the UK in Q1 2015. The Multiple Myeloma DSP is a real-world, cross-sectional survey that involves haematologists and haemato-oncologists who completed patient record forms for the next 8 multiple myeloma patients with whom they consulted. Study variables included patient demographics and background clinical information.
Results: A total of 262 physicians reported on 2,024 patients with multiple myeloma. Of these patients, 73.2% were receiving first-line treatment; the remaining 26.8% were receiving second-line treatment or later. The median age of multiple myeloma patients was 70 years, 58.4% were male, and most patients (88.5%) were white/Caucasian. Only 4.3% of patients had a family history of cancer. Patients had a mean height of 168.8 cm, a mean weight of 72.8 kg, and a mean body mass index of 25.5 kg/m^2. In terms of performance status, 79.8% of patients had an Eastern Cooperative Oncology Group (ECOG) status of 0 or 1, whereas 20.2% had an ECOG status of ≥2. While 12.9% of patients had smouldering myeloma, 47.5% of patients had advanced stage (stage III) disease. The most common symptoms experienced by patients were anaemia (31.0%), bone pain (32.4%), fatigue/weakness (28.4%), and kidney impairment or failure (12.6%). Furthermore, 34.6% of patients had bone complications at some point in time. Over half (51.1%) of patients had comorbidities; of these, 22.8% had hypertension and 12.5% had diabetes. Overall, 33.7% of patients were considered eligible for transplant. Variances in patient characteristics, both by country and by line of therapy, were observed.
Summary/Conclusions: Results from this analysis provide valuable insight into multiple myeloma patients in European countries. These findings can help to inform future treatment practices in Europe.

E1292

RAD REGIMEN AS INDUCTION BEFORE ASCT: OUTCOMES, SAFETY AND EFFECTS ON BONE METABOLISM AND ANGIOGENESIS; FINAL RESULTS OF A PHASE 2 STUDY OF THE GREEK MYELOMA STUDY GROUP

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Background: There is limited published data on the efficacy and safety of the combination of lenalidomide, adriamycin and dexamethasone (RAD) as induction therapy for newly-diagnosed myeloma (NDMM) patients who are eligible for autologous transplantation (ASCT).
Aims: The primary endpoint of this phase 2 study was the assessment of overall response rate (ORR) after 4 cycles of RAD induction in NDMM patients who are eligible for ASCT. Secondary endpoints included safety, progression-free survival (PFS), time to progression (TTP) and overall survival (OS). Exploratory endpoints included: i) the yield of stem cell collection after RAD; ii) the effects of RAD on biochemical markers of bone metabolism: CTX, TRACP-5b, bone-alkaline phosphatase (bALP), P1NP, osteocalcin, soluble RANKL, osteoprotegerin (OPG) and dickkopf-1 (Dkk-1) and iii) the effects of RAD on angiogenic cytokines: angiopoietin- (Angp) 1 & -2, angiogenin (Ang), VEGF and bFGF.
Methods: Lenalidomide was administered at a dose of 25mg, po, daily, on days 1-21 of a 28-day cycle; dexamethasone was given at a dose of 40mg, po, on days 1, 8, 15, and 22, while adriamycin was administered as IV bolus infusion at a dose of 9mg/m^2, on days 1-4 of each cycle. Serum levels of the above markers of bone remodeling and angiogenesis were measured before and after 4 cycles of RAD, using ELISA methodology.
Results: Between November 2014 and February 2016, 45 patients (median age: 56 years) were enrolled. Osteolytic lesions were present in 33 (73%) patients, while 3 (6.6%) had hypercalcemia (>11mg/dl). All but one patient completed 4 cycles of RAD. Best response included one (2.2%) CR, 8 (17.8%) VGPRs, 21 (46.7%) PRs, for an ORR of 66.7%, while 14 (31%) patients had

stable disease and one progressed during the 4th cycle of treatment. After ASCT the ORR was 84.4%: 6 (13.3%) patients achieved CR, 13 (28.9%) VGPR and 19 (42.2%) PR. Adverse events of grade 3 or 4 included mainly anemia (4 patients, 9%), neutropenia (3, 6.6%) and febrile neutropenia (one patient). After a median follow-up of 19.1 months (range: 11.0-24.9), 11 patients have progressed and 4 died (all had achieved less than VGPR post-ASCT). The PFS, TTP and OS rates at 12 months were 88.6%, 88.6% and 100%, respectively. Forty (89%) patients had adequate stem cell collection post-RAD induction (mean±SD: 8.94±6.50 x10^6/kg CD34+ cells). Patients at baseline had elevated levels of CTX, TRACP-5b, sRANKL/OPG, Dkk-1, Ang, VEGF, VEGF-A, bFGF and reduced levels of Angp-1/Angp-2, bALP and P1NP compared to 30 healthy subjects of similar age and gender (p<0.01 for all comparisons). RAD therapy resulted in a reduction of circulating CTX (p=0.03), TRACP-5b (p<0.01), Ang (p=0.02), VEGF (p=0.01) and bFGF (p<0.01). Moreover, RAD increased serum levels of bALP (p=0.036), P1NP (p=0.028) and Ang-1/Ang-2 ratio (p=0.022). These alterations occurred irrespective of response, although patients who achieved at least VGPR tended to have more profound differences in the above parameters.

Summary/Conclusions: RAD resulted in successful induction for NDMM patients with an ORR of approximately 67% pre- and 84% post-ASCT. With a median follow-up of >1.5 year, the 12-month PFS rate and OS rates are high, as expected. RAD reduced bone resorption and increased bone formation; the latter has not been previously described with lenalidomide-based regimens. Furthermore, RAD reduced angiogenic cytokines and this supports the action of the regimen also through the disruption of the interactions between myeloma and stromal cells.

E1293

MULTIPLE MYELOMA IN THE REAL WORLD: HOW THERAPEUTIC LANDSCAPE HAS CHANGED IN THE LAST 15 YEARS

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Background: Therapeutic Multiple Myeloma (MM) scenario has completely changed in the last 30 years: conventional chemotherapy (CT) has been gradually abandoned and autologous stem cell transplantation(ASCT),proteasome inhibitors as Bortezomib (Bor) and immunomodulatory drugs as Thalidomide and Lenalidomide (Len) have become the new actors in MM treatment (Tx).
Aims: aim was to outline how the management of MM patients (pts) had changed in the last 15 years reporting the experience of a single center.
Methods: Overall survival(OS) was measured from disease onset to death for any cause or last follow-up. Progression free survival (PFS) was defined as the time from first-line tx to disease progression or last-follow-up. The effect of variables on OS and PFS was evaluated by log-rank test.

Table 1.

Median age	61.3yrs (27.9-86.5)
Age	
≤ 65yrs	63.5%(371/584)
65- 80yrs	32.7 %(191/584)
>80yrs	3.8 %(22/584)
Sex	
Female	46 % (270/584)
Male	54%(315/584)
ISS staging	
(available in 447/584 patients)	
ISS 1	25.7%
ISS2	2%
ISS3	72.3%
Asymptomatic MM	59/584(10.1 %)
Symptomatic MM	525/584(89.8%)
≤65yrs	337/525(64.2%)
>65yrs	188/525(35.8%)
CRAB at diagnosis	
bone lesions	63.6%
anemia (Hgb< 10 mg/dl)	23.7%
kidney failure (creatinine >2 mg/dl)	11.6%
hypercalcemia (calcium > 11.5 mg/dl)	7.7%

Results: We analyzed 584 MM pts diagnosed in our center from 2000 to 2015. Patients' characteristics are reported in Table1. Median number of therapy lines is 2 (1-9). Among pts ≤65 yrs, 242/371 (71.8%)received ASCT as 1st line tx. Patients >65 yrs were treated as follows: 16(8.5%) received ASCT,53(28.2%) VMP,21 (11.2%) MPT,45 (23.9%) MP and 53 (28.2%) other therapies. As 2nd line tx our pts received: 27 ASCT (8.9%), 115 Bor-based tx (38.1%), 48 Len-based tx (16%), 53 CT(17.5%) and 59 other therapies(19.5%). As 3rd line tx: 5 pts received ASCT (2.8%), 65 Bor-based tx (35.9%), 42 Len-based tx (23.2%), 39 CT (21.5%) and 30 other therapies (16.6%).The percentage of pts receiving a new drug in 1st line was 64% (338/525). This percentage was significantly different in pts treated before and after 2007 (42% *vs* 87%, p=0.001). Similar results were observed in 2nd line, 75% of pts treated before 2007 received a new drug and 90% after 2007(p=0.002). Median PFS in pts >65 *vs* ≤65 yrs was 1.7 *vs* 2.4 yrs (p<0.001); median PFS in pts ≤65 yrs receiving or not ASCT was 3.2 *vs* 1.9 yrs (p=0.001); of note, PFS was not different when considering pts undergoing to ASCT after a CT-based or a Bor-based induction(3 *vs* 2.5 yrs, p=0.2).Time to next treatment (TTNT) in pts receiving ASCT or not was 30.1 months (5-122.7) *vs* 10.3 months (0.2-70.5) (p<0.001) from 1st to 2nd line tx and 11.2 months (0.3-121.9) *vs* 6.3 months (1-41.6) from 2nd to 3rd line tx (p=0.026). The early mortality (within the first year) was 5.9% (31/525), in details only 1/258 of those eligible to ASCT (0.4%) and 30/267 of those not candidate to transplant (11.2%). When considering this last group before and after the 2007, we observed a significant higher incidence of early mortality in the first period [21 (17.2%) *vs* 9 (6.2%), p=0.006]. About new drugs toxicity: with Bor-based tx 30% of pts complaint neurological, 20% gastrointestinal and 18.2% hematologic toxicity; with Len-based tx 36.4% infective events and 28.9% hematologic toxicity. Median OS in pts ≤65 *vs* >65 yrs was 7 *vs* 4.8 yrs (p=0.001), of note considering pts ≤65 *vs* >65 yrs treated before 2007 median OS was 5.5 *vs* 3.1 yrs (p<0.001)and after 2007 median OS was not reached *vs* 7.5 yrs (p=0.034).
Summary/Conclusions: Our real life data show how MM therapeutic scenario have changed during the last 15 yrs. The tremendous improvement observed in this study was mainly evident in older pts with a strong reduction of early mortality and median OS reaching, in the second time frame after year 2007, 7.5 yrs. For younger pts ASCT confirmed to be of great benefit in term of TTNT and PFS. Thus, considering the real advantage of new drugs a palliative approach is not anymore justified even in very old pts.

E1294

CUL4A EXPRESSION AS A POTENTIAL PROGNOSTIC MARKER IN MULTIPLE MYELOMA PATIENTS TREATED WITH IMMUNOMODULATORY DRUGS

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Background: Despite the clinical effectiveness of immunomodulatory drugs (IMiDs) in multiple myeloma (MM), neither their mechanisms of action nor the biomarkers that could identify patients who would benefit from IMiDs treatment are yet known. While the identification of the IMiDs action via cereblon (CRBN), Ikaros (IKZF1) and Aiolos (IKZF3) was a milestone, the role of other pathways including CRBN and E3 ubiquitin ligase complex proteins (CUL4A, DDB1, Roc1) are not fully understood so far.
Aims: The aim of this study was to: 1) evaluate CUL4A, IKZF1, IKZF3, MUM1 and IRF4 expression in bone marrow trephine biopsies obtained from multiple myeloma patients before treatment with thalidomide, 2) analyze the associations between the expression of these proteins and clinical characteristics and outcomes.
Methods: IHC staining for CUL4A, IKZF1, IKZF3, IRF4 and MYC expressions was performed in trephine biopsies obtained from 25 patients with multiple myeloma before the treatment initiation. The patients (20 females, 5 males, median age 68 years) were treated with thalidomide based regimens as a first-line treatment. The patterns of proteins' expression were scored independently by two hematopathologists on a semi-quantitative scale and the cutoff was defined as ≥ 30% positive cells. Associations between studied proteins' expression and clinical parameters were assessed using Fisher's Exact Test for categorical variables and Mann-Whitney-Wilcoxon Test U for continuous variables. Survival (PFS and OS) were estimated using the Kaplan-Meier method and compared using the log-rank test.
Results: Prior to treatment with thalidomide, 13 patients (52%) showed high expression (≥ 30%) of CUL4A protein. No associations between expression of CUL4A and other proteins were seen. Patients with high CUL4A expression more often presented low disease stage according to Durie-Salmon classification (P=0.02), beta-2-microglobulin level within normal ranges (P=0.07) and higher median platelet count (P=0.003) compared to patients with low CUL4A expression. Moreover, patients with high CUL4A expression before treatment showed longer PFS compared to those with low CUL4A expression (P= 0.03). Additionally, a significant association between high Aiolos expression and higher median of CD138+ cells in bone marrow was observed (P=0,01) compared to low Aiolos expression, however no other associations with clinical course of MM patients were seen. No associations between IKZF1, IKZF3, IRF4, MYC expression and patients' characteristics or outcome were revealed.

Summary/Conclusions: In conclusion, our results suggest that CUL4A expression could serve as prognostic marker for patients assigned to IMiDs containing regimens. Further analysis of the expression of other E3 ligase complex proteins in a larger patient cohort is in progress.

Acknowledgement: This work is funded by a grant No 2014/13/B/NZ5/03160 from National Science Centre.

E1295

MAINTENANCE THERAPY WITH BORTEZOMIB IN PATIENTS WITH MULTIPLE MYELOMA (MM) AFTER ASCT AND MINIMAL RESIDUAL DISEASE (MRD)

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Background: MRD-negativity status in patients with MM after autologous stem cell transplantation (ASCT) directly correlates with higher Relapse-Free Survival. It remains unclear whereas these patients should all receive maintenance therapy with it's toxicity and cost.
Aims: To assess efficacy of maintenance therapy with Bortezomib in patients with MM, who have achieved complete remission after ASCT with MRD positive and negative status.
Methods: From January 2014 to February 2016 52 patients with MM (19 male and 33 female) ages from 24 to 66 years (median 54 years) who have achieved complete remission after ASCT were randomized for a year-long maintenance therapy with Bortezomib. On 100th day after ASCT and after completion of maintenance therapy samples bone marrow from all patients were assessed using 6-color Flow Cytometry to detect MRD. We chose Relapse-Free Survival (RFS) as the indicator of maintenance therapy efficacy. Kaplan-Meier survival curves were compared using log-rank test. Statistical analysis was performed using SAS 9.4.

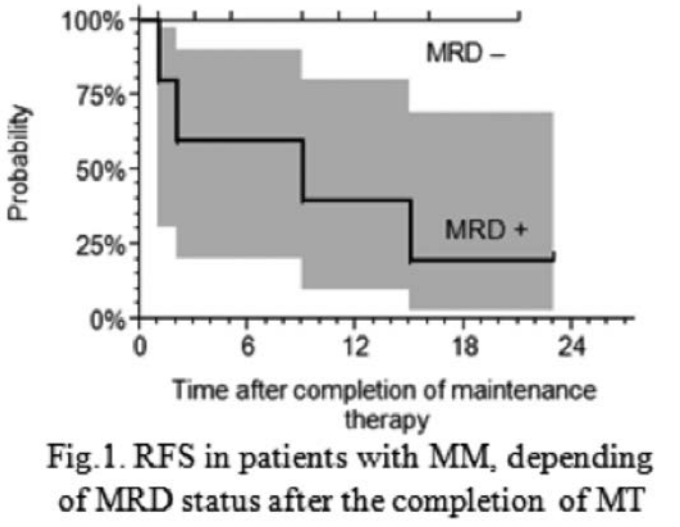

Fig.1. RFS in patients with MM, depending of MRD status after the completion of MT

Figure 1.

Results: 2-year Relapse-Free Survival in patients with MRD-negative status after ASCT was higher (p=0.05) than that in MRD-positive patients - 52,9% (95% CI: 35,5–70,5%) *vs* 37,2% (95% CI: 25,4–49,3%). The MRD-positivity significantly increases the risk of relapse (HR=1,7; 95% CI: 1,2–3,4; p=0,05). Two year cumulative probability of relapse after ASCT in patients with MRD-negative status, who had (n=15) and hadn't received (n=10) maintenance therapy with Bortezomib was not different (p=0,58). Average time of relapse in MRD-positive patients who received maintenance therapy with Bortezomib was 5 months longer than in the group of patients without maintenance therapy - 17,3 months *vs* 12,3 months. In the group of MRD-positive patients who did not completed maintenance therapy, relapse was diagnosed in 6 patients. After the end of the treatment 42% of MRD-positive patients achieved MRD-negative status. RFS in this group of patients was significantly higher than in the group of treated MRD-positive patients who retained that status after maintenance therapy (MT) - 100% *vs* 20% (p=0,02, Fig.1).
Summary/Conclusions: In cases when MRD-negative status was achieved after ASCT, maintenance therapy does not increase the RFS. In comparison – patients with positive MRD status after ASCT require maintenance therapy to improve their survival rate.

E1296

LONG-TERM OUTCOME OF MULTIPLE MYELOMA (MM) PATIENTS TREATED UP-FRONT WITH SINGLE OR TANDEM AUTOLOGUS STEM CELL TRANSPLANTATION (ASCT) - SINGLE CENTRE EXPERIENCE WITH 334 PATIENTS

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Background: ASCT after induction treatment has been standard of care for MM for almost 30 years. Some centers routinely perform two transplantation up-front (so-called tandem transplants), while others advocate postponing the second transplant until after progression. In recent years novel antimyeloma agents have significantly improved the prognosis of MM patients, thus casting further doubts on the value of the more aggressive tandem ASCT approach.
Aims: To describe long-term outcomes of MM patients treated with ASCT (single and tandem) in a single centre. alled tandem transplants), while others advocate postponing the second transplant until after progression. In recent years novel antimyeloma agents have significantly improved the prognosis of MM patients, thus casting further doubts on the value of the more aggressive tandem ASCT approach.
Methods: This was a retrospective analysis of outcomes of 334 MM patients who underwent 470 ASCT procedures at our center between 1993 and 2014. During that period treatment policies changed from single to tandem to salvage second ASCT, as data from different clinical studies became available.
Results: 296 patients received VAD (vincristine, doxorubicin, dexamethasone) as induction therapy and 38 regimens based on immunomodulatory drugs or proteasome inhibitors. All received high-dose melphalan for pretransplant conditioning, 32 in combination with total body irradiation. Tandem ASCT (defined as second transplantation performed within 6 months after the first) was performed in 136 patients, single ASCT in 168 and salvage second (after relapse/progression) in 30 patients. Transplant related mortality was 1.5%. Median follow up is 70 months (range 4 – 238). Median overall survival (OS) for the entire group is 123 months and median progression free survival (PFS) 40 months. Tandem ASCT in comparison to single and second salvage transplantation resulted in superior OS (203 *vs* 86 *vs* 68 months respectively, p<0.0001) and PFS (60 *vs* 38. *vs* 25 months respectively, p<0.0001) (figure). Thirteen percent of patients who underwent tandem ASCT are alive and progression-free more than 10 years after the procedure. Fourteen patients developed secondary malignancies.

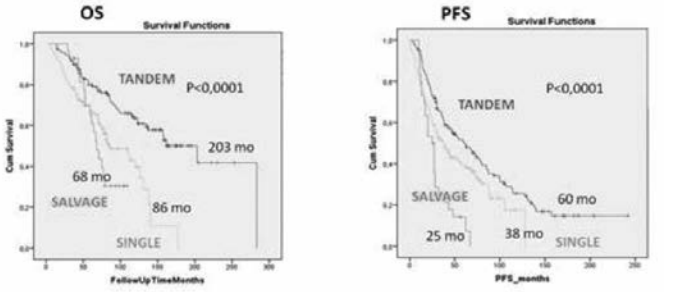

Figure 1.

Summary/Conclusions: Our results suggest that tandem ASCT is a very effective treatment modality that can partially substitute for the absence of expensive novel agents with low long-term and lethal toxicities. Tandem ASCT seems to result in superior OS and PFS in comparison to single or salvage second ASCT. More than 10% of patients treated with tandem ASCT experience very prolonged PFS.

E1297

EXTRAMEDULLARY DISEASE IN MULTIPLE MYELOMA PATIENTS UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION: CLINICAL IMPACT IN DIAGNOSIS, TREATMENT AND OUTCOME

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Background: Extramedullary disease (EMD) is defined as an infiltrate of clonal plasma cells outside of the bone marrow. The presence of EMD in multiple myeloma (MM) patients (pts) at diagnosis is a relatively uncommon presentation and accounts for about 13% (6-20%) of MM pts. Although several studies

showed an association of EMD with other adverse prognosis factors and unfavourable outcomes, reports evaluating EMD role in pts undergoing autologous hematopoietic stem cell transplantation (aHSCT) are scarce.
Aims: We aimed to evaluate the clinical and laboratorial characteristics of pts with EMD as well as its impact in outcomes of MM pts submitted to aHSCT (response to treatment, overall survival [OS] and progression free-survival [PFS]).
Methods: We analysed 155 MM pts submitted to aHSCT in our centre between January/2007 and December/2015, excluding second procedures. The assessment of response to treatment was based in the International Myeloma Working Group consensus criteria (2016).
Results: The median age of the cohort was 58 years (27-69), with 58% of males, and the most common subtype was IgGκ (45%). In our cohort, 46 pts (29.7%) presented EMD at diagnosis, which was significantly higher compared to reports in the literature (p<0.001; 95%CI 0.22-0.37). The more common involved sites were vertebral column (49%), ribs (13%) and pelvis (13%). EMD occurred more frequently in males (38 *vs* 18%; p=0.012) and in pts with bone disease at diagnosis (45 *vs* 13%; p<0.001). Surprisingly, it was more commonly observed in pts with lower International Staging System scores (I and II *vs* III) (82 *vs* 64%; p=0.022) and without anaemia at diagnosis (28 *vs* 11%; p=0.023). No other significant differences in characteristics at diagnosis were found between pts with and without EMD. Pts with EMD achieved lower complete response/ very good partial response (CR/VGPR) proportions previously to aHSCT (30.4 *vs* 53.2%; p=0.009), as well as at 100 days after aHSCT (D100) (41.3 *vs* 59.6%; p=0.037). However, no differences were found concerning refractoriness to first line therapy or proteasome inhibitor (PI) treatment, despite EMD pts received a higher mean number of therapeutic lines previously to aTPH (1.7 *vs* 1.4; p=0.025). After a median follow-up of 46.6 months, the median OS was not reached for global cohort and both groups, and there was no difference between them (p=NS). The median PFS was 51.3 months for global cohort, with no differences seen between pts with and without EMD (50.2 *vs* 54.1; p=NS). Pts with EMD treated with a PI (57%) presented a higher OS (NR *vs* 55.2 months, p=0.04), but with no impact in PFS (p=NS), and there were no differences concerning radiotherapy treatment (72%) or thalidomide maintenance after aHSCT (32%) (p=NS).
Summary/Conclusions: In our cohort, EMD prevalence was significantly higher than usually described in the literature. This observation was probably associated with a more carefully surveillance of EMD in aHSCT candidates. EMD was associated with a lower proportion of CR/VGPR previous to aHSCT and at D100 evaluation, even after a higher number of therapeutic lines, although we failed to demonstrate that EMD was an independent prognosis factor for PFS and OS. PI seem also to be the best first-line therapeutic approach for EMD pts. In conclusion, our study suggests that EMD is underdiagnosed in MM pts. It is necessary to achieve a better knowledge of the physiopathology of EMD, in order to define better treatment options that may overcome its negative impact in therapeutic response.

E1298

DIFFERENCES IN PATIENT AND DISEASE CHARACTERISTICS OBSERVED AT INITIATION OF FIRST-LINE AND INITIATION OF SECOND-LINE TREATMENT IN PATIENTS WITH RELAPSED MULTIPLE MYELOMA IN THE CZECH REPUBLIC

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Background: Tools such as the International Staging System (ISS) and the revised ISS (R-ISS) are used to stratify risk of death in patients newly diagnosed with multiple myeloma (MM), enabling assessment of survival expectations. These tools are based on factors measured at diagnosis only; understanding the role of these factors at relapse is less clear. Patient characteristics change between first-line (1L) and second-line (2L) treatment. Predicting survival using tools that rely on patient characteristics measured at diagnosis only may, therefore, become less relevant than other tools that consider factors measured at relapse. The Registry of Monoclonal Gammopathies (RMG) is a large hematological disease registry, collecting data from patients in the Czech Republic and Slovakia. Data from the RMG can be used to explore real-world characteristics of patients with MM throughout their disease course.
Aims: To explore how key characteristics of patients with relapsed MM evolve between initiation of 1L treatment and initiation of 2L treatment to better understand drivers of disease progression and death.
Methods: This non-interventional, observational, retrospective study used data collected prospectively from Czech patient charts available in the RMG. Adults (≥18 years old) initiating 1L treatment for MM between May 2007 and April 2016 were included (N=3027); those with smoldering MM were excluded. Patient and disease characteristics were extracted at initiation of 1L and of 2L treatment. Repeated measurements were available only for those who initiated 1L and 2L treatment (1L+2L group; N=1418); patients who did not start 2L treatment may have been in remission, lost to follow-up or had died.
Results: Patient and disease characteristics are summarized in the table (all patients starting 1L and those who started 1L+2L). In general, for patients who received 1L+2L treatment, their health status improved between initiation of 1L and of 2L treatment. At 2L, patients tended to have a lower ISS stage (re-measured at 2L) than when they started 1L (stage I at 1L: 26.6%; at 2L: 41.1%). Similarly, the proportion of patients with R-ISS stage III disease was lower at start of 2L (24.6%) than at start of 1L (31.1%) treatment. Eastern Cooperative Oncology Group performance status scores were also better for patients when they started 2L than when they started 1L treatment (stage 3–4 at 1L: 8.7%; at 2L: 5.5%). Laboratory measurements indicated that patients were in better health at the start of 2L treatment than at initiation of 1L treatment: median M protein levels decreased from 31.2 g/L at 1L to 17.7 g/L at 2L, and elevated calcium and creatinine levels were less common at 2L than at 1L. Median lactate dehydrogenase levels were slightly elevated at start of 2L *vs* start of 1L treatment (184.4 U/L *vs* 206.6 U/L).

Table 1.

	Characteristics at:	Initiation of 1L treatment		Initiation of 2L treatment
	Patient group:	1L (N=3027)	1L+2L (N=1418)	1L+2L (N=1418)
Patient characteristics	Age			
	<65 years	1259 (41.6%)	651 (45.9%)	551 (38.9%)
	≥65 years	1768 (58.4%)	767 (54.1%)	867 (61.1%)
	Cytogenetic risk at diagnosis[a]			
	Standard risk	230 (43.1%)	95 (35.2%)	–
	High risk	304 (56.9%)	175 (64.8%)	–
	NA	2493	1148	–
	ECOG PS			
	0	317 (14.0%)	128 (12.8%)	178 (15.2%)
	1–2	1733 (76.5%)	788 (78.6%)	927 (79.3%)
	3–4	216 (9.5%)	87 (8.7%)	64 (5.5%)
	NA	761	415	249
Disease characteristics	ISS stage			
	I	640 (28.9%)	262 (26.6%)	429 (41.4%)
	II	740 (33.4%)	361 (36.6%)	336 (32.2%)
	III	838 (37.8%)	363 (36.8%)	280 (26.8%)
	NA	809	432	373
Laboratory parameters	Calcium level, mmol/L			
	≤2.75	2088 (90.0%)	1209 (86.6%)	1141 (94.8%)
	>2.75	233 (10.0%)	187 (13.4%)	62 (5.2%)
	NA	706	22	215
	Creatinine level, mmol/L			
	≤173	1788 (76.8%)	1107 (79.2%)	1039 (86.2%)
	>173	540 (23.2%)	291 (20.8%)	166 (13.8%)
	NA	699	20	213
	Median M protein level, g/L (5th–95th percentile)	25.2 (0.0–68.8)[b]	31.2 (0.0–72.2)	17.7 (0.0–51.5)
	NA	126	62	261

Data shown are n (%), unless otherwise stated.
[a]Standard risk: negative for del(17p), t(4;14) and t(14;16); high risk: positive for del(17p), t(4;14) or t(14;16).
[b]Measured at diagnosis.
1L, first line; 2L, second line; ECOG PS, Eastern Cooperative Oncology Group performance status; ISS, International Staging System; NA, not available.

Summary/Conclusions: Patient health was better at initiation of 2L treatment than at initiation of 1L treatment. At relapse, patients are likely to be closely monitored and are able to initiate the next treatment line while in relatively good health; at initiation of 1L, patients may have experienced deterioration in health which could have triggered their diagnosis. These findings illustrate how patient characteristics change over time and indicate that factors influencing survival may evolve; therefore, restaging patients at relapse may be beneficial and could contribute to improved predictive tools that can better define survival estimations at first relapse by considering patients' experiences at 1L.

E1299

AN EARLY GOOD RESPONSE AFTER BORTEZOMIB-BASED INDUCTION REGIMENS REPRESENTS A SIGNIFICANT PREDICTOR FOR IMPROVED PFS IN NDMM PATIENTS

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Background: Introduction of triplets-based induction regimens containing proteasome inhibitors (PIs) in clinical practice have led to higher response rates and prolonged life expectancy in newly diagnosed multiple myeloma (NDMM)

patients. Different studies have linked complete response (CR) with better PFS (progression free survival), but not always with prolonged overall survival (OS), most likely due to the impact of novel agents in the management of relapsed-refractory patients. Overall, these observations suggest PFS as a more reliable predictor of clinical outcome. Also, the biological aggressiveness is emerging as a pivotal disease characteristic which affects clinical behavior and response to therapy. In this context, little is known about the association of response kinetic with survival outcomes.

Aims: In order to evaluate whether early achievement of a good quality response impacts on outcome, we retrospectively analyzed 87 NDMM patients treated at our institution with bortezomib containing regimens (BRs).

Methods: From 2004 to 2016, 87 patients with NDMM and measurable disease (serum and/or urine M protein) were treated with BRs. Both patients eligible and non-eligible to ASCT were included in the study; patients undergoing ASCT were censored at the time of transplant. Median age was 66 (range 32-87); males were 51 (59%); 72 (83%) patients were in III stage; median follow up was 30.7 months; median number of administered courses was 5 (range 2-9). PFS was defined according to IMWG criteria. Cytogenetic risk evaluation performed by a standardized FISH panel, including del17p, del13q, t(11;14), t(4;14), was available in 37 patients (42.5%). Among these high risk abnormalities were identified in 20 patients. Early good response (EGR) defined an M protein reduction ≥75% after 2 courses of therapy. Survival curves were calculated for PFS and OS by Kaplan Meyer method, using log-rank test.

Results: PFS and OS were both assessed in patients who achieved EGR as well as in patients who did not. No significant differences were observed in terms of OS between the two groups, whilst PFS was significantly longer in patients achieving EGR (p= 0.036, median PFS 44.7 *vs* 29 months, respectively). Next, we analyzed patients with high risk cytogenetic. Among these, EGR *vs* non-EGR patients reached a PFS of 43.7 and 18.7 months, respectively. Remarkable PFS differences between these two groups were not significant (p value=0.11).

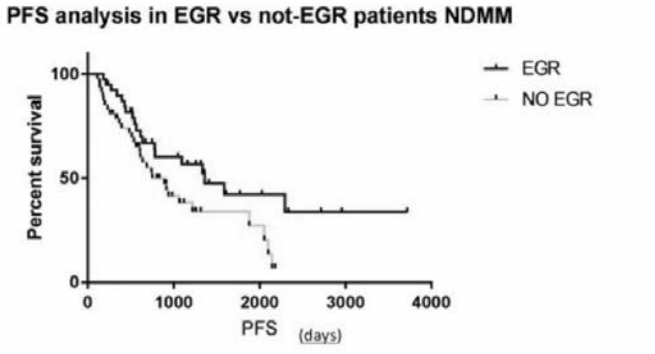

Figure 1.

Summary/Conclusions: Overall, our data demonstrate a significant impact of EGR on PFS in NDMM patients after BRs, irrespective of median age at diagnosis. In presence of high cytogenetic risk EGR is associated with prolonged PFS, although not significantly. Ongoing analysis on larger cohort of high risk patients will confirm the impact of EGR on PFS also in this group of patients. Based on our data kinetic of response, deriving from EGR assessment, may provide information on both disease aggressiveness as well as clinical outcome, thus representing a novel, surrogate marker for an early survival analysis, with favorable cost-effectiveness characteristics. In summary biological and clinical information deriving from EGR analysis combined with cytogenetic risk evaluation and patient-related (age, comorbidities) characteristics, may represent a useful tool to make clinical decisions. Further prospective evaluations are needed to include this marker in clinical practice.

E1300

RELATIVE PROGRESSION-FREE SURVIVAL OVER TIME OF NOVEL TRIPLET REGIMENS FOR THE TREATMENT OF RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background: In combination with lenalidomide (REVLIMID®, R) and dexamethasone (d), elotuzumab (EMPLICITI™, E), carfilzomib (KYPROLIS®, K), and ixazomib (NINLARO®, N) were recently approved for the treatment of relapsed/refractory multiple myeloma (RRMM). In randomized controlled trials, all three drugs showed a significant relative reduction in the risk of disease progression or death as compared to patients who received Rd. To date, there have been no head-to-head trials comparing ERd, KRd, and/or NRd.

Aims: To describe the time-specific progression-free survival (PFS) based on published Kaplan-Meier PFS curves for ERd, KRd, and NRd relative to Rd.

Methods: Individual patient-level data (IPD) were reconstructed from the published Kaplan-Meier PFS curves from the ELOQUENT-2 (ERd), ASPIRE (KRd), and TOURMALINE-MM1(NRd) randomized, controlled, Phase III trials using digitization software and the methods described by Guyot, et. al. Using the reconstructed IPD, Kaplan-Meier survival curves were estimated for each arm within each trial. PFS curves were digitized by two independent researchers and the reconstructed curves were overlaid with the published data to validate the IPD. In each trial, the relative PFS benefit over time was calculated as the difference in the Kaplan-Meier PFS estimate of each triplet regimen and the Kaplan-Meier PFS estimate of Rd divided by the Kaplan-Meier PFS estimate of Rd: $rPFS(t)=[S_{XRd}(t) - S_{Rd}(t)] / S_{Rd}(t)$. Where S(t) denotes the Kaplan-Meier PFS estimate at time t, and X denotes E, K, or N, respectively.

Results: IPD from the three randomized controlled trials was successfully reconstructed and validated. Numerically, ERd had the highest relative PFS over the initial 10 months of treatment and showed sustained benefit from month 24 onwards (**Figure 1**). At 12 months, the relative PFS benefit was 17.9% for ERd, 21.7% for KRd, and 9.7% for NRd. At 24 months, the relative PFS benefit was 45.1% for ERd, 34.3% for KRd and 24.1% for NRd. At 36 months, the relative PFS benefit was 39.9% for ERd and 19.1% for KRd. ERd had a higher relative PFS than NRd for almost the entirety of RRMM treatment. At the end of data availability, NRd and KRd showed no additional PFS benefit relative to Rd, while ERd showed a sustained benefit through 40 months. Data will be updated for the conference, where available.

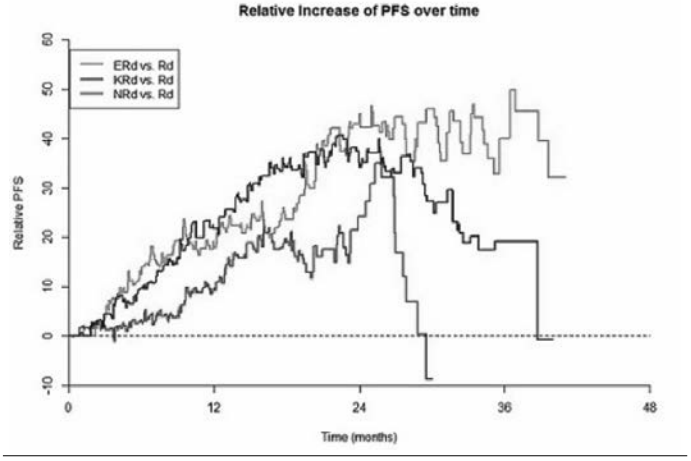

Figure 1.

Summary/Conclusions: For the treatment for RRMM, ERd showed an early and sustained benefit in relative PFS which was maintained through 40 months. KRd and NRd showed initial benefits which faded by the end of data availability.

E1301

POMALIDOMIDE WITH LOW-DOSE DEXAMETHASONE IN PATIENTS WITH RELAPSED OR RELAPSED AND REFRACTORY MULTIPLE MYELOMA: A PROSPECTIVE ANALYSIS IN A POPULATION-BASED REGISTRY

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Background: Patients with relapsed and/or refractory multiple myeloma (RRMM) have limited treatment options and a poor prognosis. Previous trials showed that pomalidomide combined with low-dose dexamethasone is effective in these patients with improvement in response and survival. These studies led to the approval of pomalidomide as third line treatment in patients with RRMM.

Aims: A prospective analysis in a population-based registry was conducted to assess response and survival in patients with RRMM treated with a pomalidomide-based regimen. Also, we defined subgroups who benefit most of this treatment regimen.

Methods: Patients were eligible for pomalidomide if they received ≥2 prior lines of therapy including bortezomib, lenalidomide and alkylator therapy and developed progressive disease on their last therapy. This is a prospective analysis of patients registered at the nationwide Netherlands Cancer Registry. Treatment consisted of 4mg pomalidomide, day 1-21, combined with corticosteroids. Treatment was discontinued in case of progressive disease or unacceptable toxicity. Primary endpoint was progression-free survival (PFS). Secondary endpoints included overall survival (OS), overall response rate (ORR), toxicity, response per risk group (based on cytogenetics and ISS at initial diagnosis) and response per age group (≤65 *vs* >65 years).

Results: A total of 82 patients (median age 68 years [range: 43-88]) were included in this analysis. CRAB criteria included anemia in 23 patients (28%), renal insufficiency in 8 (9.8%), hypercalcemia in 13 (16%) and bone lesions in 54 (66%). Median time from diagnosis to start pomalidomide was 5.75 years [range: 0.8-18.4], median number of treatment cycles was 3 [range: 1-17]. At time of analysis 59 patients had stopped pomalidomide treatment: 24 patients had progressive disease, 10 had unacceptable toxicity, 6 patients were refractory, 4 patients died during treatment and 15 patients stopped due to various other reasons. Grade ≥3 hematological adverse events occurred in 11% of patients, 4% had neutropenic fever. Grade ≥3 non-hematological toxicities occurred in 57% of patients, including infection in 22%, gastrointestinal disorders in 5% and renal disorders in 5%. Of 69 patients evaluable for response ORR was 41%, with a partial response (PR) rate and a very good partial response (VGPR) rate of 36% and 4% respectively. Response based on age was not significantly different (p=0.426). Median PFS for all patients was 3.8 months (95% confidence interval [CI] 2.3-6.6). Patients >65 years had a longer PFS of 5.7 months (95% CI 2.3-8.0) *versus* 2.8 months (95% CI 1.9-6.6) in patients ≤65, however, this was not statistically significant (p=0.427) (figure 1). For patients achieving ≥PR, median PFS was 9.6 months (95% CI 5.7-not reached (NR)). Median PFS in patients diagnosed more than ten years prior to initiation of pomalidomide treatment was 9.6 months (95% CI 1.8-NR), as compared to 2.2 months (95% CI 1.9-6.6) among patients treated within 5 years after diagnosis (p=0.05). Data about previous treatment, ISS stage, cytogenetics at diagnosis and an update of OS will be presented at EHA.

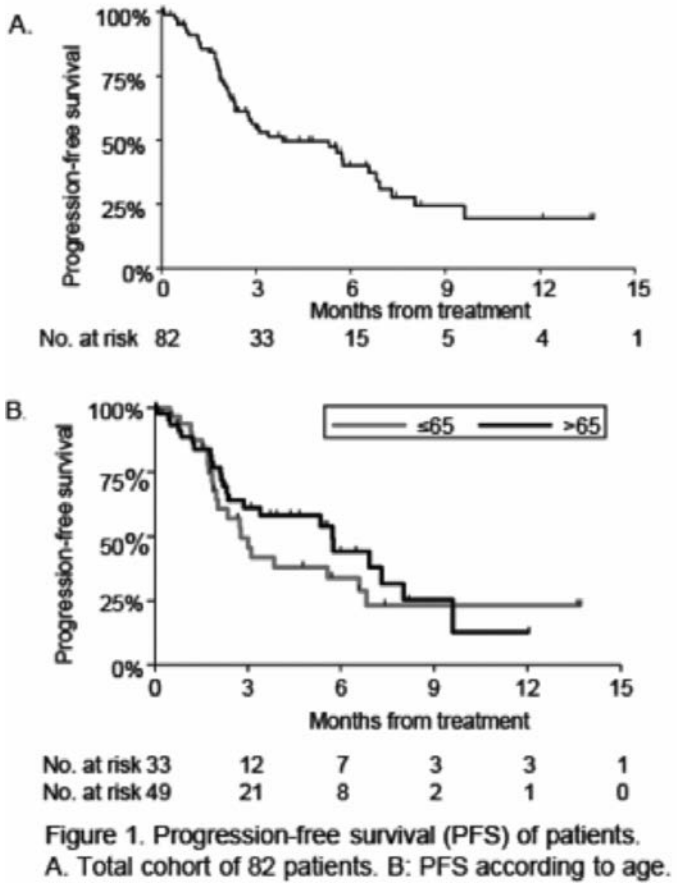

Figure 1. Progression-free survival (PFS) of patients. A. Total cohort of 82 patients. B: PFS according to age.

Figure 1.

Summary/Conclusions: In this analysis the experience in clinical practice of patients with RRMM treated with a pomalidomide-based regimen is reported. These data support results shown in clinical trials. Preliminary data presented here suggest that older patients and patients with a long interval between initial diagnosis and pomalidomide treatment (indicating a less aggressive multiple myeloma) may benefit from this treatment.

E1302

INVOLVED/UNINVOLVED HEAVY/LIGHT CHAIN INDEX CAN PREDICT PROGRESSION IN MULTIPLE MYELOMA PATIENTS AFTER AUTOLOGOUS PERIPHERAL BLOOD STEM TRANSPLANT. PRELIMINARY EXPERIENCE

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Background: High-dose therapy followed by autologous peripheral blood stem transplant (APBSCT) has demonstrated to improve overall survival and progression free survival with a high complete remission rate in multiple myeloma (MM) patients. However most patients eventually present progression or relapse (P/R). Detection of P/R is mainly based on a significant increase of monoclonal protein (MC) or free light chains (sFLC). The identification of new biomarkers to early predict P/R might be clinically useful for an anticipated therapy.
Aims: The aim of our study was to evaluate the potential role of the Involved/Uninvolved Heavy/Light Chain index (I/Ui) in this setting.
Methods: We prospectively followed 44 MM transplanted patients: 19 with IgG-kappa isotype, 11 with IgG lambda, 9 with IgA-kappa and 5 with IgA-lambda. They were followed for 29.0±3.8 months (mean±standard error (SE)). Serial serum samples from each MM patients were collected periodically after APBSCT. Relapse or progression was defined according IMWG criteria. To identify factors that predict disease progression in MM transplanted patients, we studied heavy/light chains (HLC) pair quantification, sFLC and total immunoglobulins levels in serial serum samples collected during the follow-up. Involved/uninvolved index (I/Ui) was calculated using the monoclonal chain (Involved) as numerator and the polyclonal chain of the same class (Uninvolved) as denominator. The HLC ratio (rHLC) was calculated as IgGκ/IgGλ or IgAκ/IgAλ with normal reference ranges established in 1.3-3.7 for IgG and 0.7-2.2 for IgA.
Results: In IgG MM patients, values of I/Ui were significantly increased in pre-relapse compared to basal samples (8.49±4.01 *vs* 2.23±0.67 p=0.012). By contrast, this index remained stable along follow-up in patients in complete remission (CR) or with a partial response (PR). However, the later showed higher values of I/Ui ratio, suggesting that the presence of an M-component induces a moderate immunosuppression of the uninvolved chain of the monoclonal isotype. Regarding IgA MM, we established a cut-off value of 2.0 for I/Ui that allowed the discrimination of patients at high risk of early progression (values above 2.0) from those in CR, whose levels of I/Ui are always below 2.0 (p=0.02).
Summary/Conclusions: Our results show that HLC-pair measurement could detect progression or relapse and the increase of MC in transplanted MM patients earlier than other methods. Future studies will need to demonstrate the real value of the I/Ui index as a biomarker to anticipate progression in MM patients subjected to APBSCT.

E1303

MULTIPLE MYELOMA IMMUNOPHENOTYPIC REMISSION IS A SIGNIFICANT PREDICTOR OF PROGRESSION FREE SURVIVAL AFTER FIRST AUTOLOGOUS STEM CELL TRANSPLANTATION - PILOT STUDY

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Background: Minimal residual disease in multiple myeloma assesed by multiparameter flow cytometry has become an increasingly important predictor of progression-free survival (PFS).
Aims: Our primary endpoint was to evaluate PFS in myeloma patients after stem cell transplantation who reached immunophenotypic CR (iCR) *versus* those who have not.

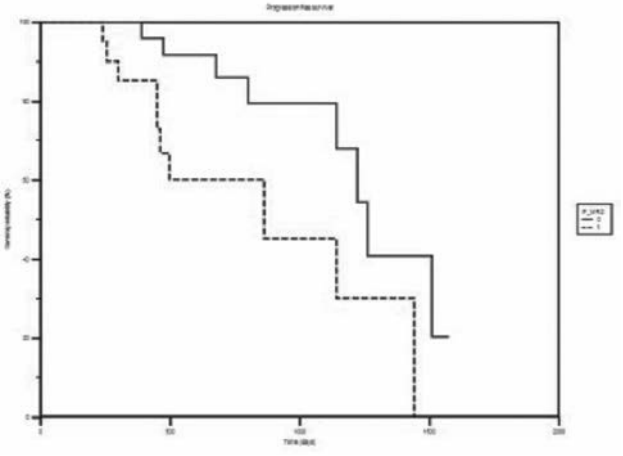

Figure 1.

Methods: We prospectively evaluated prognostic importance of minimal residual disease detection by multiparameter flow cytometry (MFC) in multiple

myeloma patients who underwent autologous stem cell transplantation from January 2014 until December 2016. All patients were uniformly treated with bortezomib based induction therapy followed by high dose chemotherapy (Melphalan 200mg/m^2) and autologous stem cell transplantation. Minimal residual disease (MRD) status was determined by 8-colour MFC 1 month after autologous transplantation from bone marrow aspirate in all patients who achieved at least conventional VGPR or CR.
Results: We identified 56 patients who fulfilled the above mentioned criteria. 30 were males and 26 females, median age was 61. 62.5% of patients (35/56 patients) achieved iCR, 37.5% of patients (21/56) did not. Median follow up of the cohort was 19 months (6-59). 32.1% of patients (18/56) relapsed during the follow-up period, 16.1% of patients (9/56) died. 22.9% (8/35 patients) in iCR and 47.6% (10/21 patients) not in iCR relapsed during the follow up. Patients in iCR showed significantly longer PFS with median 42 months than those in less than iCR with PFS median 29 months (p=0.0196, log-rank test). This was reflected by decreased hazard ratio of relapse (0.3565) for iCR group.
Summary/Conclusions: Achieving immunophenotypic CR is clearly associated with longer progression free survival compared to conventional CR. Reaching iCR should be a goal of myeloma treatment.

E1304

REGULATION OF NORMAL AND MONOCLONAL IMMUNOGLOBULIN SECRETION BY CYTOKINES (S- SYNDECAN-1, BLYS & TGF-BETA-1) IN PATIENTS WITH IG-SECRETING B-CELL DISORDERS AT PRESENTATION. PROGNOSTIC IMPLICATIONS

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Background: The most common neoplastic lymphoproliferative diseases that secrete paraprotein are multiple myeloma (MM), Waldenstrom's Macroglobulinemia (WM) and chronic lymphocytic leukemia (CLL). The two first entities secrete paraprotein by definition, while serum free light chains (sFLC) are increased in 50% of CLL cases. Microenvironmental factors, such as soluble Syndecan-1 (ssynd-1) and BlyS normally promote lymphoplasmacytic differentiation as well as their secretory activity, whereas others, like TGFbeta1, inhibit them. Determination of Ig is necessary in MM and WM for diagnostic purposes and for monitoring patients, while in CLL, sFLC has prognostic value. The total amount of secreted Ig does not really reflect disease burden. The heavy chain Ig can be accurately determined with the 'Heavylite' method (that measures separately HLC-IgA, -G, -M kappa or lambda), thus allowing exact quantification of the amount of pure monoclonal fraction but also the degree of suppression of polyclonal Igs, both being reflected by the corresponding ratios (HLCR).

Table 1.

		Stage*	iHL	iFLC	HLCR	FLCR	Difference HL	Difference FLC	TTT	OS
sSynd1 >M	MM	0,02	0,003	0,08	NS	NS	0,06	0,11	0,001	0,002
	MW	NS	0,04	NS	NS	NS	0,04	NS	0,005	0,1
	CLL	0,09	NS	NS	NS	NS	NS	NS	0,1	NS
BLyS >M	MM	NS	NS	0,07	NS	NS	NS	NS	NS	NS
	WM	0,18	0,001	0,02	0,2	0,02	0,009	0,02	0,006	0,16
	CLL	NS	-	NS	NS	0,09	0,02	0,2	0,1	0,02
TGFb1>M	MM	0,02	-0,06	-0,12	-0,005	-0,035	-0,02	-0,02	0,0001	0,0001
	WM	NS	NS	NS	NS	NS	NS	0,1	0,05	0,02
	CLL	NS	-	NS	NS	NS	NS	NS	0,017	0,07
									iHK: involved Ig iFLC: involved FLC HLCR: Igx-kappa/Igx-lambda FLCR: FLCkappa/FLClambda	
OS	MM	<0,0001	0,004	0,002	0,001	0,003	0,004	<0,0001		
	WM	0,004	NS	0,006	NS	0,2	NS	0,02		
	CLL	<0,0001	-	0,03	NS	0,2	NS	0,2		

*ISS, WM-IPSS, Binet as appropriate, NS: not significant, M: median value, OS: overall survival, TTT: time to treatment

Aims: To determine any possible relationship between the amount of Igs secreted and serum sSynd1, BLyS and TGFbeta1, as well as with disease outcome.
Methods: We studied 269 patients: 105 with MM (79 IgG and 26 IgA, of whom 33%, 31%, and 36% were staged ISS 1, 2 and 3 respectively), 64 suffering from WM (44%, 28%, 28% staged WM-IPSS 1, 2, 3 respectively), and 100 with CLL (67%, 23%, 10% staged Binet 1, 2, 3 respectively). Patients were regularly followed-up from diagnosis to last visit or death (median follow-up time: 63 months). sFLC/sFLCR and HLC/HLCR were determined by nephelometry (Freelite™ and Heavylite™, the Binding Site Birmingham, UK) while sSynd1, BLyS and TGFbeta1 by ELISA, either in fresh or in frozen sera sample drawn at the time of diagnosis. Statistical analysis was performed by standard methods using the SPSS v22.0. software.
Results: The main correlations observed between the Ig levels secreted in the 3 diseases and cytokines studied, as well as their impact, with regard to patients' outcome, are shown in table.
Summary/Conclusions: sSynd1 in MM and BLyS in WM and CLL correlated with Ig production. By inhibiting both monoclonal and polyclonal Ig, TGFb1 correlated in MM with both HLC and FLC ratios and differences. In addition, the aforementioned variables are prognostic with regard to patients' outcome.

E1305

PATIENTS WITH MULTIPLE MYELOMA (MM) IN LONG TERM COMPLETE REMISSION (LTCR) AFTER AUTOLOGOUS TRANSPLANT (APBSCT) EXPRESS A DISTINCTIVE INMUNE PROFILE WITH POTENTIAL PROGNOSIS VALUE

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Background: A small fraction of patients with MM could be considered potentially cured as long as they remain for more than six years in long term complete remission (MM-LTCR) after autologous transplant (APBSCT). The exhaustive study of the immune status of these patients could highlight interesting information.
Aims: Here we present an observational study that evaluates the numbers and phenotype of T- and B-cells subsets in the peripheral blood (PB) of MM-LTCR patients.
Methods: After approval by the ethics committee, we selected 13 patients diagnosed with MM, in sCR according to IMWG criteria for at least six years after APBSCT, and 15 healthy adults (HA) of similar ages as a comparative group. Group MM: 7 males and 6 females; median age: 61. Median follow up in sCR after APBSCT was 8 years (range 6-19). Group HA: 5 females and 10 males, median age 60 (36-78). Immunophenotype characterization was done using a comprehensive 8-color flow cytometry panel. Subpopulations of CD4+ and CD8+ T-cells from PB were quantified, including naïve, central and effector memory, regulatory T-cells, as well as subpopulations of B-cells: naïve, transitional, marginal zone-like, class-switched memory and plasmablasts. In order to confirm their specific immune signature, the analysis was repeated in the same LTCR-MM patients one year after the first analysis was done. A Kruskal-Wallis test was used to evaluate differences among the studied groups. A posteriori test was done to compare the control group with the two patient's group (patients and patients +1 year), independently of each other. A Wilcoxon matched test was used to compare a patient under group "patients" with the status of the same patient in the second group "patients +1 year". Statistical analysis was done using GraphPad Prism software.
Results: The patients had a lower percentage of total CD4$^+$T-cells (p=0.0004) together with a decrease in the naïve CD4$^+$ T-cells (CD27$^+$CCR7$^+$CD45RA$^+$; p=0.0004) and an increment of the effector memory CD4$^+$ T-cells (CD27$^-$CCR7$^-$; p=0.0028), both CD27$^-$CCR7$^-$CD45RA$^-$and CD27$^-$CCR7$^-$CD45RA$^+$ cells. Similar results were found within the CD8$^+$T-cells. No differences were observed in the Tregs defined as CD4$^+$CD25^{high}CD127$^-$. The mean percentage of total B-cells in the patients was within the normal range and no significant differences were found when compared to HA. However, naïve B-cells (CD27$^-$IgD$^+$IgM$^+$) proportion was higher in patients and a corresponding reduction of marginal zone-like B-cells (CD27$^+$IgD$^+$IgM$^+$, p=0.0047) and class-switched memory B-cells (CD27$^+$IgD$^-$IgM$^-$, p=0.0043) was observed. No differences were observed in the percentage of transitional B-cells (CD27$^-$CD10$^+$CD38$^+$) or plasmablasts (CD27^{++} CD38^{++}) in the PB of the two groups. When the analysis was repeated in the same LTCR-MM patients one year after the first analysis, no changes were detected neither when analysed as a group nor when analysed individually.
Summary/Conclusions: The MM-LTCR patients seem to express a distinctive immune "footprint" characterized by a decreased proportion of naïve T-cells and an increased percentage of effector T cells, which probably exert a competent immune surveillance. Conversely, the increase in naïve B cells may guarantee the humoral response homeostasis, including the recovery of normal plasma cells that might compete with myelomatous cells for normal bone marrow niches. The precise role of these refined immunological studies in the monitoring and therapeutic decisions in MM patients, and also in the duration of sCR, should be defined in the future.

Study partially performed with research grants from the spanish Leukemia and Lymphoma Foundation and Grant PI12/00494P from the Fondo de Investigaciones Sanitarias and FEDER funds.

E1306

IMPACT OF THE AFFORDABILITY OF NOVEL AGENTS IN PATIENTS WITH MULTIPLE MYELOMA: REAL WORLD DATA ON CURRENT CLINICAL PRACTICE IN MEXICO

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Pedraza[1], P. Santana-Hernandez[1], G. Sotomayor-Duque[1], D. Arredondo-Campos[1], C. De la Cruz-De la Cruz[1], O.G. Cantu-Rodriguez[1], D. Gomez-Almaguer[1]
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Background: The success of bortezomib and lenalidomide in improving outcomes as first-line therapies in multiple myeloma (MM) patients has been achieved at a very high cost. Treatment has become difficult to access for patients living in low to middle-income countries, as most receive assistance by public healthcare systems wherein novel drugs are unaffordable.
Aims: To compare the outcomes of MM patients who can afford private insurance and treatment in a private center (PrivC), with those managed in a public center (PubC), who do not have access to healthcare coverage and are treated on an out-of-pocket basis.
Methods: We analyzed records of 148 patients diagnosed with MM in two health sectors in Monterrey, Mexico, from October 2007 to July 2016; 77 (52%) from PubC, where the most common induction therapy was cyclophosphamide-thalidomide-dexamethasone, followed by thalidomide maintenance, and 71 (48%) from PrivC wherein bortezomib or lenalidomide-based induction and lenalidomide maintenance were used. We compared demographics, disease stage, response rate and survival among both groups.
Results: Median age, gender and frequency of immunoglobulin isotype did not differ significantly between the two groups. Patients treated in PubC were more likely to be diagnosed with advanced stage disease (ISS III 42% *vs* 26% p<0.05). Median follow-up was 36 months (range 3-120 months). Autologous transplantation was performed in 80% of the transplantation-eligible patients in PrivC and only in 31% of PubC. At least a very good partial response to induction therapy was achieved more often in the PrivC among transplantation-eligible (65% *vs.* 42%, p<0.05) and ineligible patients (66% *vs*. 41%, p<0.05). Overall survival was significantly higher in PrivC for transplantation-eligible (median 84 *vs*. 59 months, p<0.05) and ineligible groups (median 79 *vs* 46 months; p<0.05). After controlling for disease stage and transplantation factors, the risk of mortality was still higher in PubC (HR 1.49; 95% CI:1.0-2.2, p<0.05).
Summary/Conclusions: Stage at diagnosis, induction therapy and autologous stem cell transplantation were contributors to survival disparities between patients treated in public *vs* private health care facilities in Mexico. These findings underscore the need for more efforts to improve the affordability of novel agents and transplantation settings in public health services.

Myeloproliferative neoplasms - Biology

E1307

BASAL CALCIUM, AN IMPORTANT ELEMENT IN THE DEVELOPMENT OF CALR MUTANT MPNS

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Background: Calreticulin (CALR) is a calcium (Ca^{2+}) buffering chaperone mutation of which has recently being associated with essential thrombocythemia and primary myelofibrosis without *JAK2* mutations. These mutations have been suggested to impair the Ca^{2+}buffering activity of Calreticulin due to a change of the negative charge in its C-terminal domain. $Ca^{2+}$is known to be important during megakaryocyte activity; however its role during megakaryopoiesis and the possible link of *CALR* mutations and abnormal megakaryocyte production due to impaired Ca^{2+} buffering activity in myeloproliferative neoplasms (MPNs) remains unclear.
Aims: Here we aim to understand how basal Ca^{2+}fluctuates during normal megakaryopoiesis and how *CALR* mutations could affect the basal Ca^{2+}levels in megakaryocytes in MPNs.
Methods: Ca^{2+} stainning was performed using Fluo-8 dye and Ca^{2+} basal levels were measured by flow cytometry. Changes in basal Ca^{2+}during megakaryopoiesis using two cellular systems, K-562 cells and mouse bone marrow cells, were measured each 24 hours. Further studies using *CALR* mutant cellular models were performed using the same methology.
Results: Our results showed a characteristic behaviour of fluctuations of basal Ca^{2+} during this megakaryopoiesis, where Ca^{2+}levels decreasein the last stage of megakaryocyte formation. These results suggest that Ca^{2+}reduction could be essential for megakaryopoiesis. In order to understand how *CALR* mutations affect basal Ca^{2+}, Marimo cells and Dami cells expressing *CALR* mutations were analysed. Here we show a decrease in basal Ca^{2+}in Marimo cells and DAMI-*CALR* type2 mutation compared to the controls. Moreover, DAMI-*CALR* type1 did not show any significant reduction, suggesting possible differences in Ca^{2+}behaviour depending in *CALR* type mutation. We are currently working in the analysis of basal Ca^{2+}fluctuations during megakaryopoiesis in the presence of *CALR* mutations and preliminary results show abnormal basal Ca^{2+}levels throughout all the process of megakaryocyte differentiation.
Summary/Conclusions: Altogether, our findings indicate that basal Ca^{2+} could be an important element during megakaryopoiesis and *CALR* mutations found in MPN could impair the normal production of megakaryocytes due to changes in cellular Ca^{2+}. However, further analysis need to be done in order to understand the role *CALR* mutations and their effect in the Ca^{2+}buffering activity of CALR in MPNs.

E1308

THE INHIBITION OF JAK/STAT SIGNALING IS COMPENSATED BY ACTIVATION OF MAPK PATHWAY IN MYELOPROLIFERATIVE NEOPLASMS

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Background: Myeloproliferative neoplasms (MPN) remain incurable regardless of advancement in the use of JAK1/2 inhibitor Ruxolitinib, which competence is unrelated to the JAK2V617F mutation.
Aims: We want to explore JAK1/2 inhibition dependency in correlation with activated JAK/STAT3 signaling and cell cycle in MPNs.
Methods: The immunoblotting has been used to analyze activation of JAK/STAT3, PI3K/AKT and MAPK signaling in JAK2V617F mutated HEL cells and granulocytes of MPN. The cell cycle and apoptosis of granulocytes are studied by flow cytometry.
Results: Concerning myeloproliferation, JAK1/2 inhibitors reduced the percentage of cells in G2M phase and increased apoptosis in JAK2V617F mutated HEL cells. Comparing to polycythemia vera (PV), the percentage of granulocytes is decreased in S and G2M phases of essential thrombocythemia (ET) and primary myelofibrosis (PMF) that demonstrated increased apoptosis. Hexabromocyclohexane increased percentage of granulocytes in GoG1 phase of JAK2V617F positive, but reduced in JAK2V617F negative PMF, the later one similar to Ruxolitinib. JAK1/2 inhibitors reduced percentage of apoptotic granulocytes in JAK2V617F positive, but increased in JAK2V617F negative PMF. JAK1/2 inhibitors could not prevent constitutive activation of JAK/STAT3 signaling in HEL cells as well as in granulocytes of JAK2V617F positive ET and PMF. Absence of JAK2V617 mutation supported dephosphorylation of JAK/STAT3 pathway by JAK1/2 inhibitors in ET, but not in PMF. JAK1/2 inhibitor

Ruxolitinib largely activates MAPK signaling in MPN, while slightly PI3K/AKT signaling in PV and JAK2V617F negative PMF. Specific JAK2 inhibitor Hexabromocyclohexane activates PI3K/AKT signaling in JAK2V617F positive ET, but reduced in JAK2V617F negative ET and PMF.
Summary/Conclusions: This observation support cross-talk between examined pathways, where inhibition of JAK/STAT3 signaling is compensated by activation of MAPK pathway irrespective of JAK2V617F mutation, while PI3K/AKT signaling demonstrates JAK2V617F dependence in MPN.

E1309

CIRCULATING PLATELET AND MEGAKARYOCYTE-DERIVED MICROPARTICLES OF JAK2V617F MUTATED PATIENTS WITH MYELOFIBROSIS ARE DISREGULATED: A NOVEL LIQUID BIOPSY TOOL OF RESPONSE TO RUXOLITINIB?

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Background: Microparticles (MPs) are small vesicles (0.1-1 micron) deriving from plasma membrane budding during homeostasis and cell activation. MPs express antigens and contain constituents from cell of origin and are increased in conditions that are characterized by high cell turnover or death, particularly inflammatory, autoimmune and neoplastic diseases. Myelofibrosis (MF) is a clonal neoplasia of the hematopoietic stem/progenitor cells characterized by distinctive abnormalities in megakaryocyte (MKC) development and platelet (PLT) activation. Mutations in 3 genes (*JAK2, CALR, MPL*) and chronic inflammation are the main pathogenetic drivers of MF. Ruxolitinib (RUX), a JAK1/2 inhibitor, suppresses both clonal myeloproliferation and release of proinflammatory cytokines, reducing splenomegaly and constitutional symptoms in around 50% of patients (pts). We hypothesized that MPs, as mediators of inflammation, could be overexpressed in MF and possibly predict responses to RUX.
Aims: This study aims to: 1) enumerate circulating MK and PLT-derived MPs of MF pts; 2) evaluate the effect of RUX on MPs production by PLT and MK; 3) investigate whether circulating MK and PLT- MPs may be a biomarker of response to RUX.
Methods: EDTA-anticoagulated peripheral blood from healthy donors (HD, n=10) and $JAK2^{V617F}$ positive MF pts (n=12) at intermediate-2/high IPSS risk was collected at baseline and 3 and 6 months after RUX therapy and immediately centrifuged. PLT (CD61+CD62P+) and MK (CD61+CD62P-)-derived MPs were analysed in PLT poor plasma samples by flow cytometry (CytoFLEX, Flow Cytometer-Beckman Coulter). The instrument was calibrated with MEGAMIX Beads (Beckman Coulter) with various diameters to cover the MPs (0.5 and 0.9µm).
Results: At 3 and 6 months, 5 out of 12 pts achieved a spleen response (R) according to 2013-IWG-MRT criteria. At baseline, the mean percentage of MK-derived MPs was significantly decreased (29±6 *vs* 72±5; p<0.0001) while that of PLT-derived MPs significantly increased (49±7 *vs* 11±1; p<0.0001) in MF pts compared to HD. However, the mean percentage of MK-derived MPs from pts not achieving a spleen response (NR) was significantly decreased compared to R (17±6 *vs* 45±5; p<0.005) and HD (17±6 *vs* 72±5; p<0.0001). By contrast, the mean percentage of PLT-derived MPs was significantly increased in NR compared to R (64±7 *vs* 37±9; p<0.05) and HD (64±7 *vs* 11±1; p<0.001). Of note, NR pts had significantly lower PLT number as compared with R pts (220±29 *vs* 422±98 p<0.05). No correlation was observed at baseline between the percentages of MK/PLT-derived MPs and platelet number, allele burden, splenomegaly and constitutional symptoms. At 3 and 6 months, RUX did not significantly modify the mean percentages of MK- and PLT-derived MPs compared to baseline values.
Summary/Conclusions: At variance with HD, the majority of circulating MPs in $JAK2^{V627F}$ mutated MF pts at intermediate-2/high IPSS risk derived from PLTs. RUX therapy did not modify the MK/PLT-derived MPs pattern, suggesting that JAK1/2 inhibition does not seem to affect the pathways of MK/PLT MPs production or clearance. Most importantly, MPs evaluation at baseline is significantly associated with subsequent spleen response. Specifically, NR pts had increased percentages of PLT-derived MPs with a concomitant reduction of PLT number. This could be related to a state of PLT hyper-activation with hyperproduction of MPs. Further studies are needed to confirm whether MPs may actually be considered a biomarker of disease activity and response to RUX.

E1310

A COMPARATIVE FUNCTIONAL AND PHENOTYPIC PLATELET ANALYSIS AMONG GENETIC GROUPS OF ESSENTIAL THROMBOCYTHEMIA PATIENTS

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Background: Essential thrombocythemia (ET) is a myeloproliferative neoplasm (MPN) characterized by a sustained elevation of platelet counts. Patients are at risk of hemorrhagic or thrombotic complications, and, eventually, of progression to acute myelogenous leukemia or marrow fibrosis. ET patients are classified on genetic subgroups based on known driver mutations (*i.e.* JAK2V617F, MPL W515K/L, CALR Type I/II). So-called triple-negative patients (TN) do not bear any of the aforementioned mutations but many other mutations that have not been hitherto shown to be causative of the disease. Cytoreductive or antiplatelet treatment is currently being used in the clinic. However, a comprehensive study of platelet properties is lacking in these patients, with updated genetic classification.
Aims: We aim to characterize platelets of the ET genetic groups and establish a phenotypic and functional profile that will help us to understand the pathophysiology of the disease and potentially add to a better patient diagnosis/prognosis.
Methods: More than 40 ET patients (from Belgian and Spanish cohorts) and healthy donors (HD) were recruited. Since treatment with acetylsalicylic acid is common, a number of HD that had taken the drug 3 consecutive days prior blood sampling were recruited. Platelets were subjected to a functional assay (Platelet aggregation) and flow cytometry analysis of surface marker expression. A novel flow cytometry based platelet aggregation assay (de Cuyper et al, Blood 2013) has been used to measure kinetics and quantitate the responses to different platelet receptors (CLEC2, GPIIB/IIIA vWF R, and collagen receptors GPVI and GPIA/IIA) upon specific agonist stimulation.
Results: Among the TN cases we identified four MPL S204F/P cases that were analyzed separately given that part of their hematological parameters (MPV, RBC counts) were not similar to the rest of the ET cases. Additionally, flow cytometry analysis also showed that MPL S204F/P platelets are larger and they have lower expression of surface markers (CD61, CD42b, CD49b, CD31) as compared to the other ET groups and HD. On the other hand, JAK2 V617F and CALR type I ET platelets exhibited normal to increased expression density of these receptors as compared to HD. Variable patterns were observed amongst the other ET genetic subgroups, with reduced responses especially upon challenge with Aggretin A or collagen, while platelets from the JAK2 ET genetic subgroup displayed hyper-reactivity to certain agonists (*i.e.* Aggretin A and Ristocetin). MPL S204F/P platelets displayed, in general,decreased aggregation responses.
Summary/Conclusions: These preliminary results suggest that MPL S204F/P platelets are intrinsically defective (hypo-reactive), in contrast to JAK2 V617F platelets (hyper-reactive), while in other genetic subgroups, potential defects are most probably synergistic and/or acquired by treatment. Data suggests that JAK2 V617F and CALR type I platelets could also undergo basal degranulation or vesiculation in the circulation. Analysis of the platelets has identified characteristics in different genetic groups of ET that should be further investigated taking into account the different treatment conditions and using larger cohorts of patients. When specific functional and phenotypic platelet patterns are established they could contribute significantly to a better diagnosis/prognosis of the disease.

E1311

ASSOCIATION ANALYSIS OF CYTOGENETIC AND GENETIC ALTERATIONS IN PRIMARY MYELOFIBROSIS

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Background: A number of genomic abnormalities have been associated to primary myelofibrosis (PMF). Next-generation sequencing (NGS) and single-nucleotide polymmorphism array (SNP-A) methods are widely used for PMF genomic studies and certain cytogenetic and genomic associations have been determined. To better characterise the genomic landscape of PMF we performed comprehensive analysis of gene mutations and chromosomal aberrations in a population-based cohort of PMF patients.
Aims: To characterize genomic alterations in PMF using SNP-A and NGS methods.
Methods: PMF peripheral blood samples were screened by Infinium HD whole-genome genotyping assay with the HumanCytoSNP-12 BeadChips (Illumina Inc., CA). NGS analysis was performed using TruSight Myeloid 54 gene sequencing panel (Illumina). SNP-A and NGS data analyses were performed using Illumina BaseSpace Informatics suite (Illumina). *JAK2*, *CALR*, *MPL* mutations were additionally confirmed with Sanger sequencing while small indels – with DNA fragment analysis.

Results: 110 patients diagnosed with PMF according to WHO criteria between years 2013 and 2014 were included into this study. SNP-A analysis identified 77 chromosomal abnormalities in 61 patients (55,4%). These comprised the loss of heterozigosity (LOH) (59.7%), hemizygous deletions (23.4%) and copy number gains (16.9%). The most common aberrations in affected patients were: 9p LOH (55.7%), 20q deletion (11.5%), 1q duplication (4.9%), 19p deletion (4.9%), 13q deletion (3.2%), 1p LOH (3.2%) and 6q LOH (3.2%). NGS analysis detected 219 gene mutations (in a total of 27 genes) in 108 patients (98%). The most frequently mutated genes were: *JAK2* (62.9%), *CALR* (27.8%), *ASXL1* (20.3%), *TET2* (16.6%), *MPL* (7.4%), <5% *ZRSR2*, *EZH2, DNMT3A, U2AF1, ETV6, SF3B1, IDH1, IDH2*. Recurrent specific mutations were detected in 10 genes. Sixty-two patients (57.4%) had more than one somatic mutation. Six patients (5.5%) had no *JAK2*, *CALR* or *MPL* mutations and were defined as "triple-negative". Previously not described *ZRSR2* gene 12 bp insertion was indentified in four patients (3.7%). The correlation analysis showed significant associations between 9p LOH and JAK^{V617F}mutation (p<0.001); 20q deletion and *ASXL1* mutations (p=0.011); 19p deletion and *CALR* mutations (p=0.004). Notably, the affected genes are located in coresponding affected chromosome regions, indicating disruption of both alleles by different biological mechanisms. *KRAS* and *ETV6* mutations were statisticaly associated with *ASXL1* mutations (p<0.001 and p=0.005, respectively) while *JAK2* and *CALR* mutations were mutualy exclusive in all cases (p<0.001).
Summary/Conclusions: A number of associations between gene mutations and chromosomal aberations was revealed in PMF. Co-presence of 9p LOH with $JAK2^{V617F}$ and *CALR* mutations with 19p deletion indicate that further deregulation of these key signaling pathways may take place disrupting the second allele of the affected genes by different biological mechanism – LOH or deletion.

E1312

FREQUENCY OF CONCURRENT BCR-ABL1, JAK2, CALR AND MPL MUTATIONS IN A COHORT OF 5,545 CASES WITH SUSPECTED MPN BY A DEEP SEQUENCING APPROACH

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Background: Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm (MPN) characterized by *BCR-ABL1*, whereas in about 90% of *BCR-ABL1*-negative MPN a mutation in *CALR, JAK2* or *MPL* can be detected. These genetic alterations are thought to be nearly mutually exclusive, however, an accurate frequency is still missing.
Aims: To determine the incidence of genetic markers occurring in parallel in a large cohort of patients with suspected MPN and characterize double mutated cases.
Methods: From July 2016 till January 2017 5545 samples were sent to our laboratory with suspected MPN. The male:female ratio was 1:1, and the median age was 60 years (range: 18-98 years). Median white blood cell count was $9x10^9$/L, hemoglobin level (Hb) was 15g/dl, and platelet count was $328x10^9$/L. All of these cases were analyzed by an amplicon deep sequencing approach for mutations in *JAK2* (exon12, exon14), *CALR* (exon9) and *MPL* (exon10) with a sensitivity of 1%. 3070 patients were additionally screened for *BCR-ABL1* fusion by a multiplex PCR approach. Samples that were double mutated for *JAK2*, *CALR* and *MPL* were analyzed by amplicon deep sequencing for additional mutations in 13 myeloid genes.
Results: In total 1775/5545 (32%) of suspected MPN patients showed *JAK2, CALR* and/or *MPL* mutations. 1438 (26%) were *JAK2*, 267 (5%) *CALR,* and 89 (1%) *MPL* mutated. Of note, the analysis of a subgroup (n=3070) for *BCR-ABL1* fusion identified 123 (4%) as CML cases. The *JAK2* mutated cases presented mainly with Val617Phe (99%) and rarely with *JAK2* exon12 mutations (1%). *CALR* mutations were primarily type 1 (54%) and type 2 (30%). *MPL* mutations were located at amino acid Trp515 in 96% of cases. Double mutated cases were present in 19/1775 (1%) cases: *JAK2/MPL* (63%), *JAK2/CALR* (32%), *CALR/MPL* (5%). In nearly all *CALR* mutated cases (6/7) the *CALR* mutation was detected with the higher load, whereas in *JAK2/MPL* double mutated cases the ratio was equal. Most of the patients (18/19) had one mutation with a load below 10% and could have been missed by other approaches. *BCR-ABL1* together with *JAK2* or *CALR* mutation was found in one patient, each (2/123, 2%). Interestingly, out of the 267 *CALR* mutated cases, 3 patients had already received treatment for CML but were suspected to have independent *BCR-ABL1*-negative MPN. For two of these patients, samples 1 and 6 years prior to diagnosis of CML were available. Both showed *CALR* mutations already at this former time-point at high loads. In 10/19 (53%) double mutated patients 13 additional mutations were detected in 8 different genes. *SRSF2* and *TET2* were the most frequently mutated ones (n=3, each). No significant difference in mutation frequency was detected to the overall frequency in MPN patients with single mutations. The *JAK2, CALR* and/or *MPL* mutated *vs* wild-type cases showed higher age (mean: 67 *vs* 56 years, p<0.001) and higher platelet counts (646 *vs* $317x10^9$/L, p<0.001). Overall, the mean age was significantly different according to the presence of mutations as follows: triple-negative (56 years), *CALR* (63 years), *JAK2* (67 years), *MPL* (71 years) and double mutated (74 years).
Summary/Conclusions: One-third of the cases can be diagnosed having MPN according to the detection of *BCR-ABL1*, *JAK2, CALR* and/or *MPL* mutation in an unselected cohort with suspected MPN. The frequency of double mutated *JAK2, CALR* and *MPL* cases is 1%. In CML cases *BCR-ABL1* fusion and *JAK2* or *CALR* mutation were detected in 2% of the patients. The impact of these parallel genetic events on the clinical course of the disease has to be evaluated in the future.

E1313

A COMPREHENSIVE ASSESSMENT OF MOLECULAR AND CYTOGENETIC MARKERS OF PROGNOSIS IN PATIENTS WITH PRIMARY MYELOFIBROSIS

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Background: According to resent reports the data of molecular and cytogenetic analysis (type or absence of driver mutation (DM), mutations in *ASXL1, EZH2, IDH1/2* genes, karyotype) is a promising tool for prediction of survival in primary myelofibrosis (PMF). Multiple combinations of genomic aberrations lead to clinical course and survival heterogeneity. The aforementioned factors need to be considered together to evaluate their mutual influence.
Aims: The aim of the study was to evaluate a prognostic impact of DM, mutational status of epigenetic regulation (ER) genes, karyotype and their combinations for overall survival (OS) in PMF patients.
Methods: We have examined 110 patients (pts) with PMF (34.5% males). Median (Me) age was 59 years (16-82). For all pts the detection of *JAK2*V617F was done. *JAK2(-)* samples were tested for *MPL* 515 codon mutations and exon 9 mutations of *CALR* (direct sequencing). All pts except 4 underwent the analysis of mutations in *ASXL1, EZH2, IDH1/2* genes with high resolution melting method followed by sequencing of probably mutated samples. Karyotype research was done for 48 (43.6%) pts.

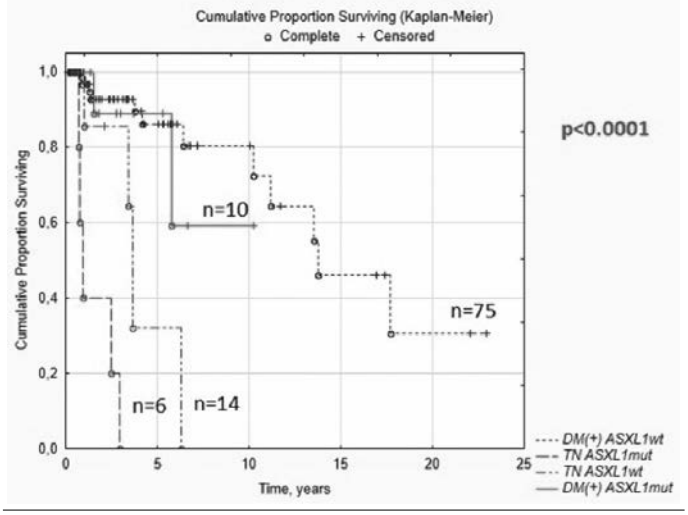

Figure 1.

Results: DM were detected in 81,8% pts: *JAK2(+)* - 50%, *CALR(+)* - 25.5%, *MPL(+)* - 6.4% cases. No DM were found in 18.2% pts considered triple-negative (*TN*). Mutations in ER genes were detected in 20.8% pts. High risk (HR) chromosomal aberrations (CA) (unfavorable CA according to DIPSS along with del(6)(q15), add(6)(p25), del(X)(q22), t(X;7)(p21;q11)) were found in 27.1% pts. Univariate analysis identified HR karyotype (hazard ratio (HR) 8.2, p<0.001), the absence of DM (HR 8.1, p<0.001) and nonsense and frameshift (hereinafter *mut*) (HR 2.9, p=0.018) but not missense mutations of *ASXL1* (p=0.378) as being prognostically detrimental for survival. *CALR* mutations had a favorable impact on survival with borderline significance (HR 0.3, p=0.052). A multivariate analysis included *TN, CALR, ASXL1* status and HR karyotype as covariates revealed an inter-independent prognostic value of HR karyotype (HR=7.4, p=0.001) and *ASXL1mut* (HR=2.8, p=0.023). In Cox regression model considering the same covariates except karyotype *TN* status (HR=2.4, p=0.050) and *ASXL1mut* (HR=3.3, p=0.012) but not *CALR* mutations (HR=0.3, p=0.075) were significant for OS. *CALR* mutations became significant (HR=0.3, p=0.075) when only *ASXL1mut* were included as covariate (HR=3.9, p=0.004). When comparing groups divided by *CALR/ASXL1* status the shortest OS was noted in *CALR(-)ASXL1mut* pts (Me 2.5 years, p=0.021). *CALR(+)ASXL1 wide type (wt)* pts seem to have better OS than *CALR(-)ASXL1wt* (median not reached (with follow up period of 10.1 years) and 13.5 years, respectively, p=0.124). Median OS estimated in pts due to presence/absence of DM and *ASXL1* status

was 0.9 years in *TNASXL1mut,* 3.6 years in *TNASXL1wt*, 13.8 years in *DM(+)ASXL1wt* and was not reached in *DM(+)ASXL1mut* (with follow up period of 10.3 years) group (p<0,0001). Differences in OS depending on the *ASXL1* status were statistically significant in the *TN* (p=0.007) but not for *DM(+)* group (p=0.788). The better OS was observed in *ASXL1wt* pts with low risk (LR) karyotype (Me 6.4 years, p=0.0005). There were no differences in OS of *ASXL1wt*-HR, *ASXL1mut*-LR and *ASXL1mut*-HR pts (1.4 *vs* 1.6 *vs* 1.2 years, p=0.493).
Summary/Conclusions: The differences in OS were more statistically relevant in groups divided by *TN/ASXL1* and karyotype/*ASXL1* status. The presence of *ASXL1mut* significantly worsens OS in the *TN* group. OS in pts with any of the findings: HR karyotype or *ASXL1mut* – was significantly shorter than in cytogenetically favorable *ASXL1wt* counterparts.

E1314

JAK2 HAPLOTYPE 46/1 (GGCC) HAS NO EFFECT ON THE PRIMARY RISK OF JAK2 V617F MUTATION, BUT IT STRONGLY POTENTIATES THE PROGRESSION OF GROWN ALLELE BURDEN IN MYELOPROLIFERATIVE NEOPLASMS

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Background: Several research groups have determined that the JAK2 46/1 (GGCC) haplotype in multiple ethnic groups is strongly associated with a predisposition to acquiring JAK2 V617F-positive MPNs. The role of the JAK2 46/1 haplotype in the natural evolution of the mutant JAK2V617F allele burden in PV but not ET or PMF has been shown [Alvarez-Larrán A e.a. Leukemia Research 2012, 36(3):324-326]. However, the data on the impact of the haplotype on the JAK2 V617F allele burden do not always agree. Using a highly sensitive test allowed to reveal a high prevalence JAK2 V617F among persons without symptoms of hematological disorders [Krichevsky S e.a. Blood Cells, Molecules and Diseases,, doi: 10.1016/ j.bcmd.2017.01.001]. Influence of haplotype 46/1 for such cases is not known. There are two competing hypotheses of "hypermutability" and "fertile ground" explaining the causes of the higher frequency of mutations of JAK2V617F in haplotype 46/1 carriers. The "hypermutability" hypothesis refers to an increased risk of a primary mutation in carriers of haplotype 46/1. In this case, the increasing frequency of the haplotype in patients with low allelic burden (<5%) must also be observed, including those individuals without evidence of hematological disorders.
Aims: Studying the relations of haplotype 46/1 and JAK2 V617F allelic burden
Methods: The diagnosis of chronic myeloproliferative neoplasms was based on the WHO (2008) criteria. The cohort included patients with JAK2 V617F mutation: 100 patients with PV, 51 with ET, 14 with MF, 41 patients with unclassifiable MPN and 47 patients with asymptomatic V617F+ carriers. Among all patients, 17 patients were treated with hydroxyurea and 20 were treated with interferon. The control group included 100 healthy donors without JAK2 V617F mutation. SNP genotyping of two 46/1 tag SNPs, rs12340895 and rs10974944 (the G-allele, and G-allele tags the JAK2 46/1 haplotypes) was performed by a polymerase chain reaction (PCR). The JAK2V617F allele burden was evaluated by a quantitative real-time allele-specific PCR test using "in-house" kit (0.01% sensitivity). Associations between JAK2 V617F mutation and haplotypes of JAK2 were assessed using the Chi-squared test. The odds ratio (OR) and 95% confidence interval (CI) were also calculated for each comparison of JAK2 46/1 haplotype frequency and ranges of allele burden.

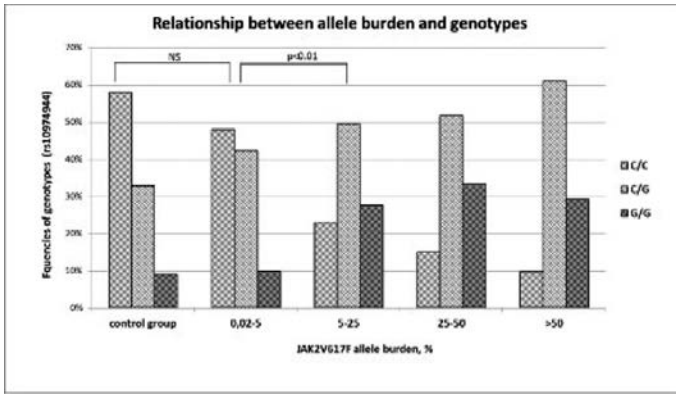

Figure 1.

Results: The JAK2 46/1 haplotype (GG and CG) was present in 170 patients (80.6%) with MPN, in 25 (52%) patients with suspected MPN, in 23 (49%) asymptomatic JAK2 V617F+ patients and in 42 (42%) cases of control group. G variant of rs10974944 was more frequent in all JAK2 V617F-positive MPNs, than in the control population (χ^2=46.5, p<0.0001). These results were similar to findings of previous studies, which have shown that the 46/1 haplotype predisposes to the acquisition of JAK2 V617F mutation. JAK2V617F allele burden was significantly higher in patients with PV than in patients with ET (p=0.001), but no differences were observed with from patients with the PMF. 46/1 haplotype was closely associated with MPN patients if the allele burden exceeds 5% (Fig. 1) regardless of the phenotype or the treatment. In this case with an increase in JAK2V617F allele burden the JAK2 46/1 haplotype frequency significantly increased. However, there was no significant difference in the JAK2 46/1 haplotype frequencies between patients with allele burden less than 5% and the control group.
Summary/Conclusions: No significant differences of the carrier haplotype 46/1 prevalence between control group and patients with minimal allelic burden (less than 5%) JAK2 2V617F have been observed. This is evidence against primary "hypermutability" hypothesis. A further increase in allelic load is more pronounced in carriers of haplotype 46/1 that supports the "fertile ground" hypothesis. We hypothesize that DNA mutation JAK2V617F repair is downgraded in 46/1 haplotype carriers.

E1315

MINIMAL RESIDUAL DISEASE MONITORING BY DIGITAL PCR FOR JAK2V617F DETECTION IN PATIENTS WITH MYELOFIBROSIS (MF) OR ACUTE MYELOID LEUKEMIA SECONDARY TO MF AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: Myelofibrosis (MF) is one of the *BCR-ABL1*-negative Chronic Myeloproliferative Neoplasms (MPNs), characterized by clonal expansion of abnormal hematopoietic progenitors and gradual replacement of normal bone marrow with fibrous tissue. MF patients' prognosis is widely variable and the median survival can vary from months to many years. At present, Allogeneic Stem Cell Transplantation (ASCT) is the only curative treatment option for these patients. The most frequent phenotype-driving mutation in MF is the V617F mutation in the *JAK2* gene. A high sensitive quantification of *JAK2V617F* mutation load can be useful to assess Minimal Residual Disease (MRD) in treatment directed to eradicate the malignant clone, such as ASCT. Droplet Digital PCR (ddPCR) is a quantitative approach for the detection of rare allele characterized by a high level of sensitivity and specificity.
Aims: To evaluate the efficacy of ddPCR *JAK2V617F* mutation detection assay in monitoring the MRD level at consecutive time-points in a small cohort patients who underwent an ASCT for MF or MF-derived Acute Myeloid Leukemia (s-AML).
Methods: DNA from 9 patients affected by primary, secondary MF or s-AML were serially collected during the follow-up after ASCT (50-2500 days). These samples were investigated for hematologic chimerism by PowerPlex System (Promega, USA) and were also analyzed for *JAK2V617F* mutation both by conventional allele specific PCR (ASO-PCR) and by a validated ddPCR mutation detection assay (Bio-rad, USA). Results were expressed as percentage of *JAK2V617F* mutated alleles on total evaluated alleles.
Results: The JAK2V617F ddPCR mutation assay was able to detect low mutation load (up to 0.006%), confirming to be much more sensitive than ASO-PCR (0.5-2%). In 4 patients, early after transplantation, we observed by ddPCR a low level of MRD that progressively increased during the follow-up and anticipated a decrease in donor chimerism level and a worsening of clinical situation. In 2 patients, who always showed a full donor chimerism and complete hematologic remission of the disease, very low levels of MRD (ranging from 1% to 0.006%) could be detected by ddPCR in the 2 years after ASCT. With a longer follow-up, a full molecular remission was achieved as demonstrated by ddPCR. In 2 other patients, we observed a very early achievement of full donor chimerism and JAK2V617F molecular negativity (within 90 days post HSCT), also when evaluated by ddPCR. These patients entered a complete hematologic remission of the disease which still persists (after 1 and 5 years after transplantation, respectively). Interestingly, in one patient whose post-transplant hematopoiesis proved full donor and negative for *JAK2V617F* mutation for 2 years, a weak positive signal revealed by ddPCR (0.075%) became apparent and anticipated an extra-hematologic relapse (skin and bone). A subsequent second allogeneic transplant from the same sibling donor restored clinical and molecular remission.
Summary/Conclusions: The ddPCR proved to be a sensitive and accurate method in detecting JAK2V617F mutation. Therefore, this assay can be a valid tool for the MRD monitoring in JAK2V617F MPN patients undergone an ASCT. However, the use of this highly sensitive PCR should be considered with caution in the clinical management of transplanted patients to avoid inappropriate use of donor leukocyte infusion (DLI) and tampering of immunosuppression. A large

number of patients have to be studied with ddPCR to better understand the clinical significance of low mutation load.

E1316

S100A8/9 ACTIVATION OF MAPK PATHWAY IS SUPPORTED BY ITS RECEPTORS RAGE AND TLR4 IN POLYCYTHEMIA VERA

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Background: S100 proteins have been shown to regulate cell proliferation, excessively augmented in myeloproliferative neoplasms (MPN). S100A8/9 is produced by cells of myeloid origin as mediator of inflammation, while AKT and MAPK pathways mediate cell proliferation.
Aims: This study analyzed activation of AKT and MAPK pathways by S100A8/9 proteins in healthy controls and MPNs: polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), according to JAK2V617F and calreticulin (CALR) mutation status.
Methods: S100A8/9 factor is examined in granulocytes of MPN using immunoblotting, while its influence on cell cycle of granulocytes is determined by flow cytometry. Mutations of JAK2V617F and CALR exon 9 are analyzed by DNA sequencing. Besides JAK2V617F+ PV patients, we formed per three groups of patients: JAK2V617F+, JAK2V617F-/CALR+, and JAK2V617F-/CALR- for ET and PMF.
Results: S100A8/A9 proteins demonstrated a common significant increase in plasma of MPN patients, whereas the presence of CALR mutation augmented S100A8/A9 levels in granulocytes of ET and PMF patients. Activation of AKT pathway is generally reduced by S100A8/9 factor, further on ameliorated by inhibition of the receptor for advanced glycation end products (RAGE) in granulocytes of JAK2V617F+ and JAK2V617F-/CALR+ groups of ET and PMF patients, while it has been prevented by Toll-like receptor 4 (TLR4) inhibition in PV patients. MAPK pathway is significantly inhibited by S100A8/9 only in JAK2V617F+ ET patients and JAK2V617F-/CALR- PMF patients, partially prevented by TLR4 inhibition in PMF. Inhibition of TLR4 reduced S100A8/9 mediated MAPK activation in JAK2V617F-/CALR+ ET. In contrast, S100A8/9 mediated MAPK activation has been significantly augmented by TLR4 and RAGE inhibition in PV patients. S100A8/9 stimulated granulocyte cycle arrest in G2M phase has been stopped by JAK1/2 inhibition.
Summary/Conclusions: S100A8/A9 protein levels demonstrated stable elevation in MPNs. Common inhibition of AKT pathway has been controlled by TLR4, whereas MAPK pathway activation by TLR4 and RAGE in PV, during treatment with S100A8/A9.

E1317

MUTATIONAL PROFILE STUDY OF DOUBLE-NEGATIVE ESSENTIAL THROMBOCYTHEMIA BY HIGH-DEPTH NEXT GENERATION SEQUENCING (NGS)

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Background: Essential thrombocythemia is one of the three classical philadelphia negative myeloproliferative neoplasms. It is frequently difficult to diagnose and some molecular markers are used as diagnostic criteria according to WHO classification. Despite this, a significant proportion of patients do not present a clonality marker.
Aims: To identify the mutational profile of ET negative for V617F and CALR mutations and to correlate it with clinical data.
Methods: A cohort of 22 ET negative for mutations in JAK2 (qPCR) and CALR (GENESCAN) was selected. Median age at diagnosis was 46 years (range: 14-88), male:female ratio 9:13; 2 patients had a record of thrombotic event prior to diagnosis, 4 patients had symptoms at the time of among diagnosis, 3 patients suffered a thrombotic event after diagnosis, 1 patient suffered transformation to AML. Median Hb, WBC and platelets at diagnosis were respectively 14.75g/dl 8,85 x10^9/L and 720x10^9/L. We performed targeted gene sequencing by NGS (Ion Torrent Proton System–Life Technologies) using a panel of 33 genes implicated in leukemia prognosis. X2 and t-student tests were used to find association between mutations and clinical data.
Results: On average, 97.94% of the target sequence showed a mean depth coverage around 2500. We discovered 17 non-synonymous mutations which 16 were somatic single nucleotide variants (SNVs) and 1 a nucleotide deletion in coding regions. No mutations were detected in 9 samples (40.9%), 10 samples presented 1 mutation (45.5%), and the other 3 samples present 2 or more mutations (13.6%). TET2 was the most frequently mutated gene (18.2% of patients, mean allele frequency of 24,45%), followed by JAK2 (13.6% V617F at a low mean allele frequency (5.8%), MPL (9.1%, one W515L, one with two mutations W515R and S505C, mean allele frequency of 21,95%), SF3B1 (4.5%), DNMT3A (4.5%), KIT (4.5%), VHL (4.5%), CBL (4.5%) and KMT2A (4.5%). The samples with more than one mutation: one presented a CBL and two TET2 mutations, one two mutations in MPL and the other one mutation in TET2 and other in JAK2. No correlation was found between mutational profile and clinical data.
Summary/Conclusions: In ET, around 60% of patients present the JAK2V617F mutation, 15-30% show CALR mutations and around 5% present MPL mutations. In spite of this, there is still a significant percentage of ET patients without a molecular marker. Our study shows that the use of a NGS panel allows identifying markers of clonality as for example TET2. NGS also makes affordable to interrogate whole genes classically associated to ET, to detect mutations that were not found by traditional approaches. Finally, we can conclude, as previously described, that ET is an entity with a low mutational burden in comparison with other MPNs as primary myelofibrosis.

E1318

TCR GAMMA CLONALITY ASSESSED BY NGS DOES NOT HELP TO DISTINGUISH EGPA FROM HES

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Background: Hypereosinophilia-associated syndromes are a heterogeneous group of diseases characterized by sustained and elevated blood eosinophilia with evidence of eosinophil-induced organ damage. Classically, Eosinophilic Granulomatosis with Polyangiitis (EGPA) and Hypereosinophilic Syndrome (HES) present several overlapping clinical and laboratory features, making it challenging to correctly insert patients in restricted and well-defined categories with specific and more effective therapeutic approaches in daily practice. Therefore, great efforts are ongoing searching for novel biomarkers able to differentiate these two disorders.
Aims: To detect T cell receptor gamma (TCRG) clonal rearrangements in EGPA and HES, comparing the frequency of distribution of the V and J region segments in 21 patients afferent to the hematology, rheumatology or pulmonology divisions.
Methods: Consecutive patients with a diagnosis of EGPA and HES were enrolled into the study. Inclusion criteria were: documentation of a persistent peripheral eosinophilic count of ≥1.5 x10^9/L and signs or symptoms of organ involvement. Clinical and laboratory data of the patients were collected. Sequence-based determination of the frequency distribution of TCRG Gene Rearrangements was performed using next-generation sequencing with the Illumina MiSeq (LymphoTrack TRG assay, Invivoscribe).
Results: We included 21 patients (9 with EGPA and 12 with HES). Four EGPA patients were MPO-ANCA positive. We detected TCRG clonal rearrangements in 44% patients with EGPA and in 42% patients with HES. No association was observed between TCRG clonal rearrangements and ANCA status in EGPA patients. The following recurrent TCRG gene rearrangements were observed: Vg10JgP1 (5 cases) and Vg4Jg1/2 (4 cases) were observed in both EGPA and HES, whereas Vg9Jg1/2 (2 cases) and Vg10Jg1/2 (2 cases) were observed only in patients with HES. The presence of TCRG rearrangement was not different according to the symptoms (asthma, vasculitis, skin, heart, gut, lung involvement, splenomegaly). IL2, IL5, IL4, eosinophil cationic protein (ECP), absolute eosinophils were measured: IL5 and ECP were higher in the polyclonal than in the clonal cases (9±2.5 *vs* 1.7±0.9; p=0.021 and 121.8±61.5 *vs* 39.5±1.5; p=0.07). On the contrary, no difference was observed in the absolute eosinophil count. Finally, the presence/absence of TCRG clonality did not significantly impact on the response to treatment (immunosuppressive or interferon) and on the progression-free survival length.
Summary/Conclusions: Conclusions: Even if preliminary, this study reveals a similar T cell receptor gamma repertoire in EGPA and HES, with recurrent rearrangements, thus suggesting a possible antigen-driven inflammatory response underlying hypereosinophilia in both EGPA and HES. Interestingly, this study confirms our previous results showing the TCR delta rearrangement (assessed by qualitative PCR) in 40% of the EGPA patients.

E1319

PROINFLAMMATORY CYTOKINE IL-6 STIMULATION OF ANGIOGENIC FACTORS AND DNA REPLICATION IS BLOCKED BY JAK-STAT PATHWAY INHIBITION IN MYELOPROLIFERATIVE NEOPLASMS

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Background: We already demonstrated augmented proinflammatory IL-6 and angiogenic vascular endothelial growth factor (VEGF), hypoxia inducible factor-1α (HIF-1α) and endothelial nitric oxide synthase (eNOS) levels in myeloproliferative neoplasms (MPN).
Aims: To observe IL-6 activated signaling pathways during stimulation of angiogenic factors and their JAK-STAT dependence in MPN.
Methods: We analyzed phosphorylation of JAK/STAT3, PI3K/AKT and MAPK signaling by immunoblotting in HEL 92.1.7 cells (with JAK2V617F mutation) and granulocytes of MPN. The granulocyte cycle phases have been studied by flow cytometry.
Results: We demonstrated IL-6 stimulated angiogenic factors in HEL cells and HEL-derived macrophages, blocked by JAK-STAT inhibition for eNOS and HIF-1α. IL-6 stimulated JAK-STAT3 and angiogenesis related PI3-AKT signaling pathways in HEL cells, the later one prevented by JAK1/2 inhibition. Opposite to primary myelofibrosis (PMF), IL-6 activation of JAK-STAT3 and PI3-AKT pathways has been prevented and enhanced by JAK1/2 inhibition, respectively in granulocytes of polycythemia vera (PV). Moreover, IL-6 inhibition of JAK-STAT3 and PI3-AKT pathways in essential thrombocythemia (ET) has been prevented by JAK2 inhibitor in JAK2V617F positive ET granulocytes. JAK1/2 inhibitor Ruxolitinib upregulated IL-6 activation of MAPK pathway in MPN, in contrast to specific JAK2 inhibitor Hexabromocyclohexane. IL-6 mediated reduction in the percentage of HEL cells in G2M phase was inversed by Ruxolitinib that potentiated apoptosis and reduced the cell percentage in G0G1 phase both in HEL cells and granulocytes of PMF. It has been detected the cell cycle arrest of MPN granulocytes in S phase (DNA replication) after treatment with IL6, completely diminished by JAK1/2 inhibition.
Summary/Conclusions: Therefore, we concomitantly revealed that inflammation stimulated angiogenic factors and signaling pathways involved in cell proliferation, apoptosis and angiogenesis are regulated by JAK-STAT inhibition.

Myeloproliferative neoplasms - Clinical

E1320

PERCEPTION OF SYMPTOM BURDEN AND TREATMENT GOALS BETWEEN PHYSICIANS AND PATIENTS WITH MPNS: AN ANALYSIS FROM THE INTERNATIONAL MPN LANDMARK SURVEY

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Background: The global MPN LANDMARK survey evaluated the patient (pt) and physician-reported impact of myeloproliferative neoplasms (MPNs), including myelofibrosis (MF), polycythemia vera (PV), and essential thrombocythemia (ET), among pts from 6 countries. We present an analysis comparing physician and pt perceptions of the impact of these MPNs.
Aims: To investigate differences between pt and physician perceptions of symptom burden, treatment goals, and disease management
Methods: This was a cross-sectional survey of pts with MPNs and physicians treating pts with MPNs. Respondents completed an online survey measuring their perception of the impact of MPNs on symptom burden, disease management, and treatment goals. Pts and physicians were recruited independently.

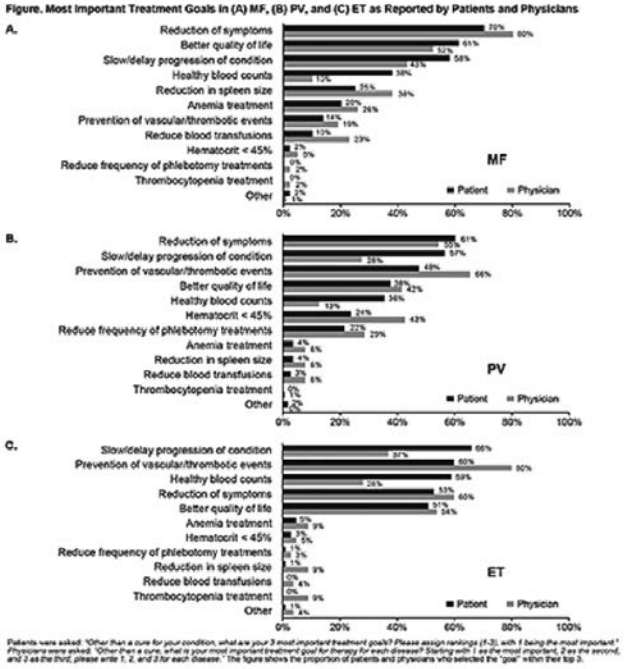

Figure 1.

Results: Pts (n=699) from Australia (n=10), Canada (n=64), Germany (n=149), Italy (n=106), Japan (n=84), and the UK (n=286) completed the survey (MF, n=223; PV, n=174; ET, n=302). Most pts had been diagnosed within ≤2 years of experiencing symptoms (73%); 56% were women. Physicians (n=219) were from the same countries; most were hematologists (54%) or hemato-oncologists (27%). Overall, 54% of pts reported having a prognostic score; however, 71% of physicians reported using a prognostic risk classification. Physicians assessed symptoms by proactively asking pts how they were feeling (43%) or asking about specific symptoms (37%); 11% waited for pts to mention symptoms. Importantly, only 26% of physicians used a validated symptom assessment form; 44% used their own rating method. Pts and physicians both agreed that pts with MPNs have a high symptom burden and that MF had a higher degree of burden on daily living. Interestingly, a higher proportion of physicians

than pts felt that MPN symptoms have an impact on pt quality of life (92% *vs* 76%) and that pts had a substantial emotional burden associated with their disease. For instance, 34%, 29%, and 26% of pts with MF, PV, or ET reported feeling anxious or worried compared with 70%, 46%, and 36% of physicians reporting that their pts experience substantial anxiety or worry. Some pts did not recognize that their symptoms could be MPN related; for example, ≈ one-fifth of pts did not think that their night sweats could result from their MPN (16% MF, 21% PV, 25% ET). Consistent with this, 60% of physicians indicated that pts could identify only few or some of their symptoms as MPN related. Pts and physicians were both concerned about reducing symptoms (pts: 70% MF; 61% PV; 53% ET; physicians: 80% MF, 55% PV, 60% ET); however, pts were also concerned about delaying MPN progression (58% MF, 57% PV, 66% ET; physicians: 43% MF, 28% PV, 37% ET; **Figure 1**). Compared with pts, physicians indicated a greater focus on prevention of vascular/thrombotic events in PV (66% *vs* 48%) and ET (80% *vs* 60%). Overall, only 27% of physicians felt they completely agreed with their pts on treatment goals; 66% felt they "somewhat" agreed. However, most pts (87%) were satisfied with their physician's disease management/communication.

Summary/Conclusions: This study revealed a potential disconnect between physician and pt perceptions relating to communication and disease management, and an apparent lack of standardization in symptom assessment. Of note, some pts did not recognize that their symptoms could be MPN related and had different treatment goals than their physicians, indicating a need for improved pt education and pt-physician communication and a treatment plan that includes standardized monitoring of symptoms and agreement on treatment goals.

E1321

BASELINE QUALITY OF LIFE INDEPENDENTLY PREDICTS OVERALL SURVIVAL IN THE MYELOFIBROSIS: KEY INSIGHTS FROM THE COMFORT-I STUDY

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Background: Quality of life (QOL) is a critical aspect of cancer treatment and survival. A strong association exists between QOL and overall survival (OS) for numerous malignancies including breast, gastro-esophageal, colorectal, lung, prostate, ovarian, and head and neck cancer (Sloan 2012, Montazeri 2009, Nilsson 2017). Healthcare organizations have used symptom burden as a primary therapeutic endpoint when assessing the benefit of JAK inhibitors in myelofibrosis (MF) in clinical trials, although QOL was also considered. To date, little is known about the association of these items in regards to overall survival in MF.

Aims: To evaluate the prognostic relevance of QOL and symptom burden among patients with MF from the COMFORT-I study.

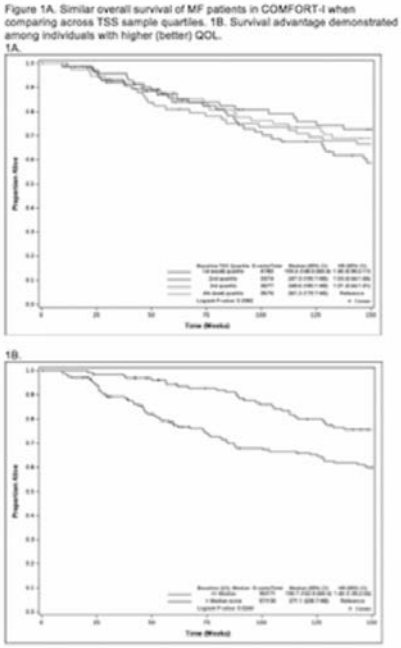

Figure 1.

Methods: Data from the COMFORT-I trial of ruxolitinib (Verstovsek 2012) *versus* placebo was obtained from Incyte© for independent analysis. Association of total symptom burden (TSS; divided by the sample quartiles) and QOL (divided by the sample median) at baseline with OS among MF patients was estimated using the Kaplan-Meier method and tested using log rank tests and Cox regression. Symptom burden and QOL were assessed using the 5-symptom MF-SAFv2.0 (Mesa 2009) and EORTC QLQ-C30 Global Health/QOL scale (Aaronson 1993), respectively. The PROMIS instrument was used to assess fatigue (Cella 2007).

Results: A total of 309 patients were available for analysis including 155 ruxolitinib-treated and 154 placebo-treated MF patients. Baseline demographics, disease-related variables, and calculated overall survival were similar to previous published results (Verstovsek 2015). *Symptom Burden*: When comparing OS by TSS quartiles at baseline, no significant associations in OS were observed (Figure 1A). Individual symptoms of bone or muscle pain, feeling full, pain under ribs on left side, abdominal discomfort, itchiness, or night sweats also did not demonstrate significant associations when comparing OS by quartile symptom score. Baseline fatigue score demonstrated no difference in OS when stratified by median or quartiles. *Global Health Status/QOL*: Intention to treat analysis demonstrated significant survival advantage for patients with higher QOL at baseline (HR 1.47, p=0.02, Figure 1B). When censoring placebo patients at crossover, this hazard ratio improved to a HR 1.79 (p=0.008). *Cox Proportional Hazards Modeling*: Cox regression for survival analysis reached significance for items of age (p<0.001), sex (p<0.009), and QOL (p=0.009) when taking into consideration TSS, IPSS prognostic risk score, age, sex, COMFORT treatment arm, and QOL. When censoring for placebo patients at crossover, this analysis demonstrated that the same items remained significant (age [p=0.001], sex [p<0.001], and QOL [p=0.002]).

Summary/Conclusions: For the patients prospectively evaluated in the COMFORT-I trial, pre-treatment QOL is strongly prognostic for overall survival and remains highly prognostic even when adjusting for symptom burden, disease risk, age, sex, and treatment. Prior literature has confirmed the importance of QOL in prognosticating survival in other cancer types. However, this is the first study that has identified the key correlation among individuals with MF. Neither individual nor combined symptom scores at baseline appeared prognostic for overall survival, emphasizing the importance of QOL assessment in addition to symptom assessment. Weight loss (a prognostic factor for DIPSS scoring) was not included in this symptom burden assessment and may represent an independent factor associated with increased survival.

E1322

CHARACTERIZATION OF DISEASE AND OUTCOMES OF PATIENTS WITH MYELOFIBROSIS: A POPULATION BASED STUDY

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Background: Myelofibrosis (MF) is a myeloproliferative neoplasm with profound negative effects on health related quality of life and survival. It is characterized by clonal myeloproliferation, ineffective erythropoiesis, bone marrow stromal changes, hepatosplenic extramedullary hematopoiesis, and aberrant cytokine expression. Although progress has been made in the understanding of the pathogenesis and management of MF, there are still unresolved issues regarding prognosis and causes of death.

Aims: This population-based study characterizes disease and outcomes in patients (pts) with MF by using the U.S. Surveillance, Epidemiology, and End Results (SEER) database.

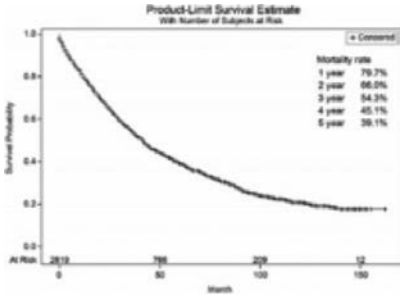

Figure 1.

Methods: We identified a total of 3,367 pts with primary myeloid fibrosis (PMF, ICD-O-3 morphology code as 9961/3 and primary site code as C420, C421 or C424) diagnosed between January 2000 to December 2013. Pts with missing survival status (n=753), pts lost to follow up (n=4), and pts with missing age record (n=1) were excluded. Kaplan-Meier analysis was performed to determine overall survival (OS) and cancer specific mortality. The effects of specific covariates on OS were analyzed using a Cox proportional hazards model.

Results: The final study cohort comprised of 2,619 PMF pts. Median follow up period was 28 months (interquartile range (IQR),11-56). Median age at diagnosis was 68 years (interquartile range 59-77 years) with 60.6% (n =1,586) ≥ 65 years old. More than half of the pts were male (58.5%; n=1,531); 82.2% (n=2,153) were white, and 16.4% (n=430) were diagnosed between 2012 and 2013. The geographic distribution was as follows: East 14.8%, South 18.4%, West 54.2% and Midwest 12.6%. Median OS was 42 months (Figure 1). The hazard ratio of all-cause mortality for age was 1.05 (95% Confidence interval (CI) 1.04-1.05), for female *vs* male was 0.72 (CI 0.64-0.80), for nonwhite *vs* white 1.01 (CI 0.87-1.16), for unmarried *vs* married was 1.04 (CI 0.94-1.16), for patients diagnosed 2012-2013 *vs* 2000-2011 was 0.95 (CI 0.75-1.20). Compared to West, the hazard ratio of OS for East, South and Midwest was 1.05 (CI 0.90-1.22), 1.28(CI 1.12-1.47), 1.03(CI 0.88-1.19) respectively.
Summary/Conclusions: This population based study showed that the overall survival of pts with PMF was short. Older and male pts were associated with higher mortality risk. There were significant differences across geographic regions of the United States. Although there is a trend of improvement in the period of 2012 to 2013, the result is not statistically significant, partially due to short follow up. These findings underscore the continuing need for effective therapies for pts with MF.

E1323

SERUM ALBUMIN IS A STRONG PREDICTOR OF SURVIVAL IN MYELOFIBROSIS, INDEPENDENT OF IPSS, DIPSS, AND DIPSS+ SCORES

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Background: Albumin is the main protein in human plasma. Serum albumin (SA) is used as a surrogate marker of nutritional status and inflammation. The prognostic role of SA has been studied in many diseases, including hematologic malignancies. In myelofibrosis (MF), ruxolitinib has been shown to improve SA levels in addition to other metabolic parameters. SA holds particular significance in MF given its ability to capture both nutritional status and inflammation level in a disease hallmarked by hyperactive inflammatory pathways and constitutional symptoms.
Aims: We aim to closely evaluate the significance of SA in MF patients as it pertains to clinical presentation, laboratory correlations, disease genomics, comorbidities and outcomes.
Methods: We retrospectively reviewed an institutional database of 376 MF patients who presented to Moffitt Cancer Center between 1/1/1998 and 12/31/2012 and had available SA levels within 30 days of presentation. Laboratory values and prognostic scores were determined at time of first presentation. Overall survival (OS) was measured from time of first presentation until date of death or censored at time of last follow-up. Progression free survival (PFS) was defined as time from first presentation to development of acute myeloid leukemia (AML).

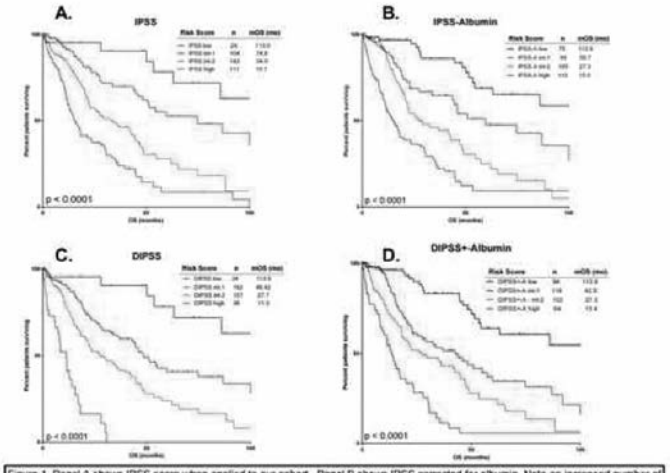

Figure 1. Panel A shows IPSS score when applied to our cohort.. Panel B shows IPSS corrected for albumin. Note an increased number of low-risk patients that are captured with equivalent median OS. Panel C shows DIPSS as applied to our cohort. Panel D shows DIPSS corrected albumin. Note, more low and high-risk patients are captured with comparable median OS.

Figure 1.

Results: Our cohort of MF patients had median age of 67 and 69 at diagnosis and presentation, respectively. Most patients had primary MF (73%) with 11% and 16% having post-PV MF and post-ET MF, respectively. First, we looked at the correlation between SA and other clinical factors. SA was positively correlated with hemoglobin (p<0.01) and platelet count (p<0.01), and negatively correlated with age (p<0.01), peripheral blast percentage (p=0.03), ferritin (p<0.01), prognostic scoring models (p<0.01 for IPSS, DIPPS and DIPSS+) and pack-year smoking history (p<0.01). SA did not correlate with spleen size or any specific somatic mutation, but negatively correlated with somatic mutation burden (p=0.03). On univariate regression, SA was associated with inferior PFS (HR: 0.31 [0.13-0.72]; p<0.01) and OS (HR: 0.25 [0.17-0.36]; p<0.01). Four cohorts were created based on SA: cohort I=SA 2.5-3.5 g/dL (n=31); cohort II=SA 3.6-4.0 g/dL (n=99); cohort III=SA 4.1-4.5 g/dL (n=182); and cohort IV=SA>4.5 g/dL (n=64). OS increased with increasing SA; with median OS (in months) of 9.34, 25.3, 48.4, and undefined in cohorts I-IV, respectively. On focused comparison, each cohort was significantly different than all others. On multivariate analysis, the influence of SA on OS remained significant after controlling for prognostic scores (IPSS, DIPSS, DIPSS+) and comorbidities. For PFS, SA remained significant when controlling for IPSS and DIPSS, but lost significance (p=0.08) when controlling for DIPSS+. Multivariate analysis was performed on a cohort of patients with available molecular data (n=138). SA significantly influenced OS after controlling for prognostic systems, comorbidities and mutations of *SRSF2* and *ASXL1*. Lastly, given its independent prognostic value, we incorporated SA into pre-existing prognostic models. For IPSS, DIPSS, and DIPSS+, patients were assigned 0, 1, 2, and 3 points for low (LR), intermediate-1 (int-1), intermediate-2 (int-2), and high risk (HR), respectively. Patients were then assigned -1, 0 and 1 point for SA >4.3, 3.8-4.3, and <3.8 g/dl, respectively. Risk groups were defined as LR (-1-0 points), int-1 (1 point), int-2 (2 points), and HR (3-4 points). Survival curves and histograms displayed better capture of LR and HR prognostic groups with median OS similar to standard prognostic modeling (see figure).
Summary/Conclusions: SA level is independently prognostic in MF and correlates with variables known to hold prognostic value. Its representation of nutritional status, inflammation, and comorbidities imbues it with special status in predicting outcome. Its incorporation into known prognostic scoring systems provides an improved ability to accurately capture low and high-risk subgroups.

E1324

CLINICAL UTILITY OF NEXT-GENERATION SEQUENCING IN THE MANAGEMENT OF MYELOPROLIFERATIVE NEOPLASMS

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Background: Although Next Generation Sequencing (NGS) has helped characterize the complex genomic landscape of myeloid malignancies, its clinical utility remains not well defined. Funding for NGS testing by healthcare systems or third party payers is variable due to the lack of data on its utility in a routine care setting. At our centre, targeted sequencing (TAR-seq) is offered to all new patients referred for myeloid malignancies as part of the Advanced Genomics in Leukemia (AGILE) project.
Aims: In this study, we evaluate the impact of TAR-seq on the management of patients with a diagnosis of MPN or post-MPN acute myeloid leukemia (MPN/AML).
Methods: All consenting patients referred to the MPN program at the Princess Margaret Cancer Centre between February 2015 and December 2016 with a suspected or confirmed diagnosis of MPN were evaluated (*n*=188). TAR-seq was performed on DNA extracted from peripheral blood (*n*=159, 85%) or bone marrow (*n*=29, 15%) using the TruSight Myeloid Sequencing Panel (Illumina), a targeted NGS panel of 54 genes (39 hotspot region; 15 complete coding region coverage) implicated in myeloid malignancies. Reporting was restricted to high quality exonic nonsynonymous, intronic splice site, frameshift, nonsense and known pathogenic synonymous variants. Variants with global mean allele frequency >1% were identified using multiple population databases (1000 genomes, ESP, ExAC) and excluded. Each patient's TAR-seq results were reviewed alongside their clinical information systematically by at least two hematologists with expertise in MPN, and disagreements were resolved by consensus.
Results: 179 patients fulfilled the 2008 WHO diagnostic criteria for MPN: 107 were diagnosed with myelofibrosis (MF), 26 with polycythemia vera (PV), 21 with essential thrombocythemia (ET), 13 with other MPNs including MPN-unclassifiable and 12 with MPN/AML. In 6 patients with 'triple negative' MPN, who lacked mutations in the driver genes *JAK2*, *CALR* and *MPL*, TAR-Seq confirmed clonal hematopoiesis through identifying other mutations. In 61 transplant-eligible patients with MF, 32 (52%) were considered to carry a *high molecular risk* (HMR) profile based on harboring at least one mutation in *ASXL1*, *EZH2*, *IDH1/2*, *SRSF2* or *TP53*; or a total of three or more mutations. Of these, 11 patients (34%) were considered for early transplant, three with Intermediate-1 and eight with Intermediate-2 risk, who were responding well to JAK 1/2

inhibitor (JAKi) therapy. All high-risk, transplant-eligible MF patients were considered for transplant irrespective of their HMR status. Nine patients with low/intermediate-1 risk MF bearing HMR mutations were considered for a clinical trial of early JAKi therapy, and one patient was successfully enrolled. Seven patients were identified with *IDH1/2* mutations (five with MF and two with MPN/AML), and therefore can be potential candidates for enrolment into clinical trials evaluating novel IDH 1/2 inhibitors. In PV and ET, TAR-seq identified HMR profiles in 6/26 (23%) and 5/21 (24%) patients, respectively. These patients are monitored closely, but no therapeutic decisions were taken based on their HMR profile. In MPN/AML, *TP53* mutations were detected in 4/12 (33%) patients. However, these patients progressed rapidly before their TAR-seq results became available to inform clinical management.

Summary/Conclusions: We have determined that TAR-seq improves the characterization of triple negative MPN patients, refines risk stratification and decisions related to the timing of transplant in MF, and can potentially identify candidates for future targeted therapies. Therefore, we suggest that NGS should be part of the standard of care in MF, and in the investigation of triple negative MPN. Based on these findings and in conjunction with ongoing studies in the MPN program, an algorithm integrating NGS in the management of MF has been developed, and will be evaluated prospectively.

E1325

IMPACT OF COMORBIDITIES AND BODY MASS INDEX ON SURVIVAL IN PATIENTS WITH MYELOFIBROSIS TREATED WITH RUXOLITINIB

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Background: Charlson Comorbidity Index (CCI) and body mass index (BMI) are significantly associated with outcome in patients (pts) who receive continue treatment with tyrosine kinase inhibitors. Ruxolitinib (RUX) is the first JAK1/2 inhibitor that may induce spleen/symptom responses and improve quality of life in pts with myelofibrosis (MF). No data are yet available on the impact of comorbidities and BMI on pts treated with RUX.

Aims: To evaluate the impact of CCI and BMI on overall survival (OS) in a cohort of RUX-treated MF pts.

Methods: A multicenter observational study on WHO-defined MF treated with RUX according to standard clinical practice was conducted in 20 Italian Hematology Centers. Response to RUX was evaluated according to 2013 IWG-MRT criteria. OS was calculated from the date of RUX start to the time of death or last follow-up. Baseline parameters evaluated for correlation with OS were: blood cell count, spleen ≥10cm, marrow fibrosis grading, time from MF diagnosis to RUX start, transfusion dependency, mutation status, Total Symptom Score (TSS), CCI, and BMI.

Results: Between June 2011 and Apr 2016, 343 pts with PMF (51.9%), or post-ET (20.1%) / post-PV (28.0%) were treated with RUX in participating Centers. At RUX start, median age was 67.6 years (range 36.5-89.0) with a male prevalence (57.1%); International Prognostic Score System (IPSS) was intermediate (intm)-1 (16.0%), intm-2 (47.5%), high (36.4%). Transfusion dependence and spleen enlargement were present in 23.9% and 97.4% of pts, respectively (62.4% with spleen ≥10 cm). TSS was <20 in 131 pts (38.2%); 62 (18.1%) pts had a BMI<21 (corresponding to lower quartile). CCI was zero in 105 pts (30.6%), one in 74 pts (21.6%), two in 58 pts (16.9%) and ≥ 3 in 106 pts (30.9%). Median follow-up from MF diagnosis was 3.6 yr (range 0.4-25.6) and median RUX exposure was 21.2 months (3-56.2). In multivariable Cox regression analysis, factors negatively correlating with OS from RUX start were: transfusion dependence (HR: 2.65; p<0.001), CCI ≥3 (HR: 1.67; p=0.031), BMI<21 (HR: 1.74; p=0.039), and IPSS (intm-2= HR: 3.19; p=0.057; high risk= HR: 6.83; p=0.001). Scoring values were assigned to each factor based on multivariable HR values (transfusion dependency=1.5; CCI≥3=1; BMI<21=1; intm-2=2 and high risk=4) and three different groups were identified: group1 (0-2 points, 137 pts), group2 (3-5 points, 144 pts) and group3 (5.5-7.5, 62 pts). OS at 3 years was 91.8%, 65.6% and 34.8% in group1, 2 and 3, respectively (log rank p<0.001) for a median OS of undefined, 56.7 and 22.8 months. Notably, while 88.7% of high IPSS risk pts clustered in group3, only 60.5% of pts in group1 were at intm-2 IPSS risk, and 48.6% of pts in group2 were at high IPSS risk. The achievement of a spleen response at 6 months (39.2% *vs* 36.4%, p=0.71) was not influenced by lower BMI. However, pts achieving a spleen response at 6 months significantly increased OS (Fig. 1A). Also, a higher CCI did not correlate with lower spleen response at 6 months (44% *vs* 34% of pts with CCI<3, p=0.11). The impact of higher CCI on survival was only mildly affected by the achievement of a spleen response at 6 months (Fig. 1B).

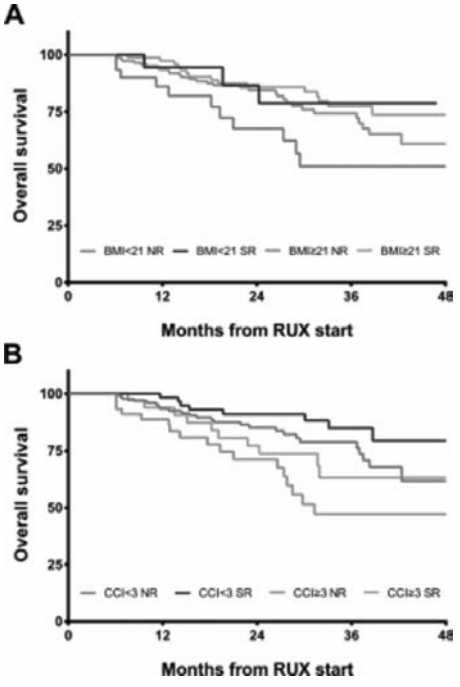

Figure 1.

Summary/Conclusions: Together with transfusion requirement, CCI and BMI may influence survival in RUX-treated MF pts. Taking into account these additional parameters may allow to better define survival probability beyond IPSS risk assessment. Unfavorable CCI and BMI did not hamper responses to RUX; also, the achievement of a spleen response counterbalanced the negative prognostic effects of a lower BMI.

E1326

ANALYSES OF 845 PATIENTS WITH PMF, PET-MF AND PPV-MF TREATED IN 35 GERMAN HEMATOLOGY CENTERS – A RETROSPECTIVE FIELD STUDY

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Background: Primary myelofibrosis (PMF) as well as secondary post essential thrombocythemia (pET)-MF and post polycythemia vera (pPV)-MF are considered rare diseases associated with significant morbidity. Diagnostics and therapeutic options have significantly improved during the last decade by development of novel drugs, improvement of allogeneic stem cell transplantation (SCT) procedures and supportive care. Whereas the characteristics of PMF, pET-MF and pPV-MF patients (pts) participating in clinical trials are well analyzed, data are rare for the general MF population including patients not included in or eligible for clinical trials.

Aims: In order to gain a broader, more comprehensive data set on the general MF population we performed a questionnaire poll in 35 German hematology centers gathering characteristics on 845 pts who were currently under care.

Methods: A questionnaire asking for general patient and disease specific data as symptoms, splenomegaly, prognostic factors, past/ current treatment and blood count, degree of MF in bone marrow and transfusion frequency was designed. It was distributed to participating centers (n=35, mostly private offices) throughout Germany and analyzed centrally. Time period of collection

was 03/2013-12/2015. 845 pts were included *i.e.* a median of 20 pts (range 6–90 pts) per center
Results: Gender was equally distributed (50%/50%). Pts ages at initial diagnosis were as follows:<50 years (y) (11%), 50–60y (19%), 61-70y (31%), and >70y (40%). Current age was >65y in 70% of all pts. PMF represented the largest MF cohort (77%), followed by pET-MF (10%), pPV-MF (7%) and unspecified (6%). Most pts had a longer disease duration (>5y (36%); 1-5y (48%); <1y (15%); unknown (1%)). Key current blood values at time of survey included abnormal thrombocyte counts (<50GPT/l (6%); 50 to <100GPT/l (10%); ≥450GPT/l (28%) and elevated WBC >25.000/µl (11%). Presence of circulating blasts in the peripheral blood was documented in 11% of pts. Hemoglobin [g/dl] was ≥10 (68%), 8–10.0 (21%), <8 (8%), unknown for 3% of the pts. Constitutional symptoms were present in 20% of the pts. Common symptoms included splenomegaly (60%), decreased fitness (41%) and weight loss (16%). Pruritus was present in 5% and night sweats in 9% of all pts. An individual Dynamic International Prognostic Scoring System (DIPPS) score was calculable in 495 pts: 19% low risk, 52% intermediate-1, 23% intermediate-2 and 5% high risk disease. Concomitant diseases were common, most often cardiac (56%). Most common medical treatments included cytostatic (37%), anticoagulation (25%), JAK-inhibitors (23%) and none (24%). Non-medical treatments were rare: stem cell transplantation (3%), splenectomy (2%) and spleen irradiation (3%). Only 31% of all pts received red blood cell transfusions, however 7% had received >50 units.
Summary/Conclusions: Daily practice MF pts share several characteristics with MF trial cohorts (e.g. COMFORT). As expected the diseases were not as progressed as in the trials. Interestingly gender was equally distributed in our study. SCT was a rarely used treatment within this cohort whereas JAK2 inhibitors were frequently used

E1327

CALR MUTATION TYPE INFLUENCES THE RISK OF THROMBOSIS IN ESSENTIAL THROMBOCYTEMIA ACCORDING TO A COOPERATIVE STUDY BETWEEN TWO SPANISH CENTERS

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Background: "Driver mutations" in Essential Thrombocitemia (ET) involve JAK2V617F, CALR and MPL genes. The mutation profile affect the hematological parameters and the risk of thrombosis being the JAK2V617F mutation the one associated with the higher risk of thrombosis. Among CALR mutations there are two main types: type -1 like and type-2 like, but it is not clear if the mutation type is associated with a different clinical feature
Aims: The objective of this study is to understand the clinical meaning of CALR mutation type in ET
Methods: We analyzed 309 ET patients from two hospitals: H.C.U. Santiago and H.U. of Gran Canaria Dr. Negrin. Dates of diagnosis were between 1-11-90 and 1-10-2016, and the median follow up was of 6.88 years. Patients were treated according to local protocols. We collected clinical data of patients at diagnosis and during follow-up as well as events such as thrombosis, transformation to myelofibrosis (MF) or acute leukemia (AL). Thrombosis associated with diagnosis refers to those events happening from two years before diagnosis until diagnosis. The statistical analyses were performed with R Core Team (2016) and IBM SPSS 21.0
Results: JAK2V617F mutation was present in 60.5% of the patients, 1.9% had MPL mutations, 14.5% were CALR type-1like, 11% were CALR type-2like and 11% were triple negative. In three cases, we were not able to classify CALR mutation as type-1/2 like. With regard to the clinical events: 21 patients (6.8%) had thrombosis associated with diagnosis, and 34 (11%) at least 1 thrombosis since the diagnosis. Twelve patients suffered more than 1 thrombotic event. MF evolution was found in 18 patients (5,6%) and 2 cases transformed to AL. In the analysis of hematological parameters according to the mutation type, when we compared **JAK2V617F *VS* CALR type-1like** we observed that JAK2V617F group showed higher hemoglobin values (mean 13.8g/dL *vs* 13.26 g/dL; p=0.014) and lower platelet counts (mean 730x10^9/L *vs* 884x10^9/L, p=0.001) than CALR type-1like group. Confronting **JAK2V617F *VS* CALR type-2like:** the JAK2V617F group showed a higher leucocyte counts (mean 9.39x10^9vs 8.44x10^9/L, p=0.039) and higher hemoglobin values (mean 13.8g/dL *vs* 12.8g/dL, p<0.001)), but lower platelet counts (730x10^9/L *vs* 1081x10^9/L, p<0.001) as compared with the CALR type-2like group. When we studied **CALR type-1like *VS* CALR type-2like:** the CALR type-2like group showed higher platelet count (1081x10^9/L *vs* 884x10^9/L, p<0.035) than CALR type-1like group. **With regard to the thrombotic events,** frequency of first thrombosis (arterial or venous) was 20.9% (n=39) in the JAK2V617F group, 15.5% (n=7) in the CALR type-1like, and 3% (n=1) in the CALR type-2like group. Frequency of thrombosis was significantly higher in JAK2V617F than in CALR group (p= 0.036) and also in JAK2V617F vs. CALR type-2like (p=0.013). When comparing CALR type-1like *vs* CALR type-2like the differences were marginally significant (p=0.06). In a multivariate analysis with the IPSET variables and CALR subtype, in our series the previous history of thrombosis (p <0.001) and the JAK2V617F status (p=0.026) were significantly associated with increased risk of thrombosis, but no the advanced age neither the presence of cardiovascular risk factors. However, presence of CALR type-2like mutation, with respect to the JAK2V617F mutation, was a protective factor of thrombosis (p= 0.06). The five year-thrombosis free survival (TFS) study was as follows: 83%, 85% and 97% for groups JAK2V617F, CALR type-1like and CALR type-2like (log rank p=0.03)(fig. 1).

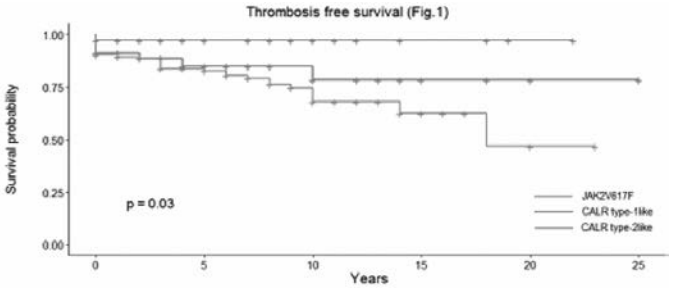

Figure 1.

Summary/Conclusions: The type of driver mutation is associated with a different risk of thrombosis. Among the two types of CALR mutantion, patients have similar clinical characteristics except for the risk of thrombosis which seems lower in CALR type-2like compared to type-1like. This finding shows the importance of studying the CALR mutation type in ET.

E1328

MONITORING OF LEUKOCYTE-PLATELET AGGREGATES AND SELECTIN LEVELS IN PATIENTS WITH PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

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Background: Although the reduction of thrombotic risk is a primary goal of therapy in Philadelphia negative myeloproliferative neoplasms (Ph$^-$MPN), even low risk patients (pts) may experience thrombotic events during the course of the disease. Some recent studies revealed a correlation between the occurrence of thrombosis and activation of blood and endothelial cells. However, not many information is available about influence of therapy on these parameters.
Aims: We prospectively analyzed the levels of leukocyte-platelet (Le-Plt) aggregates, together with levels of soluble selectins, in a group of pts with Ph$^-$MPN at diagnosis and during therapy.
Methods: Our study included 90 consecutive *de novo* Ph$^-$MPN pts (37 polycythemia vera, 27 essential thrombocythemia, 26 primary myelofibrosis), diagnosed according to WHO criteria. According to therapy, pts were assigned as: hydroxyurea (HU 7.8%), aspirin (ASP 55.6%), hydroxyurea+aspirin (HU+ASP 31.1%), although 5.6% of pts were without therapy. Neutrophil-platelet (Neu-Plt) and monocyte-platelet (Mo-Plt) aggregates were determined in whole blood samples (EDTA/CTAD) by flow cytometry. Aggregates were estimated as fraction (%) of CD42b$^+$CD61$^+$ neutrophils and monocytes. Plasma levels of E-, L-, and P-selectins were determined by enzyme immunoassay. All analyses were performed on diagnosis, and repeated 6-9 months after the start of therapy (for pts on HU after achievement of partial or complete remission).
Results: In all pts, mean levels of Neu-Plt and Mo-Plt aggregates at diagnosis were significantly elevated in comparison to control values (22.9% *vs* 8.9% and 13.0% *vs* 5.2% respectively, p<0.01). Mean concentration of soluble E-, L- and P-selectins were also significantly higher in Ph$^-$MPN than in control group (34.2 ng/mL *vs* 19.0 ng/mL; 2748.7 ng/mL *vs* 1322.0 ng/mL and 294.0 ng/mL *vs* 69.8 ng/mL, respectively, p<0.01). Mean levels of Neu-Plt and Mo-Plt aggregates in response to therapy were significantly reduced compared to baseline levels (Figure). Significant reductions were observed for E-selectin levels in HU+ASP group, for L-selectin levels in all three therapy groups and for P-selectin levels in HU and HU+ASP groups (Table). During the median follow up of 39 months from diagnosis of Ph$^-$MPN, thromboembolic events occurred in 13.3% of pts (12/90), particularly: 0/7 on HU, 3/50 on ASP, and 9/28 on HU+ASP. In this subgroup we observed increased baseline levels of Neu-Plt and/or Mo-Plt aggregates in 9/12 pts, while all 12 pts had increased at least one soluble selectin, predominantly P-selectin. Retesting revealed that all 9 pts with thrombosis and increased aggregates level at baseline, normalized those levels after therapy, while only 4/12 pts normalized soluble selectin levels.

Table 1.

Soluble selectins, ng/mL (threshold)	HU (n=7)		p	ASP (n=50)		p	HU+ASP (n=28)		p
	baseline	retest		baseline	retest		baseline	retest	
E- (<39.0)	55.8	50.3	0.285	44.1	58.8	0.001	56.1	45.8	0.046
L- (<2491.8)	3608.7	2180.2	0.043	3907.7	3023.1	0.020	3490	2220	0.001
P- (<129.2)	407.9	131.5	0.018	332.2	267.4	0.310	523.3	162.0	0.0001

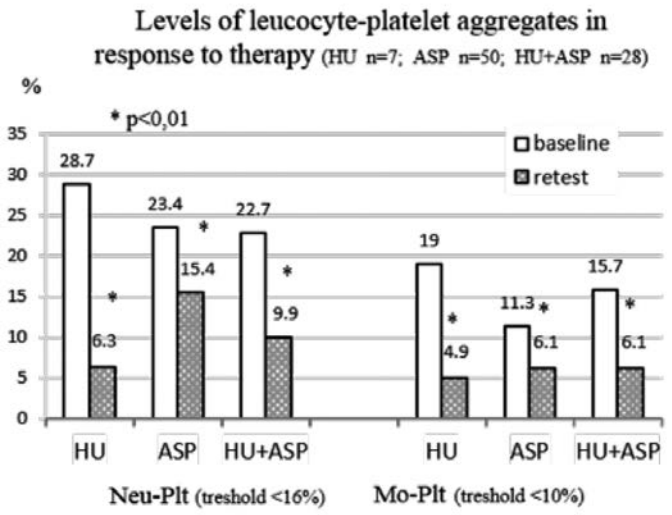

Figure 1.

Summary/Conclusions: We have found elevation of blood and endothelial cell activation markers at baseline in Ph-MPN. Cytoreductive and antiaggregatory therapy reduced the mean level of Le-Plt aggregates and concentration of soluble selectins. In subset of pts with thrombosis, therapy lead to normalization of Le-Plt aggregate levels, with incompletely normalized soluble selectin levels. Even with normal Le-Plt agregates, observed elevated selectin levels can explain persistent thrombotic risk due to intrinsic changes in relationship between blood and endothelial cells as a part of biology of Ph-MPN itself.

E1329

HEAT SHOCK PROTEIN 27 EXPRESSION IS INCREASED IN PATIENTS WITH PRIMARY AND SECONDARY MYELOFIBROSIS AND MAY BE AFFECTING THEIR SURVIVAL

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Background: Increased heat shock protein 27 (HSP27/*HSPB1*) expression and phosphorylation were observed in a large number of neoplastic diseases and they have mostly been associated with aggressive disease features and poor prognosis. There are only few reports investigating HSP27 in primary myelofibrosis (PMF), a myeloproliferative neoplasm characterized by high inflammatory state reflecting in debilitating clinical symptoms.
Aims: To analyze *HSPB1* mRNA expression in patients with PMF and secondary myelofibrosis (SMF) and to correlate it with clinical and hematological features.
Methods: We analyzed *HSPB1* relative expression in bone marrow aspirates of 26 patients with PMF, four patients with SMF and 13 controls using quantitative real time polymerase chain reaction (RT-PCR). Spleen size was assessed by palpation. Association with overall survival was analyzed in 27 PMF and SMF patients evaluated at the time of diagnosis. The Kuskal-Wallis one way analysis of variance, The Mann Whitney U test, the Chi squared test, the Spearman rank correlation, the log-rank test and the Cox regression analysis were used, cut-off point for survival analyses was determined using the ROC curve analysis.
Results: Relative expression of *HSPB1* differed significantly between diagnoses (P<0.001); it was significantly higher in patients with PMF and SMF than in control group (P<0.05 for both comparisons), but did not differ between PMF and SMF patients (non significant). Increased expression was associated with increase in the spleen size (P=0.009) and *JAK2* V617F mutation (P=0.073). We did not detect significant associations with other disease specific features. Lower *HSPB1* expression was associated with inferior overall survival in both univariate (HR 3.2; P=0.04) and multivariate analysis (HR 6.12; P=0.034) where effect was independent of age (non significant), gender (non significant) and the International Prognostic Scoring System (IPSS) score (HR 3.31; P=0.033).

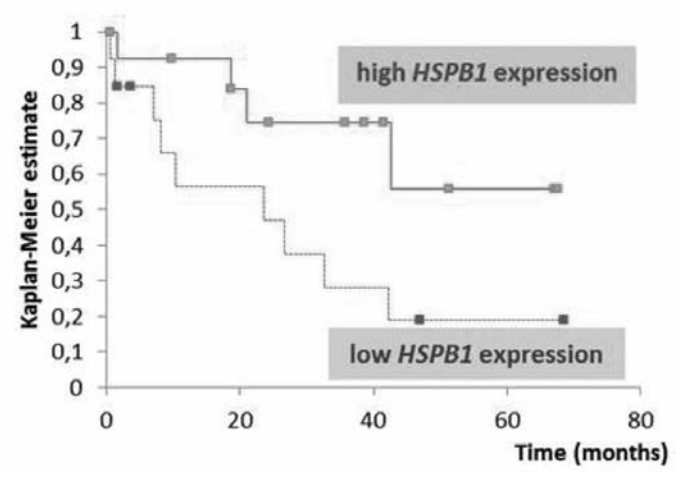

Figure 1.

Summary/Conclusions: Both PMF and SMF patients have increased *HSPB1* mRNA expression in their bone marrows which is associated with increased spleen size. Surprisingly, higher expression is also associated with improved overall survival which is independent of IPSS score. We speculate this to be due to atheroprotective properties of HSP27.

E1330

NON-DRIVER MUTATIONS IDENTIFIED BY A 190-GENE NEXT GENERATION SEQUENCING PANEL IN PATIENTS WITH PRIMARY MYELOFIBROSIS AND POST-POLYCYTHAEMIC/ESSENTIAL THROMOCYTHAEMIA MYELOFIBROSIS

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Background: It is a consensus that the driver mutation is an independent prognostic factor in PMFs. Moreover, some non-driver mutations are found associated with initiation, progression and prognosis in PMFs. However, a recent study from the AGIMM (AIRC- Gruppo Italiano Malattie Mieloproliferative) group showed that the type of driver mutation did not influence prognosis in post-PV/ET MF. These observations proved that there were indeed some differences in these two types of MF.
Aims: The aim of current study was to describe the non-driver mutation landscape and the molecular differences between the patients with PMF and those with post-PV/ET MF.
Methods: Targeted gene sequencing was carried out at diagnosis. We sequenced 190 genes across 62 patients, resulting in 229 high-confidence mutation. The average gene coverage was 99%. The average read depth was 540×. Also, 92% of targeted regions were covered with >20×. Every mutation identified in this study was then compared against these expected patterns and categorized into “oncogenic,” “possible oncogenic variants,” or “unknown significance”. Using copy number-adjusted VAF, we reconstructed the clonal architecture to establish whether a mutant gene was an ancestral or subclonal mutation. According to the statistically differences in VAF among gene mutations, subjects were classified as two different clonal architecture, namely clone+subclone(s) (P<0.05) or clonal.
Results: In PMFs, 42 (93.3%) patients had at least one non-driver mutation. Within the 17 patients lacking the driver mutations in *JAK2V617F*/exon 12, MPLW515 and CALR, 2 had mutant genes (SH2B3 and PIAS3) involving in JAK-STAT pathway, 13 had mutations in other genes and 2 had no mutations. In Post-MFs, non-driver mutations were detected in 16 (94.1%) patients. There are no differences in the median number of non-driver mutations in PMFs *vs.* post-PV/ET MFs (3 *vs.* 3.18, *P*=0.885) and PMF patients with *vs.* without driver mutations (3 *vs.* 3.18, *P*=0.668). In PMFs, 12 non-driver genes were mutated in >5% of patients, namely *ASXL1* 33.3%, *U2AF1* 22.2%, *TET2* 15.6%, *FAT1* 15.6%, *SETBP1* 13.3%, *SRSF2* 8.9%, *CUX1* 8.9%, *EP300* 8.9%, *FAT2* 6.7%, *NOTCH3* 6.7%, *EZH2* 6.7%, and *GATA3* 6.7%. In post-PV/ET MFs, *ASXL1* (41.2%) was the most frequent mutation, followed by *TET2* (29.4%). *U2AF1* and *SRSF2* mutations were significantly more frequent in PMF than in post-PV/ET MF. Moreover, *SETBP1* and *FAT1* were mutated in PMF more often and not mutated in post-PV/ET MF. Figure1 A-C show 3 illustrative patients. Clonal architecture was significantly different between PMFs and post-PV/ET MFs (Figure 1D). About 50% PMF patients were classified as clonal, however, most (87.5%) post-PV/ET MF patients were clone+subclone(s). In PMFs, driver mutation was an ancestral mutation with other non-driver mutations in 14 (31.1%) subjects as 2015-R02413 in Figure 1A. Moreover, driver mutation even was a subclonal mutation in 9 (16.7%) subjects as 2015-R02406 in Figure

1B. In post-PV/ET MFs, 11 (64.7%) subjects showed that JAK2 mutation as an only ancestral mutation as G121517R00701 in Figure 1C.

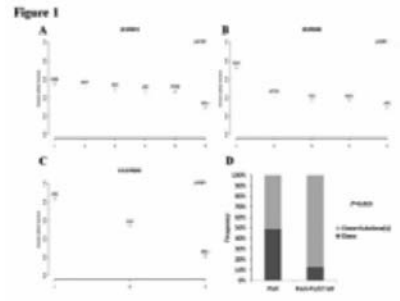

Figure 1.

Summary/Conclusions: In conclusion, we found that the differences in non-driver mutation profile and clonal architecture between PMF and post-PV/ET MF. In addition, by applying a 190-gene panel we demonstrated some variants classified as of "unknown significance". And larger sample sizes may enable some of these to be reclassified in the future. The precise role of each mutation and their impact on MPN phenotype will require further studies.

E1331

DETERMINING MEANINGFUL CHANGE IN THE MYELOFIBROSIS SYMPTOM ASSESSMENT FORM (MFSAF) V2.0 USING A COMBINATION OF DISTRIBUTION- AND ANCHOR-BASED APPROACHES IN THE COMFORT-I TRIAL

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Background: Symptom response was defined in the COMFORT-I trial as a 50% improvement from baseline at week 24 in the Myelofibrosis Symptom Assessment Form (MFSAF) v2.0 total symptom score (TSS; Mesa [J Clin Onc, 2013]; 0 to 60 scale where 60 represents the worse symptom experience imaginable) with no minimum score requirement at baseline.
Aims: In this analysis of the phase III placebo-controlled COMFORT-I study we used distribution- and anchor-based approaches to investigate whether alternative change scores in the MFSAF v2.0 TSS could be meaningful relative to patient-reported quality of life (QOL).

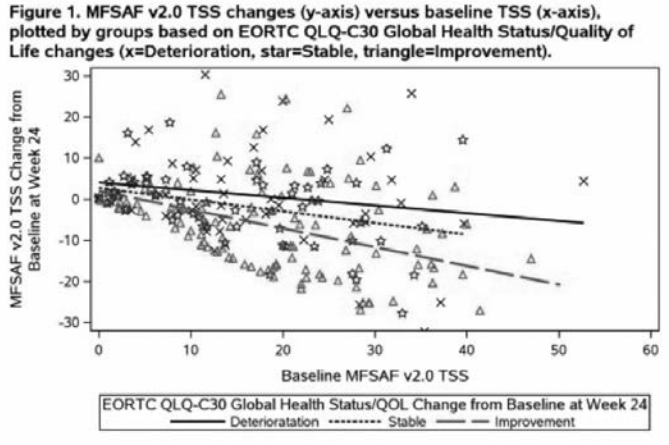

Figure 1.

Methods: One third and one half of the pooled standard deviations (SD) of scores and change scores (raw and percentage change) were used as distribution-based estimates. The anchor-based approach estimated meaningful changes (raw and percentage change) relative to the patient's change in global health status/QOL (GH/QOL; 0=worst, 100=best) as measured by the EORTC QLQ-C30 where a decrease of 12.1 or more points was considered as deterioration; an increase of 7.6 or more points was considered as improvement; and all other changes were considered as stable based on change scores established in a multiple myeloma population (Kvam et al., Eur J Hem, 2011). Analysis of covariance (ANCOVA) was used to investigate whether estimated meaningful changes were consistent across the spectrum of observed baseline TSS. This model of TSS changes at week 24 included a continuous term for baseline TSS, a 3-level grouping factor for GH/QOL change (deterioration *vs* stable *vs* improvement), and an interaction term between baseline TSS and the GH/QOL grouping factor.
Results: 301 patients randomized to ruxolitinib [N=149] or placebo [N=152] completed TSS at baseline (45% female, median age 68 [range 40-91]). Median baseline TSS was 16.6 (range 0 to 52.7). Pooled SD at baseline and week 24 in TSS was 11.4 and 11.6, respectively, resulting in estimated meaningful changes of 3.8-5.8 points. For change and percentage change from baseline at week 24 in TSS, the pooled SDs were 9.8 and 75%, respectively, resulting in estimated meaningful changes of 3.3-4.9 points or 25%>38%. Among patients with TSS and QLQ-C30 data at baseline and week 24, 51 (23%) patients had deterioration, 61 (27%) were stable, and 110 (50%) had improvement based on QLQ-C30 GH/QOL changes. Mean (95% CI) changes in TSS for the three groups were 0.8 (-2.5 to 4.2), -1.4 (-3.6 to 0.8), and -6.8 (-9.0 to -4.6), and for percent changes 20% (-6% to 46%), 17% (-11% to 44%) and -34% (-45% to -22%). ANCOVA revealed that baseline TSS statistically significantly impacted meaningful change estimates (p=0.02, Figure 1) resulting in estimated mean (95% CI) changes in TSS for the improved group of -20.8 (-26.4 to -15.1), -11.7 (-14.3 to -9.0), and -2.6 (-5.1 to -0.1) for baseline TSS of 50, 30, and 10.
Summary/Conclusions: Distribution- and anchor-based approaches suggest that changes as small as 3-6 points on a 0-60 scale of the MFSAF v2.0 TSS may be meaningful to patients. However, estimates of meaningful change appear to increase in magnitude for higher baseline scores, though in a way that a static percentage change criterion would either require too much change for lower baseline TSS or not enough change for higher baseline TSS. All analyses suggest that some changes in symptoms which do not meet a 50% improvement may still be meaningful to patients.

E1332

ERYTHROPOIESIS STIMULATING AGENTS CAN IMPROVE ANEMIA IN PATIENTS WITH MYELOFIBROSIS TREATED WITH RUXOLITINIB

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Background: Anemia is common in patients with myelofibrosis (MF) and it is one of the main cause of symptoms in this setting. Erythropoiesis stimulating agents (ESA) have been used in MF but mostly small series and no randomized trials have been published so far. Anemia response rate ranged between 23 and 60% in different reports (Cervantes et al, BJH 2004;Cervantes et al, BJH 2006;Tsiara et al, Acta Haematologica 2007) and a larger study recently published by Cervantes group on 163 patients (Hernandez-Boluda JC. E et al, EJH 2016) showed a response rate of 50%. Ruxolitinib is currently approved for the treatment of intermediate 2 or high DIPSS/IPSS risk MF and it is highly effective in reducing spleen size and controlling the symptoms of MF, thus resulting in a marked improvement in the patients' quality of life (Verstovsek S. et al, NEJM 2012; Harrison C. et al, NEJM 2012) and possibly a prolonged survival (Cervantes F. et al Blood 2016). However, one of ruxolitinib main side effects is anemia, which occurs in 40% of the patients and can be a limiting factor for treatment tolerability and thus compliance and optimal dosage, mostly in the first weeks of treatment.
Aims: To evaluate the efficacy and safety of combination therapy with ruxolitinib and ESA.
Methods: We retrospectively evaluated 32 patients who received concomitant therapy with ruxolitinib and ESA. ESA (epoetin alpha or zeta or darbepoetin) were given off-label after obtaining patient written consent and local pharmacy approval. Erythroid response was defined as transfusion independence with normal haemoglobin (HB), transfusion decrease of >50% or sustained HB increase of >2g/dl, partial response as a sustained HB increase of 1-2g/dl.
Results: We included 32 patients diagnosed with MF, 23,1% primary,34,6% secondary to PV and 42,3% to TE. 20 patients (62,5%) were male and median age at ESA start was 70 years (range 41-80). 87% of patients were at intermediate 2 and 13% at high risk according to DIPSS. Fifty-nine% of patients received epoetin alpha, 28% darbepoetin and 13% epoetin zeta. Median dose for epoetin alpha/zeta was 40000 U/week and for darbepoetin 150 mcg/week. Seven patients had started ESA treatment before ruxolitinib therapy, whereas twenty-five patients received erythropoietin after ruxolitinib start for persisting or worsening of anemia. In particular, 5 were already RBC transfusion dependent before commencing ruxolitinib while 13 patients required red blood cell (RBC) transfusions only after treatment start. Overall ruxolitinib treatment worsened anemia leading to RBC transfusion requirement in 52% of patients. Median Hb at ESA start was 8 g/dL(range 6,2-10g/dL) and 62,5% of patients were transfusion dependent. Median basal endogenous erythropoietin level was 58 UI/l (range 8-146 UI/l). Overall response rate was 87,6%, with 68,8% of erythroid response and 18,8% of partial response. Median time to response and median

response duration were 4 and 31 months respectively. 23% of patients lost response after a median time of 16 months. Seventy-five% of patients responded to ruxolitinib in terms of spleen size, of whom 86,4% also achieved an erythroid response to ESA. A spleen increase during ESA treatment in patients responding to ruxolitinib was observed in 2 patients only.
No thrombotic events and no toxicity were reported over treatment with ESA.
Summary/Conclusions: ESA were effective in improving anemia in MF patients treated with ruxolitinib. We observed a high response rate in this patients series without significant toxicities. In particular no thrombotic event e no negative impact on response to ruxolitinib was reported. This results may be partially explained by the selection of patients with endogenous erythropoietin level below 250 UI/l, but they could also suggest synergistic activity of ESA and ruxolitinib.

E1333

COMPARING THE SAFETY AND EFFICACY OF RUXOLITINIB (RUX) IN PATIENTS (PTS) WITH DIPSS LOW/INTERMEDIATE-1–, INTERMEDIATE-2–, AND HIGH-RISK MYELOFIBROSIS (MF) IN JUMP, A PHASE 3B, EXPANDED-ACCESS STUDY

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Background: RUX is a potent JAK1/JAK2 inhibitor that led to improvements in splenomegaly and symptoms and increased overall survival in pts with intermediate (Int)-2– and high-risk MF by the International Prognostic Scoring System (IPSS) in the phase 3 COMFORT studies. JUMP is a large, phase 3b, expanded-access trial in countries with no access to RUX outside a clinical trial and includes pts with IPSS Int-1–, Int-2–, and high-risk MF. To further evaluate RUX, we conducted an analysis assessing safety and efficacy of RUX by Dynamic IPSS (DIPSS) prognostic risk.
Aims: To compare the safety and efficacy of RUX in pts with DIPSS low/Int-1–*vs* Int-2– *vs* high-risk MF
Methods: Eligible pts had IPSS high- or Int-2–risk MF, or Int-1–risk MF and a palpable spleen (≥5 cm). Starting dose was based on baseline platelet (PLT) count (5mg bid [≥50 to <100×10^9/L], 15mg bid [100-200×10^9/L], or 20mg bid [>200×10^9/L]) and could be titrated during treatment. The primary endpoint was safety and tolerability of RUX. Changes in palpable spleen length and symptom scores were also assessed. DIPSS scores were determined using pt characteristics at baseline.
Results: Based on available pt data, DIPSS status was determined for 1840 of 2233 enrolled pts. JUMP included 893 low/Int-1–, 754 Int-2–, and 193 high-risk pts (primary MF, 57%, 63%, 62%) who started treatment ≥1 y before data cutoff (01 Jan 2016). Pts with higher-risk MF were older (62, 68, and 72 y), had lower Hb (<10 g/dL, 3%, 64%, 100%), and had higher blast counts (≥1%, 18%, 44%, 84.5%). Disease duration (50, 51, and 55 mo) and spleen size (12, 13, and 14.5 cm) were similar in all 3 groups. Most pts started at 20mg bid (68%, 57%, 59%) or 15mg bid (26%, 32%, 33%). Median exposure was 16, 11, and 9 mo; mean average daily dose was 30, 28, and 29mg. At data cutoff, most pts remained on treatment or had completed per protocol (70%, 56%, 45%). Main reasons for treatment discontinuation included adverse events (AEs; 15%, 17%, 27%), disease progression (6%, 11%, 11%), and death (2%, 5%, 11%). The most common hematologic grade 3/4 AEs were anemia (22%, 44%, 55%) and thrombocytopenia (11%, 18%, 25%), but these rarely led to discontinuation. Overall rates of nonhematologic grade 3/4 AEs were <2%, except for pneumonia (4.5%), pyrexia (2.3%), asthenia (2.2%), and dyspnea (2.2%). Infections in ≥5% of pts were pneumonia (7.3%), urinary tract infection (6.1%), and nasopharyngitis (5.3%). Herpes zoster was reported in 4.8% of pts. At wk 48, 64% (226/355), 52% (121/232), and 50% (26/52) of pts had a ≥50% reduction from baseline in spleen length; 19% (68/355), 19% (43/232), and 23% (12/52) had 25%-50% reductions. Best response in spleen length by wk 72 is shown in the **Figure**; 69%, 57%, and 51% of pts achieved ≥50% reductions. Median time to response was 4.7 wk (2.9-75 wk), 5.3 wk (2.6-80 wk), and 8.1 wk (3.1-72.3 wk). From wk 4 to 48, 39%-43%, 41%-44%, and 48%-54% of pts achieved a clinically meaningful response on the FACT-Lym TS; proportions of responders on the FACIT-Fatigue were 42%-49%, 46%-49%, and 55%-61%.

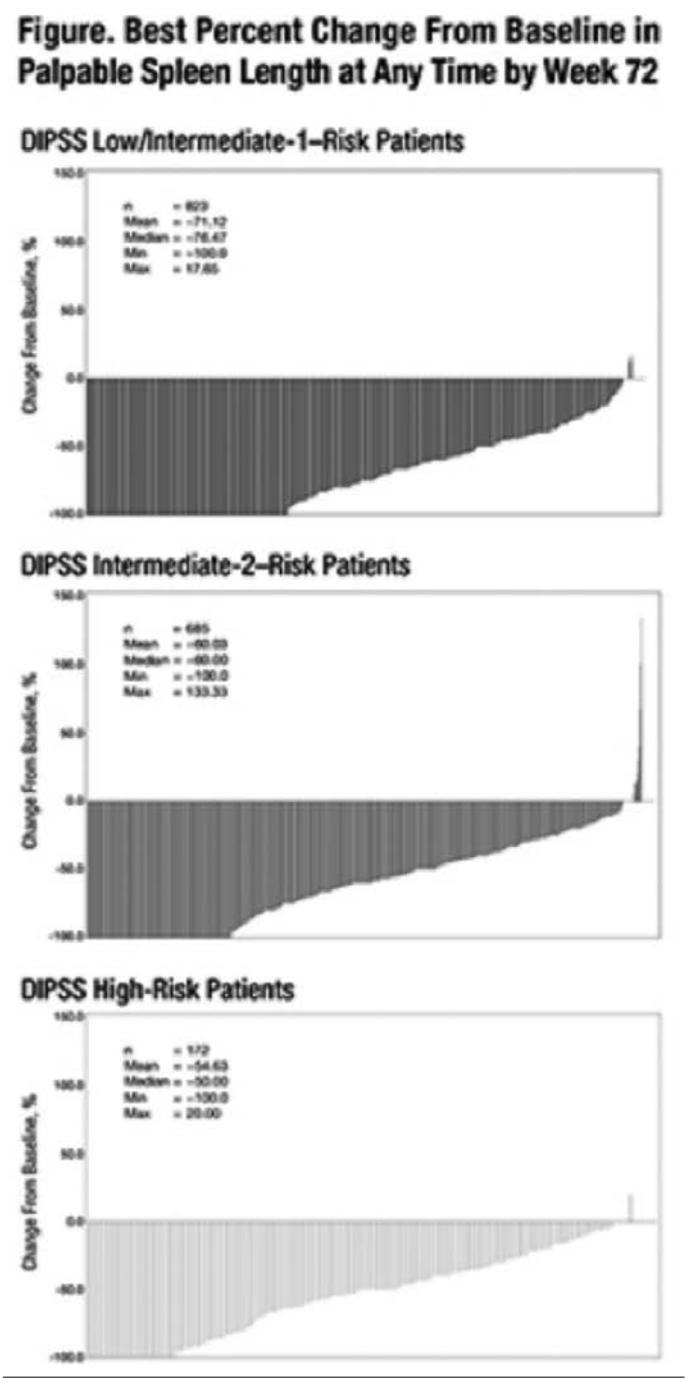

Figure 1.

Summary/Conclusions: RUX was safe and generally well tolerated. Interestingly, lower-risk pts received higher starting doses yet had lower rates of hematologic AEs. Additionally, lower-risk pts remained on treatment longer than higher-risk pts, with fewer discontinuations due to AEs. Lower-risk pts also achieved slightly better spleen size reductions and symptom improvement than higher-risk pts, suggesting that earlier RUX treatment may lead to greater benefits in pts with MF.

E1334

SAFETY AND EFFICACY OF RUXOLITINIB (RUX) IN PATIENTS WITH MYELOFIBROSIS (MF) WHO STARTED TREATMENT AT 10mg BID AND HAD THE DOSE UPTITRATED IN THE PHASE 3B EXPANDED-ACCESS JUMP STUDY

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Background: JUMP is a phase 3b, expanded-access trial that assessed the safety and efficacy of RUX in pts with no access to RUX outside of a clinical trial. Pts received RUX at 5, 15, or 20mg bid per protocol. Increasing evidence from clinical practice suggests that starting RUX at 10mg bid and subsequently uptitrating may reduce the risk of cytopenia development. An *ad hoc* analysis of a subset of JUMP pts provides information on this approach.
Aims: To assess the safety and efficacy of RUX at a starting dose of 10mg bid in pts with MF.
Methods: Pts with high-, Int-2–, or Int-1–risk MF were eligible. Int-1–risk pts had a palpable (≥5 cm) spleen. Protocol starting doses (5, 15, or 20mg bid) were based on baseline platelet (PLT) counts (≥50 to <100×10^9/L, 100 to 200×10^9/L, >200×10^9/L, respectively). Although not per protocol, some pts started RUX at 10mg bid. The primary endpoint was safety. Secondary endpoints included changes in spleen length and symptoms.
Results: A total of 48 pts (primary MF, 60%) started RUX at 10mg bid ≥1 y before data cutoff (01 Jan 2016). Mean baseline characteristics were: median age, 65.5 y (range, 20-83 y); male, 44%; spleen length, 12.3 cm; time since diagnosis, 56.6 mo; hemoglobin (Hb), 112.1 g/L (<100 g/L, 33.3%); PLT count, 351×10^9/L (<100×10^9/L, 10.4%). Pt characteristics were similar to those of the overall population and did not indicate an increased risk of developing cytopenias. At data cutoff, most pts remained on treatment or had completed treatment per protocol (58.3%). Primary reasons for treatment discontinuation included adverse events (AEs), disease progression, and death (8.3% each). Overall, 41.7% of pts had dose modifications (AEs, 33.3%); 20.8% had interruptions (all due to AEs). Median exposure was 14.4 mo. The mean average daily dose was 25.8mg/day (SD, 10.1) and was comparable to those (33.2 and 23.3mg/day) of patients starting at higher doses (20 and 15mg bid; **Figure**). The most common hematologic grade 3/4 AEs were anemia (27.1%; overall, 34.1%) and thrombocytopenia (14.6%; overall, 16.3%). Hb and PLT dynamics were also similar. AEs (all-grade [grade 3/4]) in >10% of pts included pyrexia (14.6% [4.2%]), asthenia (12.5% [0%]), weight increase (12.5% [0%]), abdominal pain (10.4% [0%]), headache (10.4% [2.1%]), and peripheral edema (10.4% [0%]). Infections in >2 pts included herpes zoster (8.3%), gastroenteritis, nasopharyngitis, and septic shock (6.3% for each). At wk 24, 60.9% of pts (14/23) had a ≥50% reduction from baseline in spleen length and 26.1% (6/23) had 25%-50% reductions; rates were similar at wk 48 (58.3% [7/12] and 25.0% [3/12]). Most pts (56.3%) achieved a ≥50% reduction at any time. Pts also experienced significant improvements in symptoms. From wk 4 to 48, 43%-59% and 45%-68% of pts achieved a clinically meaningful response on the FACT-Lym TS and FACIT Fatigue, respectively.

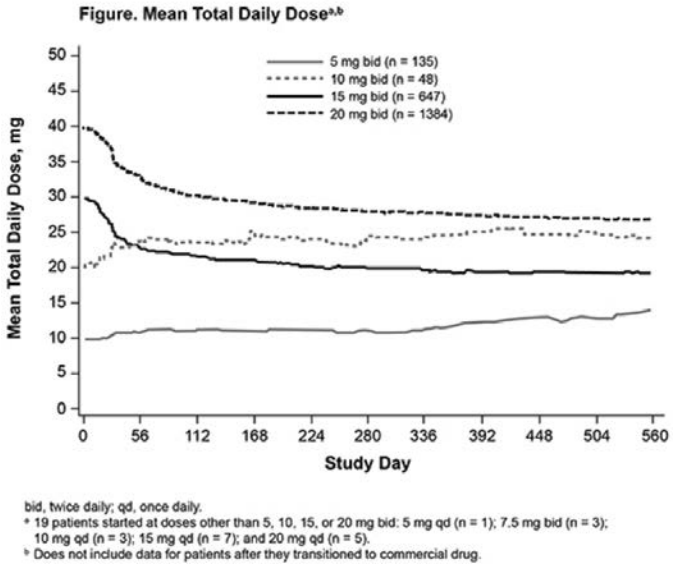

Figure 1.

Summary/Conclusions: A small cohort of pts in JUMP started at 10mg bid, and had the dose uptitrated during the first 8 wks to a mean average daily dose comparable to those of pts starting at higher doses, leading to safety and efficacy outcomes consistent with those in the overall JUMP population. This alternative approach will be prospectively evaluated in anemic MF pts in the REALISE study (NCT02966353).

E1335

HYDROXYUREA IS ASSOCIATED WITH SKIN TOXICITY IN MYELOPROLIFERATIVE NEOPLASMS: RESULTS FROM A PROSPECTIVE NON-INTERVENTIONAL STUDY

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Background: Until today, hydroxyurea (HU) remains the most commonly used cytoreductive drug in patients (pts) with classic myeoproliferative neoplasms (MPN), *i.e.* essential thrombocythemia (ET), polycythemia vera (PV), and myelofibrosis (MF). However, mucosal lesions, cutaneous ulcers, and pre-carcinomatous skin alterations such as actinic keratoses are being considered as potential side effects of HU.
Aims: We sought to investigate the occurrence of skin toxicity in MPN pts under HU compared to other (non-HU) cytoreductive drugs in routine clinical practice.
Methods: Classic MPN pts regularly presenting at the outpatient centers of the University Hospital of Ulm and Johannes Wesling Clinic Minden were included in our non-interventional study after having given informed consent. Skin alterations were evaluated prospectively between December 2010 and November 2016.
Results: In total, 151 MPN pts under cytoreductive therapy were included (ET, n=55; PV, n=55; MF, n=41). Primary MPN diagnosis was made between 1979 and 2012 at a median age of 55 years (range, 22-82). Median duration of the disease until baseline of the study was 6.3 years (0-32.6). Median prospective observation time for the total cohort within the study period was 5.3 years (0.4-6.2). Most frequently used cytoreductive drugs were HU in 120 pts, followed by ruxolitinib in 59, anagrelide in 39, and pegylated Interferon-alpha (IFN-a) in 28 pts. Median cumulative HU exposure was 46 months (1-252), while the median cumulative treatment time for the corresponding drug in the 126 non-HU pts was 24 months (1-267) [ruxolitinib: 22 months (2-64); anagrelide: 19 months (1-216); IFN-a: 64 months (1-267)]. Of 120 pts exposed to HU, 52 pts (43%) presented with skin abnormalities during the observational period occurring after a total HU treatment time of median 46 months (1-252). Sixteen of 120 pts (13%) discontinued HU due to skin toxicity such as skin ulcers (n=6), phototoxicity / erythrodermia (n=5), actinic keratoses (n=3), dry skin / xerostomia (n=2). Of note, four malignant skin diseases were reported under HU therapy (basal cell carcinoma, n=3; malignant melanoma, n=1). Although pts of the HU cohort were exposed longer to the drug compared to pts of the non-HU group, numbers of skin events in non-HU treated pts were as following: n=5 under anagrelide (skin ulcers, n=2; allergic reaction, n=2; basal cell carcinoma, n=1), n=4 under IFN-a (local reaction after subcutaneous injection, n=3; actinic keratosis, n=1), and none under ruxolitinib. In 3/126 (2%) non-HU treated pts, occurrence of skin toxicity led to discontinuation of the corresponding cytoreductive drug. Interestingly, both skin ulcers as well as the single events ´basal cell carcinoma´ and ´actinic keratosis´ occurred under combination therapy with HU. Taken together, skin alterations occurred more frequently under HU compared to non-HU treatment (52/120 [43%] *vs* 9/126 [7%]; p=0.0001), and the same was true for treatment discontinuations due to skin toxicity (16/120 [13%] *vs* 3/126 [2%]; p=0.014).
Summary/Conclusions: According to our prospective observation, skin toxicity was clearly associated with HU treatment compared to other cytoreductive drugs. This resulted in a higher rate of HU treatment termination due to skin toxicity. However, median exposure time to HU was longer compared with non-HU treatment, and controlled clinical trials are necessary to provide more precise data on the occurrence and severity of skin toxicity under HU.

E1336

THE NEGATIVE PROGNOSTIC IMPACT OF BASOPHILIA, EOSINOPHILIA AND MONOCYTOSIS AT DIAGNOSIS IN PRIMARY MYELOFIBROSIS

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Background: Primary myelofibrosis (PMF) is a myeloproliferative neoplasm (MPN) with a variable clinical presentation, from asymptomatic disease to rapidly progressive bone marrow failure and/or leukemic transformation; prognostic stratification using the DIPSS-plus score isolates patient cohorts with median survivals ranging from 16 months to 185 months. The development of monocytosis during the course of PMF has been associated with a worse outcome, and absolute monocyte counts have been shown to be of prognostic value in other MPNs. Basophilia and eosinophilia are frequent findings in BCR-ABL-

positive MPNs, where they associate with an accelerated phase of disease, and seem to correlate with worse survival in myelodysplastic syndromes. However, the impact of these three findings at diagnosis in PMF remains unclear.
Aims: The aim of this work is to evaluate, at diagnosis, the prognostic impact of basophilia, eosinophilia and monocytosis in patients with PMF.
Methods: We identified all PMF patients diagnosed and followed-up in our Center between January 1st 2005 and August 31st 2016 who still fulfill PMF criteria under the WHO 2016 diagnostic revision, have synchronous bone marrow (BM) and peripheral blood (PB) analyses dating from the time of diagnosis, and have complete charts with no missing data. After the exclusion of reactive causes, monocytosis was defined as an absolute count (AC) >1.0 G/L, eosinophilia as an AC>0.6 G/L and basophilia as an AC >0.2 G/L.
Results: We studied 55 evaluable patients (73% male) with a median age at diagnosis of 70.1±11.7 years old. At diagnosis, 20% of patients had **monocytosis**, with no significant differences according to gender or age. The median overall survival (OS) in PMF patients with monocytosis was 27.3 months, and twice as long (46.4 months) in patients without. In this cohort, a new calculated cut-off of 0.75 G/L was better able to stratify patients according to survival with a specificity of 74.1% (95% CI: 53.7-88.9%); 32.7% of patients had an AC above the cut-off, with a median OS of 27.9 months, compared to 64.4 months for patients under the cut-off. We identified 12.7% of patients with **eosinophilia** at diagnosis, with no differences according to gender or age. PMF patients with eosinophilia had a five-fold lower median OS compared with patients without (6.1 *vs* 32.5 months, respectively). We obtained a new cut-off of 0.25 G/L of eosinophils, which separated patients with a specificity of 77.8% (95% CI: 57.7-91.4%); 29.1% of patients had an eosinophil AC above the cut-off, with a median OS of 27.3 months, compared to 43.6 months for patients under the cut-off. A total of 30.9% of patients had **basophilia** at diagnosis, with no differences according to gender or age. The median OS in patients with basophilia was 25.6 months, and 32.5 months in patients without. With a new cut-off of 0.25 G/L of basophils, with a specificity of 88.9% (95% CI: 70.8-97.64%), 20.0% of patients had a basophil AC above the cut-off and a median OS of 19.7 months, compared to 46.4 months for patients under the cut-off. Considering the whole cohort, 61.8% of patients had normal monocyte, eosinophil and basophil ACs; the median OS in these patients was 56.1 months, compared to 28.5 months in patients with an increase in at least one AC. Applying the new cut-offs, this difference in OS increased to 27.9 *vs* 64.4 months. Progression-free survivals were not calculated, since only 2 patients had BM- or PB-documented progression during follow-up.
Summary/Conclusions: We observed that the presence of monocytosis at diagnosis in PMF was associated with a halving of the median OS, while eosinophilia decreased the median survival to one-fifth; basophilia also associated with a reduction in survival, of approximately 20%. The application of specific cut-offs calculated for the cohort improved the differentiation and stratification of patients, with moderate to high specificity, further clarifying the negative prognostic impact of these three variables, at diagnosis, in PMF. Our results show that even simple, inexpensive and readily available parameters can be used to predict survival in PMF patients, and suggest that their integration into established scores could further increase the prognostic accuracy of the latter.

E1337

BLAST PHASE IN PH-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS: A SINGLE INSTITUTION RETROSPECTIVE ANALYSIS OF 85 PATIENTS

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Background: Classic Ph-negative myeloproliferative neoplasms (MPN) include essential thrombocythemia (ET), polycythemia vera (PV) and primary myelofibrosis (PMF). Clonal evolution can lead MPN patients in chronic phase (CP) to develop acute myeloid leukemia (AML), called blast phase (BP): this event occurs at rates of approximately 1% in ET, 4% in PV and 20% in PMF over the first decade from MPN diagnosis.
Aims: To evaluate differences in clinical features and outcome in 85 patients with MPN in blast phase, according to MPN diagnosis and mutational profile.
Methods: We identified in our database all patients affected with ET, PV and PMF who developed acute myeloid leukemia according to 2016 WHO criteria (≥ 20% blasts in bone marrow or peripheral blood) and for whom at least one DNA sample to define the mutational status of the three MPN driver genes (*JAK2*, *CALR*, *MPL*) was available. Treatment used in blast phase was classified as palliation (supportive care only or low intensity chemotherapy) or induction chemotherapy (de novo AML-like therapy).
Results: We retrospectively identified among 2902 consecutive patients affected with Ph-negative MPN 85 patients who progressed to BP, with a known molecular profile: 26 ET patients of whom 15 presented *JAK2 V617F* mutation, 8 *CALR* mutation, 1 *MPL* mutation, 1 *JAK2/MPL* mutation and 1 was triple-negative, 36 PV patients all *JAK2*V617F mutated, and 23 PMF patients of whom 17 were *JAK2* mutated, 2 *CALR* mutated, 2 *MPL* mutated and 2 triple-negative. Median age at BP was 71,3 years (range 46,3-86), being higher in PV (median 73 years, range 46,3-84,7) compared to ET (median 68,7 years, range 54,4-86, *P* 0,318) and PMF (median 67,9 years, range 48,1-84,9, *P* 0,016). The complete blood count at leukemic evolution was not influenced by the initial diagnosis. At the time of BP, 31 out of 44 patients (70%) for whom cytogenetic analysis was available showed an abnormal karyotype (22 patients with complex karyotype or high risk aberrations). *JAK2* mutated MPN can evolve into *JAK2* wild type AML (9 of 28 patient with blasts DNA available), while *CALR* mutation was identified also in AML blasts in all 6 patients for which DNA was available. Time to leukemic evolution was shorter in PMF (35,3 months, range 3,6-141,1) compared to ET (176,7 months, range 14,4-362,3, *P* <0,001) and PV (129,1 months, range 17-367,6, *P*<0,001). According to chronic phase driver mutation, time to leukemic evolution was shorter in *JAK2 V617F* mutated PMF compared to *CALR* mutated PMF (30,6 *vs* 138 months, *P* 0,024), but not statistically different in *JAK2* mutated ET compared to *CALR* mutated ET (123,4 *vs* 203,2 months, *P* 0,121). Outcome was dismal, independently from MPN diagnosis (P 0,278), with a median overall survival (OS) of 3,9 months (range 3,3-5,6). OS was not influenced by treatment during blast phase (4,5 months with induction chemotherapy *versus* 4,6 months with palliation; P 0,865). Of the whole series, only one patient is alive and in complete clinical and molecular remission, after 11 months from allogeneic bone marrow transplantation.

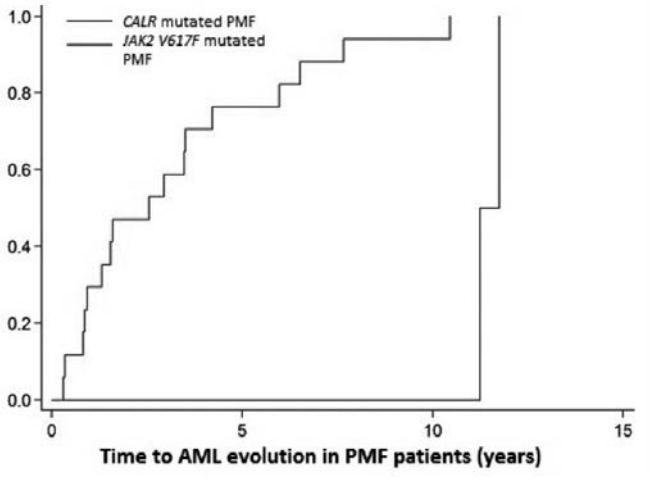

Figure 1.

Summary/Conclusions: Clinical phenotype and outcome of BP is not influenced neither by the diagnosis in chronic phase nor by the driver mutation; moreover the outcome is poor irrespective of treatment. PMF patients have a shorter time to BP than ET and PV patients; in PMF *JAK2 V617F* mutation is associated with a shorter time to BP compared to *CALR* mutation. The only potentially curative treatment is represented by allogeneic stem cell transplantation, but only a few patients can actually undergo this procedure.

E1338

TELOMERE LENGTH IS REDUCED IN ESSENTIAL THROMBOCYTHAEMIA PATIENTS COMPARED TO AGE AND GENDER MATCHED HEALTHY CONTROLS

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Background: Essential thrombocythaemia (ET) is a clonal stem cell disorder, commonly diagnosed in the 6th or 7th decade of life. ET is associated with risk of thromboembolic events, hemorrhage, constitutional symptoms, progression to myelofibrosis and acute myeloid leukemia. In over 85% of patients a clonal driver can be identified with mutations in *JAK2* (50-60%%), Calreticulin (*CALR*) (25-30%) or the thrombopoetein receptor (*MPL*) (3-5%); the remainder of patients are termed "triple negative" (TN). Telomeres are non-coding regions of DNA consisting of thousands of repeated sequences (TTAGGG) and are considered central to chromosomal integrity and genomic stability. In healthy adults, telomere length (TL) progressively shortens with age; therefore, TL is considered a marker of aging and genome instability. Hematopoietic cells in several hematological malignancies have been shown to be characterized by shortened TL.
Aims: Determine if there is TL shortening in patients with ET when compared to age and gender matched controls and establish the effects of cytoreductive on TL in ET patients.
Methods: 100 patients were included in the study (27 with *CALR*, 35 *JAK2*V617F and two MPL515W mutations. 36 patients were TN). Most patients were female (70% 70/100); median age was 45 years (range 20 - 86 years).

TL was determined in peripheral blood mononuclear cells using a monochrome multiplex quantitative PCR based on the original methods described by Cawthon. All results were corrected for age and gender.
Results: Regardless of driver mutation status ET patients had significantly shortened TL compared with age and gender matched controls, p=<0.0001. Considering individual mutation status these differences remained significant *e.g. CALR* positive, p=0.0009; *JAK2*V617F positive p=0.007 and p=0.012 in TN patients. TL appeared more markedly short in the *CALR* cohort; for the 18 patients, whose TL was below the first centile, 55% (10/18) were *CALR* positive *vs* 28% (5/18) *JAK2*V617F positive *vs* 17% (3/18) who were TN. Concerning the potential impact of therapies 31/100 patients were treated with hydroxycarbamide (HC); 24/100 interferon (IFN) (eight of these had prior exposure to HC); 34/100 were not on cytoreductive therapy. Remaining treatments were ruxolitinib (5), busulphan (4), anagrelide (1) and vorinostat (1). Independent of mutation status there was significant TL shortening in untreated patients, p=0.05; however, upon evaluating the impact of cytoreductive therapy on TL we noted that ET patients with either current, or prior HC treatment had significantly shortened TL, p= 0.0015 and p=<0.0001 respectively. Strikingly, there was no significant difference in TL in IFN patients who had no previous exposure to HC, p=0.2 but those ET patients currently on IFN but with prior HC exposure still had shorter TL, p=<0.0001.
Summary/Conclusions: We document for the first time that ET patients, when compared to age and gender matched healthy controls, have shortened TL. This shortening is more pronounced in *CALR* and *JAK2* V617F positive patients. Concerning therapy whilst present in untreated patients TL shortening was more pronounced in HC treated patients indicating that there may be a therapy effect as has been observed after HC treatment in sickle cell disease. Of note IFN treated patients had more normal TL suggesting that the disease related TL effects may be reversed by this agent.

E1339

NUTRITION IN MYELOFIBROSIS: CORRELATES FROM THE COMFORT-1 STUDY

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Background: Nutritional status declines in most patients with myelofibrosis (MF). Sixty-seven percent of patients with MF lose weight over time and 27% of patients have a BMI decrease of at least one body mass index (BMI) category (Mesa *et al.* Blood. 2008;112(11):5224). MF also leads to deficient LDL and cholesterol levels compared to age matched controls (Mesa R A *et al.* Blood. 2007;110(11):2548). Both hypocholesterolemia (p<0.001) and weight loss>10% (p<0.001) have been associated with decreased survival in PMF patients (Mesa *et al.* Blood 2009 114:3918) JAK inhibitor therapy has been found to improve nutritional markers including weight, cholesterol, albumin, and leptin compared to placebo in the COMFORT-1 study (Mesa *et al.* Clin Lymphoma Myeloma Leuk. 2015 Apr; 15(4): 214–221; Verstovsek *et al.* N Engl J Med 2012; 366:799-807). However, the corelation of these factors with other disease related variables and overall survival has not been established.
Aims: To evaluate the correlation, if any, between nutritional markers other variables collected in the COMFORT-1 study.
Methods: Data from the COMFORT-1 trial of ruxolitinib *versus* placebo was obtained from the Incyte for independent analysis. Data was analyzed for correlation with symptom burden and survival along with other variables. Symptom burden was assessed by the MF-SAF v2.0 (Mesa *et al.* Leuk Res 2009) for individual items and total symptom score (TSS).
Results: A total of 309 patients were available for analysis including 155 ruxolitinib-treated and 154 placebo-treated MF patients. At baseline, the average BMI was 24.9 (SD=4.5). Baseline demographic and other disease-related variables can be found in previous publications (Verstovsek *et al.* N Engl J Med 2012; 366:799-807). Correlatives: *Baseline*: For all patients at baseline, numerous correlations between baseline nutritional markers and markers of nutrition (Figure 1) were identified. Total Symptom Scores (TSS) inversely correlated with albumin, cholesterol, alpha-feto protein, HDL, and serum erythropoietin levels. Baseline leptin levels correlated with many items including BMI, albumin, cholesterol, LDL, erythropoietin, insulin and CRP. *Placebo*: For patients treated with placebo, changes in BMI inversely correlated with changes in CRP (r=-0.22, p=0.04). Positive correlations were observed between changes in LDL with cholesterol (r=0.87, p<0.001) and HDL (0.41, p<0.001). In addition to LDL, HDL change inversely correlated with TSS score (-0.24, p=0.02), and positively correlated with changes in bone pain (0.23, p=0.02), abdominal fullness (r=0.22, p=0.02), erythropoetin levels (0.27, p=0.01) and cholesterol levels (r=0.39, p<0.001). *Ruxolitinib*: Most correlations with nutritional and metabolic markers mirrored with baseline scores (Figure 1b). For ruxolitinib-treated patients, change in JAK2V617F mutational status inversely correlated with changes in serum cholesterol (-0.26, p=0.008), leptin (-0.38, p<0.0001), and LDL (-0.23, p=0.02). CRP changes were inversely correlated with change in cholesterol levels (-0.18, p=0.03).

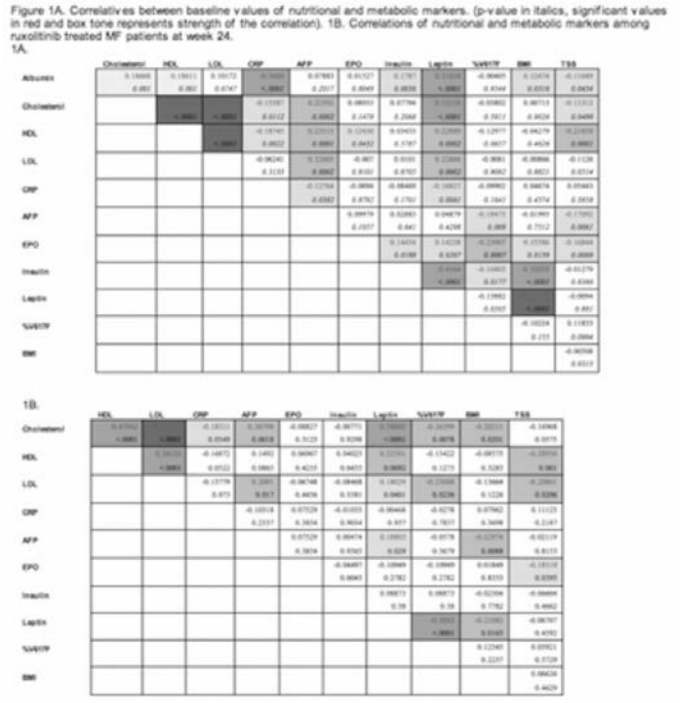
Figure 1A. Correlatives between baseline values of nutritional and metabolic markers. (p-value in italics, significant values in red and box tone represents strength of the correlation). 1B. Correlations of nutritional and metabolic markers among ruxolitinib treated MF patients at week 24.

Figure 1.

Summary/Conclusions: Nutrition decline remains an unmet need for many MF patients. JAK2 inhibition represents a potential source to improve symptom burden in those who qualify for therapy. Leptin closely correlated with many other nutritional values suggesting this may be a good marker of nutritional status in MPN patients. CRP was inversely correlated with BMI, suggesting the importance of inflammation as a contributor to weight loss. Further study into the unique nutritional needs of myelofibrosis patients is warranted.

E1340

IS THE SURVIVAL OF PATIENTS WITH ESSENTIAL THROMBOCYTEMIA BETTER IN THE LAST DECADE? RETROSPECTIVE ANALYSIS OF DATABASE OF LATIAL GROUP FOR THE STUDY OF NMP, PH NEGATIVE

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Background: To evaluate the prognosis of patients with Essential Thrombocythemia (ET) in the first decade of the century we assessed retrospectively the thrombosis free survival (TFS) and the overall survival (OS) of the patients diagnosed from 01/01/2000 to 31/12/2009 and collected on the database of our group.
Aims: Diagnosis of ET was performed with PVSG, WHO 2001 or 2008 criteria, according to the date of the first observation. The whole population of 757 patients was then divided in two groups: the first (group I) with the diagnosis performed between 01/01/2000 to 31/12/2005 (334 patients), presented a median follow-up of 111,9 months, the second (group II) diagnosed between

01/01/2006 to 31/12/2009 (385patients), with a median follow-up of 58,2 months.
Methods: The characteristics of two groups of patients are reported in the Table 1. No differences could be found between the two groups according age, gender, platelet and WBC count and Hb level, Cardio-Vascular Risk Factors (CVRF), spleen enlargement and the occurrence of previous thrombotic events. The frequency of the JAK-2 ^{V617F} mutation resulted significantly different (49.1% *vs* 68.4%) but in the group I the search of the mutation was never performed at the diagnosis. TFS and OS were calculated from the date of diagnosis of ET to the date of event with Kaplan-Meier product limit method; the comparison of proportions and median values was computed with the Chi-squared and the Mann-Withney tests, as indicated.
Results: No significant differences emerged neither for TFS (p= 0,09, HR 1,42, 95% C.I. 0.89-2.30) nor for OS (p= 0,15, HR 1,34, 95% C.I. 0,87-2,06). We also evaluated the type of treatment used in the two groups to assess the potential link between the therapy and TFS or OS (Table 2). No difference emerged between the two groups as for anti-aggregating (mainly ASA), equally utilized in both groups, 287/369, 77,8%, and 330/383, 78,3%, respectively (p=0,95). As for the cytoreductive therapy, Hydroxyurea was used in 74.8% *vs* 67.9% (p= 0.60) and alkylating agents in 1.9% *vs* 2.1% (p= 0.85), whereas the Anagrelide resulted utilized in 10,6% *vs* 3,9% (p= 0,001) and Interferon in 9,5% *vs* 5,2% (p= 0,037), respectively. The more frequent use of Anagrelide and Interferon in the first group (2000-2005) didn't modify the prognosis (as for TFS and OS) of the patients.

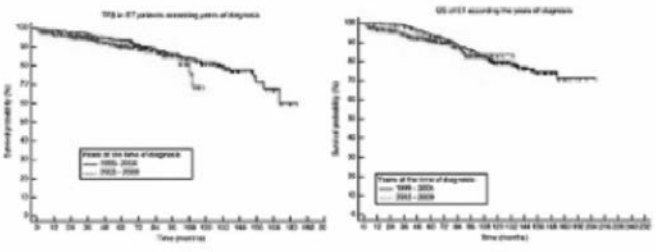

Figure 1.

Summary/Conclusions: Unfortunately, no improvement, neither as the TFS nor the OS was observed (Fig. 1 and 2): more efforts to better identify the groups at risk and, hopely, the introduction of new drugs as JAK-2 inhibitors could change the prognosis of ET patients.

E1341

CUTANEOUS INVOLVEMENT IN PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS-SINGLE-CENTER EXPERIENCE

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Background: Philadelphia-negative chronic myeloproliferative neoplasms (MPNs) may present clinical dermatological manifestations at the time of diagnosis, as well as during the course of the disease. On the other hand, also its treatments can present skin side effects.

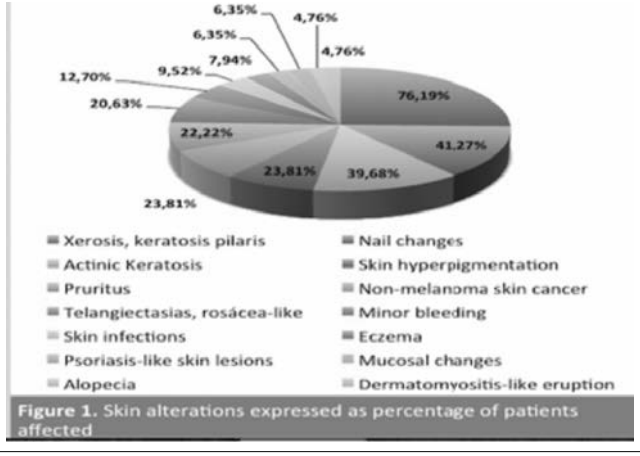

Figure 1. Skin alterations expressed as percentage of patients affected

Figure 1.

Aims: We have performed a dermatological review of a cohort of patients we follow-up at our center with the aim of assessing the cutaneous manifestations.
Methods: A randomized selection of patients with a diagnosis of essential thrombocythemia and polycythemia vera was performed. We create a specific consultation in which a detailed history of each patient (sex, age, diagnosis, signs and symptoms, treatments and its duration) as well as a deep dermatological examination was done. All data was collected in an Excel database and analyzed using the SPSS system.
Results: 63 patients (54 ET and 9 VP) were reviewed. The most frequent skin lesions were xerosis and/or keratosis pilaris (76.2% patients), nail changes (41.3%), actinic keratosis (39.7%), hyperpigmentation of the skin (23.8%), pruritus (23.8%) and non-melanoma skin cancer (22.2%). In figure 1 we detail all the skin alterations that we have found.
Summary/Conclusions: Cutaneous involvement in MPNs is more frequent than expected and it is usually underdiagnosed. Some of these lesions could be prevented with the correct treatment of their pathology and adequate photoprotective measures. The results obtained support the recommendation of an annual review by a dermatologist in a systematic way, especially in patients with higher risk factors: low phototype, high sun exposure, past dermatological history and prolonged cytoreductive therapy.

E1342

HEMOGLOBIN AND WHITE CELL COUNT IN PATIENTS CLINICALLY SUSPECTED TO HAVE ESSENTIAL THROMBOCYTHEMIA MAY HELP IN PREDICTING EARLY PRIMARY MYELOFIBROSIS OR UNCLASSIFIABLE MYELOPROLIFERATIVE NEOPLASM

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Background: Classification of myeloproliferative neoplasms (MPN) in patients presenting with thrombocytosis can be challenging. Relying only on clinical features may lead to misclassification of patients in the early stages of primary myelofibrosis (PMF) as essential thrombocythemia (ET). Although bone marrow (BM) biopsy examination is the gold standard necessary for accurate classification, in clinical practice it might be helpful to identify among patients with a working diagnosis of ET those most likely to have early PMF or unclassifiable MPN (MPN-U). To this end, Carobbio *et al. (Am J Hematol. 2012;87:203-4)* developed a simple algorithm based on presence of anemia (hemoglobin <120 g/L for females and <130g/L males) and/or leukocytosis (leukocytes ≥13x10^9/L) or elevated LDH (>200 mU/mL). For an accurate classification, the clinical and laboratory features need to be correlated with BM findings, thus collaboration between hematologists and pathologists is essential.
Aims: To examine applicability of the Carobbio algorithm in routine practice and its potential use in identifying among patients presenting with thrombocytosis and clinically suspected to have ET, those with early PMF or MPN-U. To identify unmet needs in the diagnosis of MPNs in daily practice upon which further educational initiatives can be built which stress the importance of hematologist-pathologist collaboration.

Figure. Program Overview

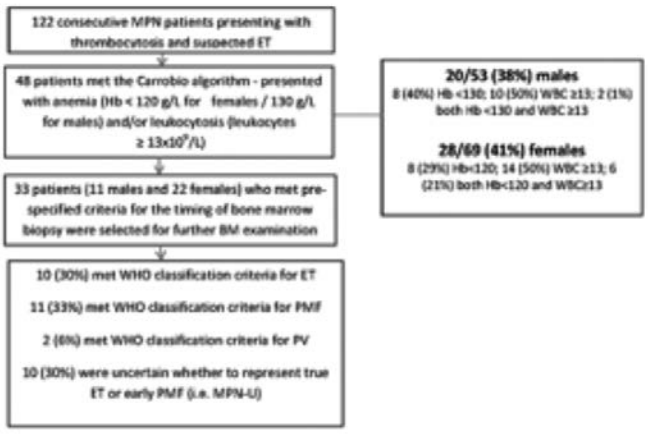

Figure 1.

Methods: A retrospective Personal Practice Assessment Program was conducted at 8 Canadian institutions. Eight hematology/pathology pairs reviewed charts of about 20 consecutive examined patients who presented with thrombocytosis and suspected to have ET. The first 5 out of 20 cases who met the Carobbio algorithm were selected for BM evaluation. To avoid the impact of treatment and/or natural disease evolution on accurate classification, the requirement was for the BM biopsy to be collected within a year of patient's

presentation with thrombocytosis. No central pathology review was planned for this stage of the study.
Results: A total of 122 patients (58 males and 69 females; 54% >60 years of age; 65% with LDH ≥200 mU/mL) with a clinical history indicative of ET were initially assessed. A majority of patients (76%) presented with suspected ET within the last 5 years, likely because it was more difficult for clinicians to identify patients with BM biopsy collected within a year of presentation with thrombocytosis if they presented more than 5 years ago. Out of 122 patients, 48 met the hemoglobin and/or leukocytes criteria outlined in the Carobbio algorithm, Figure. The BM examination was performed on 33 patients who met pre-specified criteria for the timing of bone marrow biopsy. About one third of the 33 patients met the WHO classification for ET and one third for PMF. While 2 of the remaining patients met criteria for PV, the rest were uncertain whether to represent true ET or early PMF, *i.e.* represented MPN-U (Figure 1).
Summary/Conclusions: Despite its methodological limitations, this initiative confirms that in real world clinical practice the Carobbio algorithm can be used to identify possible cases of early PMF and MPN unclassifiable among patients clinically suspected to have ET. It suggests a need for educational initiatives on using diagnostic algorithms to separate ET from PMF. It confirms the importance of hematologist-pathologist collaboration in reaching a final integrated diagnosis based on the WHO classification. These findings warrant further investigation in larger prospective studies.

E1343

PK/PD MODELING COMPARING DIVIDED DOSING (200mg TWICE-DAILY [BID]) *VS* SINGLE DOSING (400mg ONCE-DAILY [QD]) OF PACRITINIB (PAC) IN PATIENTS WITH MYELOFIBROSIS (MF) ON THE PERSIST-2 PHASE 3 TRIAL

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Background: MF is a life-threatening hematologic malignancy characterized by splenomegaly and debilitating constitutional symptoms. At the present, the JAK inhibitor ruxolitinib is the only therapy for patients (pts) with MF that has garnered regulatory approval. Although ruxolitinib has been shown to reduce splenomegaly and symptoms in pts with MF, it is associated with dose-limiting cytopenias, and not indicated for pts with platelets <50,000/μL. PAC is an oral kinase inhibitor with specificity for JAK2, FLT3, IRAK1, and CSF1R. Using data from early-phase PAC studies, population PK modeling and simulations predicted that BID dosing would result in higher steady-state AUC and lower Cmax *vs* QD dosing, which may be associated with increased efficacy and comparable or improved safety. Thus, the phase 3 PERSIST-2 trial of PAC *vs* BAT (including ruxolitinib) in pts with MF and platelet counts ≤100,000/μL evaluated both PAC 400mg QD and 200mg BID dosing schedules. Data previously presented (Mascarenhas, J. *et al. Blood*, 128(22), LBA-5.) showed significantly higher spleen volume reduction (SVR) with PAC (QD and BID pooled) *vs* BAT (P=0.001); despite identical cumulative dosing, pts had improved SVR and total symptom score (TSS) reduction with PAC 200mg BID *vs* PAC 400mg QD, with numerically fewer adverse events and a trend towards improved survival.
Aims: Validate the clinical utility of PK/PD modeling to select the PAC 200mg BID regimen in pts with MF treated in the PERSIST-2 trial.
Methods: Pts with MF and baseline platelet count ≤100,000/μL were randomized 1:1:1 to PAC 400mg QD, PAC 200mg BID, or BAT. Blood samples were collected from PAC-treated pts for PK and PD analysis at a prespecified subset of trial sites. Blood samples were collected on day 1 of week 1 (4 h post-dose), week 3 (pre-dose and 4 h post-dose), week 12 (pre-dose), and week 24 (pre-dose). At the remaining sites, blood samples were collected from PAC-treated pts for PK analysis only at weeks 12 and 24 (pre-dose).
Results: In total, PK samples were collected up to week 24 from 144 PAC-treated pts (78 BID, 64 QD). The PK of PAC was described by a 2-compartment model with first order absorption, first order elimination from the central compartment, and an absorption lag time. PAC QD was associated with higher Cmax and lower Cmin *vs* PAC BID (Table). Median PAC plasma concentrations during week 1 were 47% higher with QD *vs* BID dosing. At steady-state, median Cmin ($Cmin_{ss}$) at weeks 12 and 24 were higher with BID *vs* QD dosing by 10% and 15%, respectively. Also, median observed steady-state 4-h concentration at week 3 (coincides with $Cmax_{ss}$) was 12% higher with QD *vs* BID dosing. In an exposure-response analysis, with QD or BID dosing, no trends were detected for a relationship between observed $Cmin_{ss}$ and death, cardiac death, hemorrhagic death, hemorrhagic events, thrombocytopenia (grade ≥2 or ≥3), anemia (grade ≥2 or ≥3), or gastrointestinal events (any grade, grade ≥2, or ≥3). Eleven (15%) and 13 (17%) PAC QD pts achieved SVR ≥35% and TSS reduction ≥50% at week 24, respectively, *vs* 16 (22%) and 24 (32%) PAC BID pts. Treatment with PAC BID but not QD showed a trend of increased SVR *vs* $Cmin_{ss}$.

Table 1.

Table. PAC Exposure

	PAC Regimen	n	Geometric Mean (ng/mL)	Ratio	90% CI
$Cmin_{ss}$	200 mg BID	83	6783	1.12	0.95-1.31
	400 mg QD	76	6076		
$Cmax_{ss}$	200 mg BID	94	8290	0.93	0.77-1.12
	400 mg QD	86	8930		

Summary/Conclusions: As predicted by PK modeling and simulations analyses, PAC 400mg QD was associated with higher Cmax and lower Cmin *vs* PAC 200mg BID in pts with MF from the PERSIST-2 trial. These differences appear to translate into an improved benefit/risk profile of PAC BID *vs* QD regimens.

E1344

ZMYM2-FLT3 IS A RARE, RECURRENT, CYTOGENTICALLY CRYPTIC FUSION IN MYELOID/LYMPHOID NEOPLASMS WITH EOSINOPHILIA THAT IS RESPONSIVE TO FLT3 INHIBITION

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Background: Myeloid/lymphoid neoplasms with eosinophilia are characterised by diverse tyrosine kinase (TK) fusion genes, many of which can be effectively targeted by small molecule inhibitors. More than 70 TK fusions have been described, most of which are associated with visible cytogenetic abnormalities. However these fusions are rare, and the pathogenesis of the great majority of cases presenting as myeloproliferative neoplasm with eosinophilia (MPN-eo) remains unexplained. We hypothesized that some MPN-eo cases may be driven by hitherto undetected cryptic TK fusion genes.
Aims: To screen cases with MPN-eo for TK fusion genes and evaluate the significance of any novel fusions
Methods: PolyA RNA extraction from MPN-eo cases, RNA-Seq library preparation and 100bp paired-end sequencing was performed with multiplexing for a minimum of 75 million reads/sample using an Illumina HiSeq 2000. Bowtie, TopHat and TopHat-Fusion were used to align reads, resolve splice junctions, identify and filter potential TK fusions. Confirmation and screening of fusions was performed by RT-PCR and Sanger sequencing.
Results: Of 20 cases tested by RNAseq analysis, just one cryptic TK fusion was identified: *ZMYM2-FLT3*, predicted to arise as a consequence of an 8Mb inversion at 13q12. Unusually, both breakpoints fell within exons (*ZMYM2* exon 20 and *FLT3* exon 14, respectively) resulting in an in frame fusion. To test if this fusion might be recurrent, we analysed 105 additional cases by RT-PCR. One additional positive case was detected, with similar but not identical breakpoints to the initial case. Case 1, a 48 year old female, presented with leukocytosis ($30x10^9$/L), eosinophilia ($2x10^9$/L, elevated serum tryptase (37μg/l), splenomegaly and a hypercellular bone marrow (BM). Cytogenetics was normal and *FIP1L1-PDGFRA*, *KIT* D816V and *JAK2* V617F were all negative and no pathogenetically relevant mutations were identified by a myeloid NGS panel (28 genes). After 10 months, she progressed to myeloid blast phase. Because the disease was resistant to AML-induction chemotherapy (FLAG-Ida), an allogeneic PBSCT from an unrelated donor was performed 13 months after diagnosis. As a consequence of chronic GvHD and septic shock, the patient died 6 months after allogeneic PBSCT. The *ZMYM2-FLT3* fusion gene was identified post mortem. Case 2, a 47 year old male, presented with eosinophilia ($4.7x10^9$/L, 47%) elevated serum tryptase (42μg/l) and a hypercellular BM Cytogenetics was normal and *FIP1L1-PDGFRA*, *KIT* D816V and *JAK2* V617F were all negative. There was no response on steroids or hydroxyurea. Following the finding of *ZMYM2-FLT3* positivity, treatment with sunitinib was commenced. Blood counts started to improve from day 4 and normalized after 3 weeks. During a pause of 3 weeks due to pulmonary infection, leukocytes/eosinophils rapidly increased, but normalized again within weeks after restart of sunitinib. The patient has been maintained on sunitinib for 10 months (since re-start) and remains in complete hematologic remission.
Summary/Conclusions: *ZMYM2* is the fourth gene reported to fuse to *FLT3* in myeloid neoplasms but the first *FLT3* fusion that is cytogenetically cryptic. Patients with *ZMYM2-FLT3* may be amenable to treatment with FLT3 inhibitors and thus, although very rare, this fusion should be considered in the work up of MPN-eo cases. Due to their extensive diversity, we anticipate that RNAseq will become the method of choice to detect rare TK fusions.

E1345

COMPLETE HEMATOLOGIC AND CYTOGENETIC RESPONSE IN A PATIENT WITH FIBROBLAST GROWTH FACTOR RECEPTOR 1 ACTIVATED MYELOPROLIFERATIVE NEOPLASM RECEIVING INCB054828

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Background: Fibroblast Growth Factor Receptor (FGFR) inhibitors have demonstrated efficacy in solid tumors with FGFR pathway activation. INCB054828, a novel, highly selective FGFR1, FGFR2, and FGFR3 inhibitor, is being assessed for the treatment of several advanced malignancies (AACR 2015; Abstract 771). 8p11 myeloproliferative syndrome is an aggressive myeloproliferative neoplasm **(**MPN) associated with *FGFR1* translocation on chromosome 8p11.
Aims: To describe the characteristics of a patient with FGFR1 activated MPN who achieved a complete hematologic and cytogenetic response with INCB054828 in an ongoing phase 1/2 trial (NCT02393248)
Methods: In this 3-part, phase 1/2 dose-escalation and expansion trial, eligible adults had any advanced solid tumor (parts 1 and 3) or malignancy with FGF/FGFR alteration (part 2), had Eastern Cooperative Oncology Group performance status score ≤1 (part 1) or ≤2 (parts 2 and 3), and were refractory to prior therapy with no known effective standard therapy available to them. Patients received INCB054828 orally on a 21-day cycle (2-weeks on/1-week off) starting at 9mg QD and increasing to 13.5mg QD.
Results: This 51-year-old male patient with 8p11 translocated MPN diagnosis (currently the only patient with MPN enrolled in this trial), presented with abnormal white blood cell (WBC) count (eosinophils, 15%; peripheral blood [PB] blasts, 4%) and abnormal platelet count (68×10^9/L). The patient had prior therapy with hydroxyurea. Bone marrow (BM) biopsy at study entry showed 95% cellularity, 4% BM blasts, decreased megakaryocytes, t(8,9)(11.2,q33) in 19 of 20 metaphases, and European Myelofibrosis Network grade MF-1. After 6 weeks of treatment with INCB054828 at a dose of 9mg QD in part 2 of the study, WBC count normalized with disappearance of eosinophilia and PB blasts. BM biopsy demonstrated a normalization of bone marrow differential with 50% cellularity, 1% BM blasts, adequate trilineage hematopoiesis, MF-1 fibrosis, and a **complete cytogenetic response**. After 4 months of treatment the patient was hospitalized for pneumonia and study treatment was held. The patient progressed to AML shortly after therapy interruption, with BM blasts increasing to 83% and evidence of clonal evolution (47,XY: +8 t(8,9) (11.2;q33) [3]/48 idem, +19 [17]). The patient was taken off study at this time (end of cycle 6) and subsequently achieved a complete remission on intensive chemotherapy with fludarabine, cytarabine, idarubicin, and allogeneic BM transplantation. The patient is currently alive and in complete remission.
Summary/Conclusions: INCB054828 showed efficacy in this patient with FGFR1 activated MPN using a 21-day (2-weeks on/1-week off) regimen. Continuous treatment may be necessary to sustain response and avoid rebound as has been seen with other kinase inhibitor therapies. A phase 2 trial has been initiated to evaluate INCB054828 in patients with myeloid/lymphoid neoplasms with FGFR1 rearrangement (NCT03011372).

E1346

THE GRADE OF STROMAL CHANGES IMPACTS ON PROGNOSIS IN PATIENTS WITH PRIMARY MYELOFIBROSIS

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Background: Recently, a detailed grading system for the assessment of bone marrow stromal changes has been proposed in primary myelofibrosis, proved to be reproducible and adopted by the updated WHO 2016 classification.
Aims: In this study, we aim to evaluate any possible prognostic implications of this grading system in a series of patients with primary myelofibrosis.
Methods: The study involved 122 consecutive patients with primary myelofibrosis diagnosed between 1998 and 2015 at the Oncohematology Division of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico of Milan, for which an adequate bone marrow trephine biopsy (more than 1 cm in length) performed at the time of first observation was available, together with complete clinical, laboratory and follow-up data.
Results: Reticulin myelofibrosis (MF), collagen deposition (Co) and osteosclerosis (Ost) were evaluated and graded from 0 to 3 in the bone marrow trephine biopsies for each patient at diagnosis. In detail, the stromal changes were graded as follows: bone marrow fibrosis: MF-0 in 9 cases, MF-1 in 60, MF-2 in 31 and MF-3 in 22; collagen deposition: Co-0 in 64 cases, Co-1 in 23, Co-2 in 21 and Co-3 in 14; osteosclerosis: Ost-0 in 72 cases, Ost-1 in 24, Ost-2 in 19 and Ost-3 in 7. Patients' population was composed of 56 males and 66 females (M/F=1/1,2) with a median age at diagnosis of 68 years (range 30–85). Clinically, at presentation, anemia with hemoglobin values less than 10 g/dL was present in 20 (16%) patients, leukocytosis more than 25 $x10^9$/L was identifiable in 4 (3%) patients, and platelets count less than 100 $x10^9$/L in 7 (6%) cases. *JAK2*V617F mutation was detected in 81 cases (66%). Among the remaining 41 *JAK2*-negative patients, 4 and 27 carried *MPL* and *CALR* mutations, respectively; 10 out of 122 resulted "triple-negative". According to the International Prognostic Scoring System, 38 cases were stratified as low risk, 51 as intermediate-1 risk, 21 as intermediate-2 risk, and the remaining 12 as high risk. By the time of the analysis, 21 (17%) patients had died: leukemic evolution occurred in 14 (11.5%) patients, whereas thrombotic or hemorrhagic events occurred in 25 (20.5%). Subsequently, a comprehensive grade of bone marrow stromal changes ranging from 0 to 9 allows us to distinguish 88 (72%) cases with low-grade stromal changes (total score: 0-4) and 34 (28%) with high-grade stromal changes (total score: 5-9). Clinically, patients with high-grade stromal changes presented more frequently with anemia, thrombocytopenia, leukocytosis, peripheral blood blasts and increased lactate dehydrogenase levels. The grade of bone marrow stromal changes resulted strictly associated with the International Prognostic Scoring System and the overall mortality (low-grade: 10 dead out of 88 *vs* high-grade: 11 dead patients out of 34; p=0.013). Finally, the grade of bone marrow stromal changes was effective in discriminating the overall survival of the patients with low-grade and high-grade stromal changes (Log-Rank test: p=0.0002).
Summary/Conclusions: A detail evaluation of the bone marrow stromal changes has important prognostic implications and can be used at diagnosis in the clinical stratification of the patients affected by primary myelofibrosis. Further studies are needed to test if the prognostic significance of this grading system remains during the follow-up.

E1347

INCREASED RISK OF INFLAMMATORY BOWEL DISEASE IN PATIENTS WITH PHILADELPHIA NEGATIVE CHRONIC MYELOPROLIFERATIVE NEOPLASMS

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Background: Studies reveal that patients with inflammatory bowel disease (IBD) may have increased risk of haematological cancers. Moreover, Philadelphia negative chronic myeloproliferative neoplasms (MPNs) have previously been associated with autoimmune diseases, including IBD. Nevertheless, to our knowledge, the risk of IBD has not been investigated in patients with MPN.
Aims: We undertook a nationwide population-based matched cohort study, and estimated the risk of IBD in patients with MPN.
Methods: We used valid Danish national registries, covering more than 5 million individuals, and included all patients diagnosed with either essential thrombocythemia (ET), polycythaemia vera (PV), myelofibrosis (MF), or unclassifiable myeloproliferative neoplasm (MPN-U) between 1994 and 2013. For each patient, 10 individually age- and sex-matched comparisons were included. Patients and comparisons were followed until first occurrence of any IBD diagnosis (ulcerative colitis or Crohn's disease), death, emigration or end of 2013. Patients and comparisons with prior IBD were excluded from the analysis. Hazard ratios (HRs) between MPN patients and comparisons were estimated using cox regression models, and used as measure of the relative risk. The risk was only calculated if five or more individuals were diagnosed with IBD.
Results: Of the 8,210 MPN patients, 80 individuals were diagnosed with IBD during the study period; including 37 ET patients, 28 PV patients, 1 MF patient and 14 MPN-U patients. During a total risk time of 45,241 years, the rate of IBD per 1000 person years at risk was 1.8 (95% confidence interval [95% CI]:1.4-2.2) for the MPN patients. The corresponding rate for the 81,326 comparisons was 0.8 (95% CI: 0.7-0.8).The 10-year risks of IBD for MPN patients and comparisons were 0.8% (95% CI: 0.6-1.0) and 0.4% (95% CI: 0.4-0.5), respectively. The overall HR of IBD was 2.4 (95% CI: 2.1-2.9) for MPN patients, with HRs of 2.6 (95% CI: 2.1-3.2) for ulcerative colitis and 2.4 (95% CI: 1.7-3.4) for Crohn's disease. The risk of IBD was increased 2 to 3 fold among ET, PV and MPN-U patients, with HRs of 2.8 (95% CI: 2.1-3.7) for ET patients, 2.1 (95% CI: 1.6-2.7) for PV patients and 2.2 (95% CI: 1.3-3.7) for MPN-U patients.
Summary/Conclusions: Patients with MPN are at increased risk of IBD compared to the general population. The absolute risks of IBD are low, but abdominal discomfort may in few patients be caused by underlying IBD.

E1348

ESSENTIAL THROMBOCYTHEMIA WITH AQUAGENIC PRURITUS: AN ENTITY WITH MORE AGRESSIVE CLINICAL AND BIOLOGICAL PROFILE AT THE DIAGNOSIS AND A HIGH MORBIDITY DURING THE FOLLOW-UP

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Background: Polycythemia vera (PV) and essential thrombocythemia (ET) are Phi-negative myeloproliferative neoplasms in which arterial or venous thromboses and phenotypical evolutions (leukemia, myelofibrosis) are the most recurrent complications. Aquagenic pruritus (AP), induced by water contact, is a typical symptom of PV. However, we showed recently that ET patients also suffered from AP with clinical characteristics quite different from those observed in PV patients. In 2008, the presence of AP was associated with a lower risk of arterial thrombosis in PV patients.
Aims: It seemed particularly interesting to analyse the clinical relevance and the prevalence of the presence of AP in ET patients for such a risk.
Methods: In this study, we used the OBENE observatory (Observatoire Brestois des NEoplasies myéloprolifératives), a register of MPN patients followed in our University Hospital in which biological and clinical data of 396 ET patients have been collected. This register was approved by a local ethical committee and registered in clinicaltrials.gov (NCT02897297). To avoid masked polycythemia Vera diagnostics, all JAK2 positive cases were tested for isotopic red mass cells if appropriate.
Results: Among our 396 ET patients, 42 (10,6%) suffered from AP. Interestingly, the median age at diagnosis of these patients was lower (51.6 *vs* 63.8%, p<0.0001). Furthermore, they presented more symptoms as erythrocytosis, hyperviscosity, constitutional symptoms and splenomegaly (p<0.01). ET patients with AP were more proliferative (more polycythemic but less thrombocythemic, p<0.04 each) and were more difficult to treat (2.2 *vs* 1.1 treatment lines, p=0.005). Concerning the occurrence of thrombotic events (arterial or venous) at diagnosis, no significant difference between patients with or without AP was found. In contrast, the presence of AP induced an increase of thrombotic events during the follow-up (30.9 *vs* 17.2%, p=0.03). But surprisingly, these events appeared in the delayed timing. The arterial/venous rate of thrombotic events was also different with 50/50 *vs* 2/3:1/3. Furthermore, we observed that about one-third of the patients with AP had phenotypic evolutions against 13.3% in the other group (p=0.0007); the most frequent evolutions were PV and secondary myelofibrosis (16.7 *vs* 5.4%, p=0.005 and 19 *vs* 4.8%, p=0.0003, respectively). Concerning the overall survival of the patients, we have noter that there was less death in the group with AP than without AP (11.9 *vs* 32.5%, p=0.006) in spite of a longest follow-up (12.1 *vs* 7.7 years, p=0.002).
Summary/Conclusions: AP is classically associated to PV. But we confirmed here that AP is also present in ET. Furthermore, ET patients suffering from AP were more proliferative, more symptomatic at diagnosis but had also higher risk of thromboses and phenotypic evolutions than ET without AP. Despite that these patients have a higher overall survival. So, the presence of AP in patients with ET characterizes patients with high risk of morbidity (thromboses, phenotypic evolutions).So as in PV, the presence of AP in ET patients at the time of diagnosis should be systematically identified.

E1349

ANAGRELIDE RESPONSE ACCORDING TO THE MOLECULAR PROFILE: SOMETHING TO CONCLUDE ON THE MECHANISM OF ACTION OF THE DRUG IN MYELOPROLIFERATIVE NEOPLASMS (MPN)?

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Background: Anagrelide is a useful drug in the control of thrombocytosis in MPN. Although it is known that in therapeutic levels it primarily influences in the post-mitotic phase of megakaryocytic development interfering with its complete maturation, its mechanism of action is not well known.
Aims: The progress in the diagnosis of MPN due to the discovery of driver mutations (JAK2, calreticulin and MPL) leads us in the present study to correlate them with the response to anagrelide in a group of patients treated with this drug, investigating the possible interference in the referred biological pathways.
Methods: a total of 56 patients with MPN diagnosed in our centre between 1993 and 2015 were studied. The median age was 49 years, with 19 patients older than 60 years. 83% were female and 17% were male. The diagnosis was initially carried out based on the WHO criteria 2008 and subsequently reviewed the medical records with the new criteria of 2016. A molecular study on peripheral blood samples was carried out using quantitative allele-specific PCR techniques for JAK2, qualitative for MPL (L515V mutation) and Sanger sequencing of exon 9 for calreticulin. Type 1 mutation was considered at 52 bp deletion and type 2 at 5 bp insertion. In all patients, the goal of anagrelide therapy was to control thrombocytosis (platelet count below 600x10e9 /L), with dosage within the range of efficacy and safety recommended in the datasheet. The results were analysed with the statistical software SPSS *vs* 15.0
Results: 80.5% of the patients were diagnosed with ET, 12.5% of PV, 3.5% of myelofibrosis and 3.5% of unclassifiable MPS. 59% of the patients had a V617F JAK2 mutation, with allelic load higher than 20% in 47.5% of the cases. 28.5% presented mutation in calreticulin; of which 50% were type 1 and 50% type 2. Only one patient had a mutation in MPL (2%), the remaining 6% being classified as “triple negative”. The median daily dose of anagrelide received was 1.5mg. 17.5% of the patients required more than 2mg for an adequate control, half of them being positive for mutations in calreticulin and the other 50% of the mutation V617F JAK2 with allelic load higher than 20%. 26% of the patients received daily dose of 1mg, being 70% positive for the mutation V617FJAK2 with allelic load lower than 20%, although there were no statistically significant differences between the groups according to the mutational profile. 16% of patients discontinued treatment due to toxicity, with the most common adverse effects being mild (headache and palpitations).
Summary/Conclusions: Patients requiring higher doses of anagrelide present mutations in calreticulin or JAK2 V617F allelic load higher than 20% and patients with lower allelic load having greater sensitivity to the drug, with no statistically significant differences. It is possible that the first situation is associated with a greater pre-mitotic deregulation in the megakaryocyte where the drug does not interfere whereas the second one could be related to anagrelide interference through the JAK2 pathway in post mitotic maturation although larger exploratory studies are required.

E1350

THE DELAYED DIAGNOSIS OF PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS (MPN) IS COMMON AND RESULTS IN A HIGH INCIDENCE OF POTENTIALLY PREVENTABLE THROMBOTIC COMPLICATIONS

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Background: Ph-negative MPNs are a heterogenous group of stem cell derived, clonal bone marrow disorders characterised by increased production of mature blood cells. Patients with MPNs are at significantly increased risk of thrombotic and haemorrhagic complications which are a major cause of morbidity and mortality. The early diagnosis and treatment of MPN may reduce the incidence of thrombotic complications and the associated morbidity and mortality.
Aims: We performed a study to determine if the delayed-diagnosis of MPN was common and the implications of any such delay.
Methods: The medical records of patients treated at our centre with a new diagnosis of MPN between January 2010 and June 2016 were audited. We determined the duration from first appearance of a full blood count (FBC) abnormality consistent with the diagnosis of MPN until the time of formal diagnosis. The occurrence of any thrombotic or haemorrhagic complications during this time was recorded.
Results: 143 patients were diagnosed with MPN; 35 with polycythemia vera, 70 with essential thrombocythemia, 25 with primary myelofibrosis and 13 with MPN-unclassifiable. Patients with PV had a median diagnosis delay of 456 days (range 0-2650 days) and 26% had potentially preventable events. Patients with ET had median diagnosis delay of 823 days (range 0-8731 days) and 23% had potentially preventable thrombotic events including 2 patients with multiple events. Patients with PMF had a median diagnosis delay of 196 days (range 0-3684 days) and 12% had potentially preventable thrombotic events. In MPN-U the median diagnosis delay was 1371 days (range 42-3255) and 31% of patients had potentially preventable adverse events.
Summary/Conclusions: Over 5.5 years we identified 143 patients with a new diagnosis of Ph-negative MPN within our centre. The overall median diagnosis delay was 723 days (0-8731) with delays of more than 12 months in ET, PV and MPN-U, and more than 6 months in PMF. 21% of patients had potentially preventable thrombotic events and 2.8% had potentially preventable haemorrhagic events. Earlier recognition of FBC abnormalities consistent with MPN should result in earlier referral for specialist haematological management which, with earlier intervention, would be expected to prevent many thrombo-haemorrhagic complications and reduce MPN-associated morbidity and mortality.

E1351

LONG-TERM AND LOW-DOSE BUSULFAN IS SAFE AND EFFECTIVE IN ELDERLY PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

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Background: Therapeutic options for elderly patients (pts) with Essential Thrombocythemia (ET) resistant or intolerant to hydroxyurea (HU) are limited. Busulfan (BU) is a possible second-line treatment, but conventional schedule

(starting dose of 14mg/week up to obtain the complete hematological response CHR) is associated with high risk of leukemic transformation and second malignancies.
Aims: We analysed efficacy, toxicity, risk of Myelofibrosis (MF) and leukemic evolution in 31 of 352 ET pts collected in our database, treated with an alternative long-term schedule of BU, defined by low-starting dose (4-6mg/week) up to CHR (evaluated according to ELN response criteria), followed by dose de-escalation overtime.
Methods: Non parametric tests, such as Mann-Whitney, Pearson Chi-square and Fischer's exact tests, were used for statistical analysis of continuous and categorical variables. Survival curves were calculated by Kaplan-Meier method and compared with Log-rank (Mantel-Cox) test.
Results: 27/31 pts were evaluable for analysis (8 male, 19 female). Median age at diagnosis and at BU start were 71,3 and 79 years (yrs) respectively. We found these driver mutations: JAK2V617F in 15 pts (55.6%), Calreticulin in 8 pts (29,6%) and MPL in 1 patient (3,7%); 3 pts (11,1%) were triple negative. IPSET score at diagnosis was low-intermediate in 17 (63%) and high in 10 (37%) pts. 26 pts started BU as 2nd line treatment: 11 (42,3%) were intolerant and 15 (57,7%) were resistant to HU respectively. Only one received BU as 1st line treatment. They received BU for a median time of 47,67 months (range: 1,48 – 94,42). The median cumulative BU dose was 453mg (range: 32-1032). 25/27 pts (92,6%) obtained CHR, after a median time of 191 days. 6 pts (22,2%) presented hematological (5) and extra-hematological (1, cutaneous) side effects. Overall, 12 pts (44,4%) stopped BU: 4 for hematological toxicity, 4 for disease progression, 2 for drug intolerance/resistance; the remaining 2 not for drug-related side effects. After a median follow-up of 9,74 yrs (range: 1,82-27,05), 9 (33,3%) and 2 (7,4%) pts presented MF evolution and leukemic transformation respectively. The MF-free-survival (MFS) was 48,8% at 15 yrs and appears to be significantly lower than the entire series of ET pts (77,4% at 15 yrs; p=0,002; figure 1). Median MFS was 12,7 yrs for pts treated with BU, whereas it was not reached at 15 yrs in the entire series of ET. There were no statistically significant differences in principal hematological and clinical features between "evolving-MF pts" and "not evolving-MF pts", apart from lower hemoglobin value at BU start (11,5 *vs* 13,05 g/dl; p=0,05) and lower time of exposition to BU in MF subgroup (16 *vs* 53,7 months; p=0,026). Drug cumulative dose was the same in the two subgroups. Thrombotic complication after BU start were observed in 3 pts (11,1%). During time of analysis 5 pts (18,5%) died.

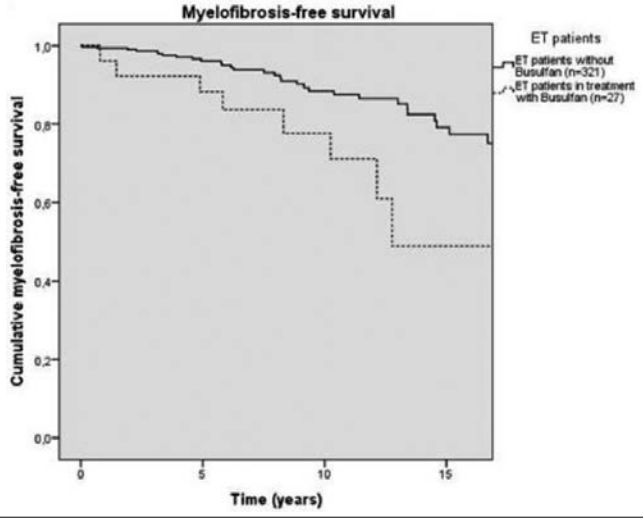

Figure 1.

Summary/Conclusions: Our experience with an alternative long-term and low-dose BU administration is safe and effective in elderly patients with ET. 92.6% of them obtained CHR, with acceptable hematological and extra-hematological toxicity. We noticed a high rate of MF evolution with respect to global ET population, while the risk of leukemic transformation seems to be limited, considering that these pts were elderly and previously treated. Predictive factors for MF evolution should be analysed and confirmed in larger series.

E1352

DIFFERENCES IN JAK2V617F POSITIVE PATIENTS WITH AND WITHOUT THROMBOSIS ACCORDING TO DIAGNOSIS, AGE, SEX AND V617F ALLELE BURDEN

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Background: Thrombosis is one of the most frequent events in Ph(-) myeloproliferative neoplasms and the reasons for that are still under investigation.

Aims: The aim of this study was to find out if there is difference in frequency and type of thrombosis in JAK2 V617F positive patients according to their diagnosis, age, sex and V617F allele burden.
Methods: One hundred and eighty two JAK2 V617F positive patients diagnosed with polycythemia vera (PV) N=63, essential thrombocythemia (ET) N=83, and primary myelofibrosis (PMF) N=36 were included in the study. Patients in each group were additionally divided according to sex, age at diagnosis and first thrombosis. V617F allele burden was quantified in peripheral blood granulocyte DNA by real time PCR established by Larsen *et al.* Br J Haematol 2007;136:745.
Results: Among 182 patients observed, 66 (36%) experienced thrombosis, with arterial thrombosis being twice more frequent than venous thrombosis in all 3 studied groups. In ET group there was statistically significant difference in sex distribution (proportion of females=0.71), p<0.001. Statistically significant difference in age at diagnosis was observed between ET and PV/PMF patients without thrombosis (p<0.001); the youngest patients were those in ET group. The age at diagnosis of ET patients with thrombosis (65 years, range 23-92) was statistically different compared to ET patients without thrombosis (50 years, range 21-83), p=0.002. Our study showed that V617F allele burden in patients without thrombosis was statistically significantly different between ET (17.2%, range 4.2-55.2) compared to PV (43%, range 1.7-99.9) and PMF (37.1%, range 1.4-99.7), p<0.001. The same statistically significant difference for V617 allele burden was established in patients with thrombosis between ET patients (19%, range 1.4-84.5) and PV and PMF patients (42.5%, range 8.9-97.2 and 48.8%, range 1.6-99.8, respectively), p<0.001.
Summary/Conclusions: Our results confirm that arterial thrombosis is more frequent than venous thrombosis in JAK2 V617F positive patients. Female sex was prevalent only in ET group. The age at diagnosis in all studied groups was similar except for ET patients without thrombosis. There was no difference in the frequency and type of thrombosis among ET, PV and PMF patients with high heterogeneity in V617F allele burden between all studied groups regardless of the occurrence of thrombosis.

Non-Hodgkin & Hodgkin lymphoma - Biology

E1353

PROTECTION AGAINST DEVELOPMENT OF B CELL LYMPHOMA BY TETRASPANIN CD37

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Background: B cell non-Hodgkin lymphoma, worldwide the most common hematological malignancy, remains a clinical problem. The molecular events leading to B cell lymphoma are only partially defined. CD37 is a member of the tetraspanin superfamily that is highly expressed on mature B cells and is required for optimal GC function and long-lived antibody production.
Aims: We investigated the function of tetraspanin CD37 in the development of B cell lymphoma.
Methods: A combination of studies was performed in mouse models (CD37/IL-6-deficient mice), and studies of DLBCL patient material using biochemical, immunological, genetic and microscopical approaches.
Results: We provide evidence that deficiency of CD37 induces the development of B cell lymphoma in vivo. *Cd37*-deficient mice develop germinal center-derived B cell lymphoma in lymph nodes and spleen with higher incidence than *Bcl2*-transgenic mice. We discovered that CD37 interacts with SOCS3, and when absent drives tumor development through constitutive activation of the IL-6 signaling pathway. The importance of the IL-6 pathway was confirmed by investigating *Cd37*x*Il6* double knock-out strains that were fully protected against lymphoma development. Our unpublished data shows discovery of inactivating *CD37* mutations in patients with DLBCL. Importantly, loss of CD37 on neoplastic cells in patients with diffuse large B cell lymphoma (DLBCL) is directly correlated with activation of the IL-6 signaling pathway and with worse progression-free and overall survival.

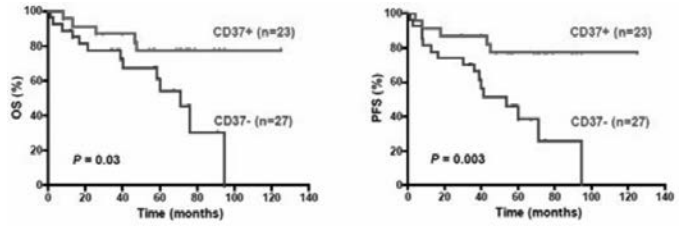

Figure 1.

Summary/Conclusions: Together, this study identifies tetraspanin CD37 as a novel tumor suppressor that directly protects against B cell lymphomagenesis, and provides a strong rationale for blocking the IL-6 pathway in patients with CD37-negative B cell malignancies as therapeutic intervention.

E1354

CONCOMITANT DUAL ABLATION OF BLIMP1 AND P53 IN B-CELLS AS A NOVEL IN VIVO MODEL FOR HIGH-GRADE B-CELL LYMPHOMA

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Background: B-Lymphocyte-Induced Maturation Protein-1 (BLIMP-1)- and p53-inactivation contributes to the pathogenesis of a wide spectrum of malignancies, including diffuse large B-cell lymphomas. Nevertheless, there is lack of *in vivo* models that may be used for a better understanding of the biology and genomics of high-grade B-cell lymphomas characterized by dual loss of both BLIMP-1 and p53.
Aims: 1) To develop and characterize a transgenic mouse model of BLIMP-1/p53 dual loss in B cells; 2) To provide an *in vivo* model that mirrors human ABC-DLBCL phenotype.
Methods: Cre recombinase under the control of CD19 promoter (C57BL/6 $CD19^{Cre/Cre}$) mice were crossed with either C57BL/6 $BLIMP^{flox/flox}$or C57BL/6 $p53^{flox/flox}$mice to achieve deletion of BLIMP or p53, respectively, in B cells. Secondly, CD19Cre/Cre $BLIMP^{flox/flox}$mice were crossed with $CD19^{Cre/Cre}p53^{flox/flox}$to achieve dual deletion of BLIMP and p53 in B cells ($CD19^{Cre/Cre}BLIMP^{flox/flox}p53^{flox/flox}$: referred as CD19/Bl-/p53-). Transgenic experimental mice (CD19/Bl-/p53-) where characterized for clonal B cell infiltration using immunohistochemistry, flow cytometry, Southern Blotting, whole exome sequencing. MTT assay was used to test BTK-inhibitor-dependent cytotoxicity using CD19/Bl-/p53-derived B220 cells.
Results: CD19/Bl-/p53- mice presented with diffuse lymphadenomegalies, splenomegaly, hepatomegaly (100%, 90.3% and 77.4%, respectively). Other clinical manifestations included presence of ascites and hind lymb paralysis (12.9% and 19.3%, respectively). The CD19/Bl-/p53- showed worse survival compared to Bl-/p53- mice non-expressing the CD19/Cre recombinase, CD19/p53-, or CD19/Bl- (363, 469.5, 460.5, and 770 days, respectively). H.E. staining of CD19/Bl-/p53—derived lymph nodes, defined a nodal architecture with a monomorphic population of large sized atypical lymphoid cells, multiple basophilic medium sized, paracentrally situated nucleoli. A "starry sky" pattern was also observed. Features were compatible with a high-grade lymphomas. IHC analysis confirmed positivity for B220 staining (TdT, Bcl6, CD138 and CD4, CD8 negative). Tumors were confirmed to be B220+/IgM+, with either Igk- or Ig-lambda-restriction as demonstrated by flow cytometry; and either mono- or bi-clonal, as demonstrated by Southern blotting. Whole exome sequencing was performed from B220+ selected cells obtained from pathological lymph nodes of CD19/Bl-/p53- mice and identified 143 SNVs. Non-synonymous somatic mutations were mapped on genes involved in the regulation of focal adhesion, PDGF signaling, p53-downstream pathway, and lipoprotein metabolism. B220+ cells selected from CD19/Bl-/p53—derived lymph nodes were implanted s.q. into recipient SCID/Bg mice, and presented with 100% engraftment, with a monomorphic lymphoid infiltration of B220+ and IgM+ cells. B220 positive cells were selected from the s.q. tumor and intravenous injected into recipient SCID/Bg (n: 10) and BL/6 mice (n: 10). Engraftment was demonstrated in all the mice, where hepatomegaly, splenomegaly and hind limb paralysis were observed. Infiltration of B220+ cells was documented within bone marrow, liver and spleen. Finally, we found that B220+ cells selected from lymph nodes harvested from CD19/Bl-/p53-mice were sensitive to ibrutinib.
Summary/Conclusions: Dual inactivation of p53 and BLIMP in B-cells supports a novel *in vivo* model that recapitulates what seen in patients with ABC-DLBCL, thus providing a novel model for studying high-grade B-cell lymphoma driven by BLIMP-1/p53 dual loss-induced c-Myc expression.

E1355

IDENTIFICATION AND CHARACTERISATION OF THE LYMPHOMA INITIATING CELL (LIC) POPULATION IN AN ALCL MOUSE MODEL

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Background: In 60% of anaplastic large cell lymphoma (ALCL) patients a translocation t(2;5) (p23;q35) is found, which results in NPM-ALK fusion gene expression and constitutive activation of the ALK tyrosine kinase. Immunophenotypic characterization of human ALCLs revealed highly CD30-positive cells of T- or Null-cell-origin.
Aims: However, the origin of the lymphoma initiating cell population as well as NPM-ALK signal transduction in course of the disease remains unclear and needs to be characterized.
Methods: In this regard, we established a retroviral murine bone marrow transplantation model resembling human ALCL. Therefore we use an inducible Cre/loxP system where NPM-ALK expression is restricted to early T-cells. We infected bone marrow of Lck-Cre transgenic mice with our MSCV-Stop-NPM-ALK-IRES-EGFP vector and transplanted it into lethally irradiated recipient mice. With a latency of 4-5 months, mice developed CD30-positive lymphomas and died from neoplastic T-cell infiltration of lymphatic organs and bone marrow.
Results: Immunophenotypic analysis confirmed T-cell origin of the lymphomas with a heterogeneous compound of all T-cell stages with mainly $CD4^-/CD8^-$ double negative (DN) T-cells including all DN T-cell stages as well as hematopoietic stem cells and lymphatic precursors. Staining of the T-cell subpopulations demonstrated high NPM-ALK expression in immature $CD4^-/CD8^-$ double negative T-cells and undifferentiated $CD4^+/CD8^+$ double positive T-cells with highest expression of proliferation marker Ki67 as well as the activation marker CD30 in the $CD4^-/CD8^-$ double negative T-cells. Interestingly, the $CD4^-/CD8^-$ double negative lymphoma population further more aberrantly expressed the T-cell receptor alpha/beta chain, which may allow these early T-cells to establish a systemic lymphoma. To further proof this hypothesis and identify the LIC population we performed secondary transplantations with sorted DN and T-cell subpopulations. Indeed, only mice transplanted with DN3 and DN4 lymphoma cells could give rise to secondary lymphomas, whereas sorted DN1, DN2, $CD4^+$, $CD8^+$ or $CD4^+/CD8^+$ transplanted lymphoma cells failed to established serial lymphomas in recipient mice. Immunophenotypic analyses of secondary lymphomas caused by transplantation of the DN3 and DN4 lymphoma subpopulation demonstrated double positive $CD4^+/CD8^+$ cells as well as single positive $CD4^+$ and $CD8^+$ cells next to the DN3/DN4 population. However, we were not able to detect redifferentiation of the DN3/DN4 cells to more immature DN1/DN2 lymphoma cells. To substantiate our findings, we performed microar-

ray analyses. Indeed, heatmap analyses revealed wide pattern similarities in the DN3 with DN4 lymphoma subpopulation in contrast to the DN1 and DN2 lymphoma cells. Interestingly, DN3 and DN4 cells show different expression profiles of stemness genes resembling early progenitor cell distribution patterns.
Summary/Conclusions: In summary, our results highlight the existence of a lymphoma initiating stem-cell-like population originated within the DN3/DN4 lymphoma cell population in a highly relevant NPM-ALK positive CD30-expressing ALCL mouse model, thereby giving the opportunity to test the eradication of the LIC with established and new therapeutical approaches.

E1356

HSP110 SUSTAINS MYD88-DEPENDENT NFKB SIGNALING IN ACTIVATED B CELL DIFFUSE LARGE B CELL LYMPHOMA

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Background: Diffuse large B cell lymphoma (DLBCL) is an aggressive lymphoproliferative disorder of B lymphocytes accounting for 30% of adult Non Hodgkin Lymphoma (NHL). Among DLBCL, Activated B Cell – DLBCL (ABC-DLBCL) is the most aggressive form and has a poor prognosis. Heat-shock proteins (HSPs) are molecular chaperons highly expressed in cancer cells and implicated in resistance to radio- and chemotherapy. Therefore, HSPs are envisioned as therapeutic targets in many cancers. Among the different HSPs, HSP110 has been recently identified as a pro-survival factor in germinal center-derived DLBCL (GC-DLBCL), through stabilization of the GC-DLBCL oncogene Bcl-6.
Aims: Here, we have explored if HSP110 could also be involved in the survival of the most aggressive form of DLBCL
Methods: The study was performed with ABC-DLBCL patient samples and several cell lines. ShRNA specific for HSP110 was introduced through a lentiviral vector designed to infect highly efficiently non-permissive B cell lines.
Results: We observed a high HSP110 expression in all ABC-DLBCL patient samples, compared to normal reactive lymph nodes by using IHC staining of ABC-DLBCL tumor sections and transcriptional analysis of ABC-DLBCL patient tumors. Furthermore, shRNA silencing of HSP110 decreases the survival of several ABC-DLBCL cell lines, and downregulates the expression of pro-survival factors such as BcL2 and BcL-XL. SiRNA silencing of HSP110 abrogates NF-kB signaling, which is the major oncogenic pathway in ABC-DLBCL cell lines. In accord with these results, over-expression of HSP110 in DLBCL and non-DLBCL cell lines increases NF-kB signaling, indicating a tight interplay between HSP110 and the NF-kB pathway. Using immune-precipitation in DLBCL cell lines and Duolink™ assays, we identified an *in vitro* and *in cellulo* interaction between HSP110 and Myd88, a critical protein of the NF-kB pathway that bears an activated mutation in many ABC-DLBC patients and that is responsible for lymphoma aggressiveness. Finally, we demonstrate that HSP110 stabilizes the wild type as well as the mutated form of Myd88, therefore facilitating the chronic NF-kB pathway activation in those cells.
Summary/Conclusions: In conclusion, we identified HSP110 as a regulator of NF-kB signaling through MyD88 stabilization in ABC-DLBCL. This finding highlights HSP110 as a new potential therapeutic target in DLBCL and potentially in other hematological malignancies driven by mutated MyD88 as Waldenstrom macroglobulinemia.

E1357

STAT3 ACTIVATION MEDIATES CD8+/CD16+/CD56- T-LGLL NEUTROPENIA THROUGH FAS LIGAND SECRETION

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Background: T-cell large granular lymphocyte leukemia (T-LGLL) is a rare chronic lymphoproliferative disorder characterized by the clonal expansion of CD3+ Large Granular Lymphocytes (LGL). In addition to the most common CD8+ T-LGL leukemia, less frequent LGL proliferations with CD4+/CD8$^{-/+dim}$ phenotype (CD4+ T-LGL leukemia) exist, which are characterized by indolent clinical course. Somatic *STAT3* mutations determining constitutive activation have been recently reported in a proportion of approximately 40% of patients, with no clear correlation with the occurrence of neutropenia, whose pathogenesis is likely to be multifactorial, comprising both humoral (*i.e.* soluble FAS ligand secretion) and cell-mediated mechanisms.
Aims: The aim of this work was to evaluate whether 1) *STAT3* mutations might be associated with a distinctive LGL immunophenotype and/or indicative for symptomatic disease and 2) STAT3 activation is directly related to the development of neutropenia.
Methods: A cohort of 101 patients affected by T-LGLL according to WHO criteria were screened for *STAT3* mutation by Sanger sequencing and PCR ARMS assay. All the samples were analysed by flow for CD3, CD4, CD8, CD16, CD56 and CD57 antigen. STAT3 tyr 705 levels were studied by Western blot. FAS ligand mRNA levels were analysed by RT-PCR Assay.
Results: By flow we observed that 68 out of 101 patients (67.3%) were characterized by CD3+/CD8+/CD4- expression (CD8+ T-LGLL), while the remaining 33 patients (32.7%) were CD3+/CD4+/CD8+^{dim}/neg (CD4+ T-LGLL). All *STAT3* mutated (*n*=38) and almost all neutropenic (38 out of 39) patients belonged to CD8+ T-LGL leukemia (*n*=68), while among CD4+ T-LGL leukemia (*n*=33) no STAT3 mutated and only one neutropenic patient (1 out of 33, 3%) was found. Among CD8+ T-LGLL, immunophenotypic signature CD16+/CD56- was both associated to the presence of neutropenia and *STAT3* mutation (37 out of 41, 90.2%; c^2=49.5, p<0.0001 and 37 out of 41, 90.2%; c^2=49.5, p<0.0001 respectively). Furthermore, by western blot we showed that high STAT3 tyrosine phosphorylation observed in LGL obtained by CD8+ T-LGLL patients belonging to CD16+/CD56- subgroup was significantly higher as compared with other immunophenotypic groups. Provided this relationship between *STAT3* mutation/activation and neutropenia, by RT-PCR we analysed Fas ligand expression, showing higher transcription levels in CD16+/CD56- CD8+ T-LGLL patients as compared to the not neutropenic patients belonging to the other immunophenotypes, both CD8+ T-LGLL and CD4+ T-LGLL (7.66±0.87, 2.45±0.22 and 2.35±0.28 arbitrary units, respectively; p<0.01). To confirm this relationship, in patient's PMBCs treatment with *STAT3* inhibitor Stattic decreased both STAT3 phosphorylation and Fas ligand transcription as compared to the untreated conditions. In addition, IL-6 and IL-15 stimulation (which are known STAT3 activator) increased Fas ligand transcription levels (1.59- and 2.01-fold after IL-6 and IL-15, respectively) which is prevented by concomitant Stattic treatment.
Summary/Conclusions: Our results provide evidence that STAT3 mutation and activation is mostly restricted to neutropenic CD8+ T-LGLL patients equipped with the CD16+/CD56- signature. The relationship between STAT3 activation and neutropenia FAS ligand related further supports to approach STAT3 inhibition as therapeutic strategy in symptomatic CD8+/CD16+/CD56- LGLL patients, obtaining the dual results of inducing apoptosis in leukemic LGL together with inhibition to FAS ligand mediated neutropenia.

E1358

CYCLIN D2 OVEREXPRESSION RECAPITULATES MANTLE CELL LYMPHOMA IN MICE

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Background: Mantle cell lymphoma (MCL) is a highly aggressive subtype of B-cell lymphoma that is characterized by a poor response to current treatment regimens. Most MCLs carry a prototypical translocation, t(11;14), which juxtaposes the *CCND1* gene towards the immunoglobulin heavy chain *(IGH)* locus, resulting in cyclin D1 overexpression. Notably, a subset of MCL patients are cyclin D1 negative but instead overexpress cyclin D2 (encoded by *CCND2*) as a consequence of recurrent genomic rearrangements involving the *CCND2* locus.

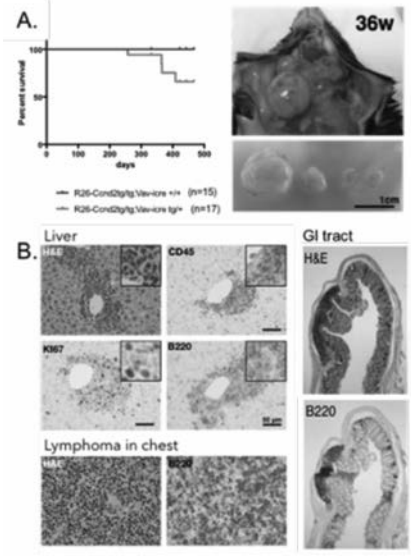

Figure 1.

Aims: Here, we aim to recapitulate MCL in a mouse model of hematopoietic-specific overexpression of cyclin D2. Next, we want to use this preclinical mouse model to evaluate novel therapeutic strategies for the treatment of MCL.
Methods: To evaluate if cyclin D2 could act as a bona fide oncogene in the pathogenesis of MCL, we developed a conditional R26-driven Ccnd2 overexpression mouse model. For this, we used a targeting vector that contained a floxed stop cassette followed by the *Ccnd2* gene and a EGFP/luciferase reporter, which was subsequently targeted in mESCs using recombinase-mediated cassette exchange (RMCE).
Results: The resulting R26-Ccnd2 mice were crossed to VaviCre mice to enable biallelic R26-driven overexpression of Cyclin D2 in the entire hematopoietic system. Interestingly, these mice developed large lymphomas starting from 36 weeks of age (Figure 1A), with tumor cells showing characteristic MCL immunophenotype (CD19+,CD5+,CD23-). Of note, these malignant B-cells were monomorphic small-sized cells with slightly irregular hyperchromatic nuclei and disseminated into other organs such liver, spleen and the gastrointestinal tract (Figure 1B). In addition, the infiltrating B-cell lymphoma cells were SOX11 positive, as evaluated by IHC, suggesting that these tumors indeed reflect a murine form of MCL. Noteworthy, the MCL cells from this mouse model also contain a luciferase reporter, allowing accurate *in vivo* tracing of tumor cells in xenograft experiments. These xenograft experiments can be used as preclinical models, in which bioluminescence is used to asses the tumor burden and to monitor tumor regression upon drug treatment.
Summary/Conclusions: In conclusion, our preliminary data suggest that modeling cyclin D2 in mice, mimicking the elevated cyclin D2 levels of human MCL patients with translocations involving the *CCND2* locus, is sufficient to form MCL.

E1359

HDAC6 INHIBITION INCREASES CD20 LEVEL BY STIMULATING TRANSLATION OF CD20 MRNA

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Background: HDAC6 (histone deacetylase, isoform 6) is a novel promising target in hematological malignancies. HDAC6 is an atypical member of HDAC family that regulates the acetylation status, and thus the functionality of cytosolic proteins, and has been explored therapeutically for its role in the process of protein degradation. HDAC6 mediates the transport of protein aggregates to the autophagic machinery to diminish their cytotoxicity. Thus, the disruption of the aggresome pathway, similarly to proteasome inhibition, results in a massive accumulation of misfolded protein aggregates and apoptotic cell death. As this strategy holds a considerable potential in aggressive B-cell tumors with a high rate of protein synthesis, HDAC6 inhibitors - are currently being tested in Phase I and II clinical trials in multiple myeloma and non-Hodgkin lymphoma. The results of preclinical studies show the increased efficacy of the combination of HDAC6 inhibitors with proteasome inhibitors in inducing stress-related cell death. The results of our studies show that HDAC6 inhibition in non-toxic concentrations significantly increases CD20 level on a protein level.
Aims: The aim of this study was to elucidate the mechanism of the regulation of CD20 expression by HDAC6.
Methods: We used qRT-PCR and Dual Luciferase Assays in order to determine the influence of HDAC6 on CD20 transcription. We used pulse-chase assays using widely used translation inhibitors – cycloheximide and homoharringtonine. In order to study the effect of HDAC6 inhibition on global as well as specific *de novo* synthesis of CD20 we optimized Click-IT chemistry methods. In order to study CD20 translation on polysomes we performed polysomes profiling followed with qRT-PCR. To get an insight into molecular mechanism of increased translation of CD20 after HDAC6 inhibition we studied the formation of stress granules (SG).
Results: We show that HDAC6 inhibition regulates CD20 level without affecting its transcription. Moreover, we demonstrate that HDAC6 inhibition increases translation of CD20 mRNA on polysomes without affecting general synthesis of novel proteins in the cell. However, our experiments suggest that the increased translation of CD20 mRNA is not a result of resumed translation of mRNA stalled in SG.
Summary/Conclusions: Our study shows a new mechanism of the regulation of CD20 expression by increasing its translation. Moreover, we demonstrate a new role of HDAC6 protein. This finding has a potential clinical application, as HDAC6 inhibitor are being widely tested in different hematological malignancies. Further studies in order to identify other targets for HDAC6 are required.

The work was supported by National Science Center 2013/09/N/NZ3/01407 (MB), 2015/16/T/NZ6/00034 (MB), 2014/13/N/NZ6/02081 (MS), 2015/18/E/NZ6/00702 (MW), 2013/11/B/NZ5/03240 (BP), Polish Ministry of Science and Higher Education grant IP2011 060271 (MW), DI2011 021241 (MB), DI 2014007344 (NM) and DI2013 006643 (AD), the Medical University of Warsaw grant 1M19/PM/112D/14/14 (MB) and 1M19/PM1/16 (AGJ) and Horizon 2020 Programme, project 692180-STREAM-H2020-TWINN-2015 (JG).

E1360

CARD11 DUPLICATION AT DIAGNOSIS IDENTIFIES VERY LOW-RISK MANTLE CELL LYMPHOMA PATIENTS:RESULTS OF THE LYMA-GENOMIC PROJECT CONDUCTED ON BEHALF OF THE LYSA GROUP

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Background: Mantle cell lymphoma (MCL) is an incurable heterogeneous disease with a median overall survival (OS) of around 4-6 years. There are 3 prognostic groups of patients: a high-risk (HR) group of 15-20% of patients having a duration of response <1yr after end of treatment (EOT); an intermediate-risk (IG) group that includes patients remaining in response one year after EOT but with an incidence of relapse of 10-15%/yr thereafter, other patients defining the low-risk (LR) group remain in response three years at least. The MIPI score (age, leukocytosis, PS, stage) helps to classify patients according to their risk of relapse but it is not currently possible to treat patients according to risk factors. Investigation of the MCL genomic landscape could help to understand MCL biology complexity and build biology-driven medical decisions.
Aims: In the present work, we report a whole-genome copy number analysis performed with OncoScan® FFPE Assay, a new robust and validated single nucleotide polymorphism (SNP) array (Foster *et al.* BMC Med Genomics 2015). We investigated the prognostic value of somatic recurrent copy number alterations (CNA) detected in 96 young MCL patients treated in the LyMa trial (Le Gouill *et al.* Abstract 145, ASH 2016).
Methods: Samples were selected according to material availability. Lymph node biopsies collected at diagnosis, formalin-fixed and paraffin-embedded were used to extract DNA, usable even when highly degraded since the OncoScan® FFPE Assay is optimized for highly degraded FFPE samples. Whole-genome copy number profiling was analyzed with 50 ng of genomic DNA. TuScan algorithm (Affymetrix) was used to analyze data. The frequency and prognosis impact of CNAs were evaluated with univariate analysis of survival data.
Results: Characteristics of the 96 patients were as follow: median age 57y (41-65), 82% of males, MIPI-low/intermediate/high respectively 19%, 51% and 30%, blastoid morphology in 10%. No significant difference was observed between these patients and the LyMa patients (n=299). Among the 96 patients, 9 were HR patients with primary refractory disease or early relapse within one year post-diagnosis while 87 patients remained in response more than one year after diagnosis (including 64 LR patients who were still in complete remission more than 30 months after diagnosis). After ASCT, 41 patients (43%) were randomized in the rituximab maintenance arm and 40 (42%) in the observational arm. Median follow-up from diagnosis was 61.3 months (1.4-83.2). Overall, 68 recurrently altered regions were observed in 98% of patients. Deletions were more frequent than amplifications, at 9 *vs* 3 by patient respectively. HR patients were associated with *TP53*del (44%vs14%;p=.04), *CDKN2A*del (56%vs22%;p=.04), 8p11del (44%vs15%;p=.05). Interestingly, we identified in 10 patients duplication of a minimal common region of 5,3 Mb located on chromosome 7p22 and including *CARD11*. This lesion was associated with low MIPI (80%vs12%;p<.001), and other gains such as 21q21, 10q11 and 6p21 which together define a favorable subgroup (24% of the cohort). These anomalies were significantly more associated with LR patients (87%vs60%;p=.02). None of the patients with *CARD11* duplication (n=10) had relapsed despite the presence of *TP53* in 2 patients or *CDKN2A* deletion in 3 patients. This translates into a longer PFS (100%vs70%;p=.02) (fig.).

Figure 1.

Summary/Conclusions: Our study confirms the worse impact of *TP53* and *CDKN2A* deletion on early relapse in MCL. By contrast, the *CARD11* duplication

is associated with an absence of relapse and thus defines a new group of very low risk patients. These findings provide important clues for future theranostic-driven therapies in MCL.

E1361

CLINICOBIOLOGICAL FEATURES OF B-CELL NEOPLASMS WITH CDK6 TRANSLOCATIONS: FREQUENT ASSOCIATION WITH MARGINAL-ZONE LYMPHOMA, CONTINGENT OF PROLYMPHOCYTIC CELLS AND TP53 ABNORMALITIES. A GFCH STUDY

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Background: Translocation involving the *CDK6* gene is a rare but recurrent abnormality in B-cell neoplasms. Three different translocations have been described: t(2;7)(p11;q21), which is the most frequent, t(7;14)(q21;q32) and t(7;22)(q21;q11), leading to juxtaposition of *CDK6* gene with *IGK*, *IGH* or *IGL* locus respectively.
Aims: The Groupe Francophone de Cytogenetique Hematologique (GFCH) collected 35 chronic B-cell disorders with *CDK6* translocation in order to document the clinicobiological features of this uncommon aberration.
Methods: Clinical and biological data were gathered at diagnosis when available. A cytological review was performed by 3 experts in 27/35 cases. FISH was used to detect *IG* or *TRAD* and *CDK6* rearrangements, and recurrent abnormalities frequent in SMZL and CLL (trisomy 3, 12, 18, deletions of *ATM*, *TP53*, 13q14 and 7q22/7q36 loci). *TP53* (exons 4-9), *NOTCH2* (exon 34), and *IGHV* genes were analyzed by Sanger sequencing. Detection of *MYD88 L625P* was performed by real-time AS PCR.

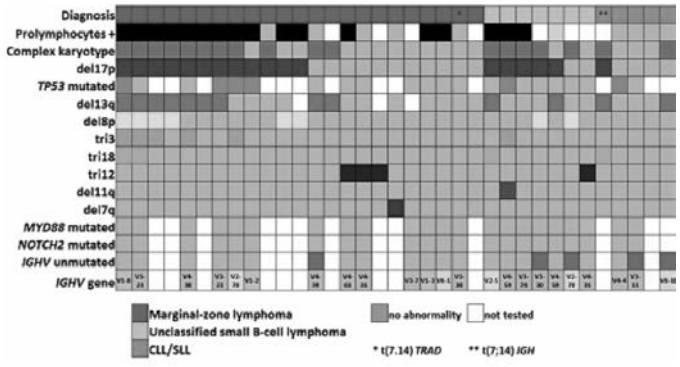

Figure 1.

Results: Our cohort included 22M and 13F, with a median age of 71 years. The involvement of *CDK6* was confirmed in all cases. A t(2;7) *IGK/CDK6* was found in 33/35 patients. One case had a t(7;14) *IGH/CDK6*, and one had a t(7;14)(q21;q11) involving the *TRAD* locus. There were 23 (66%) marginal-zone lymphoma (MZL), including 22 splenic MZL (SMZL) (including the t(7;14) *TRAD*), and 1 bronchus MALT type, 7 (20%) unclassified small B-cell lymphomas (USBCL) and 5 (14%) chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) with Matutes score 4/5 (including the t(7;14) *IGH*). Morphological review showed a contingent of prolymphocytic cells (median: 10% of lymphoid cells) in 17/27(63%) cases, including 14/19 MZL and 3/4 UBCL and 0/4 CLL/SLL. The *CDK6* translocation was the sole aberration in 9/35(26%). K was complex in 18/35(51%). The most frequent additional abnormalities were: del17p (*TP53*) (51%), del13q (41%), del8p (23%), trisomy 18 (22%), trisomy 3/3q (17%) and trisomy 12 (11%). Deletion of 7q and 11q were rare (one case of each). *TP53* was mutated in 6/22 patients (27%), including 5 with del17p. Overall, 19/29 (66%) tested cases had a *TP53* abnormality (del and/or mutated), which was significantly associated with complex karyotype (p=0.016) and del13q (p=0.042). *MYD88 L625P* was detected in 2/22 patients. No *NOTCH2* mutation was found. *IGHV* analysis showed a preferential usage of *VH4* (8/23, 35%), while *VH1* was rare (3/23, 13%, including one *VH1-2*). Most carried *IGHV* with some impact of somatic hypermutation (85%). Median follow-up was 28 months [0-192]. The median survival was not reached, only 4/32 (12.5%) died. A treatment was undertook in 15/32(47%) cases, with a median time to first treatment of 13 months. In our series, the *CDK6*+ MZL cases differed from classical SMZL by frequent prolymphocytic differentiation (14/19, 74%), very low incidence of 7q deletion (1/23, 4%), high frequency of *TP53* abnormality (12/23, 52%), absence of *NOTCH2* mutation (0/13, 0%), and a different *IGHV* repertoire with low frequency of *VH1-2* (1/13). The *CDK6*+ USBCL also had frequently a contingent of prolymphocytes (3/4, 75%), and showed a genetic profile similar to the *CDK6*+ MZL (see figure).
Summary/Conclusions: These results, obtained on the largest series to date, suggest that *CDK6* translocation is associated with indolent small B-cell lymphomas, mostly SMZL, with distinctive features. However, *CDK6* translocations can also be rarely observed in CLL/SLL. We describe one case involving the T-cell receptor (*TCR*) locus, which is a rare event in a B neoplasm. Finally, it is intriguing that this abnormality involves almost exclusively the *IGK* locus, and not the other *IG* loci, especially *IGH* which is usually the most frequently rearranged.

E1362

PRIMARY CUTANEOUS DIFFUSE LARGE B-CELL LYMPHOMA, LEG TYPE, EXPRESS STEREOTYPED B-CELL RECEPTORS WITH UNIQUE NONSYNONYMOUSLY MUTATED CONSTANT REGIONS

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Background: Primary Cutaneous Diffuse Large B-Cell Lymphoma, Leg Type (DLBCL, LT) is a rare and aggressive neoplasm with a primary cutaneous presentation that shares genetic and phenotypic characteristics with DLBCL of activated B-cell subtype (ABC-DLBCL). Although receptor stereotypes have been observed, the role of the B-cell receptor (BCR) in DLBCL, LT is largely unknown. Previous studies on small cohorts suggested that DLBCL, LT expresses IgM with overrepresentation of IGHV3 alleles and high rates of somatic mutations.
Aims: We aimed to elucidate the stereotype of the BCR in DLBCL, LT and to test for autonomous antigen-independent signaling as described for CLL (Dühren-von Minden, Nature 2012) and non leg-type ABC-DLBCL (Koning, AACR 2016 & ASH 2016).
Methods: 8 cases of DLBCL, LT were subjected to RNAseq. Additional RNAseq data from 6 healthy volunteers (GEUVADIS project), 10 non leg type DLBCL, and 16 follicular lymphoma were obtained from NCBI publicly available datasets and collaborators. VDJ/VJ rearrangements and IgM constant regions were Sanger sequenced for all cases and two granulocyte controls. Lymphoma-derived, clonal BCR were tested for autonomous signalling activity in the murine TKO pre-B-cell system (Dühren-von Minden, Nature 2012).
Results: RNAseq analysis demonstrated an IgM isotype in all eight and VJ-kappa in seven DLBCL, LT cases. IGHV3 usage was observed in 7/8 cases; 4 cases expressed the IGHV3-7 gene. DLBCL, LT BCR were strongly mutated (range: VDJ 3.1-22.2%; VJ 0.6-13.5%). No intraclonal sequence variation was observed. Non-synonymous single nucleotide variants (SNV) were observed in the constant regions of four cases and in IGKC of one additional case, but not in available granulocyte DNA of two cases with C region mutations or in the other 32 RNAseq libraries. Constant region mutations were highly specific to DLBCL, LT as compared to other DLBCL (p=0.0018) and follicular lymphoma (p=0.0013). In contrast to ABC DLBCL, V(D)J BCR of DLBCL, LT on a murine constant region backbone did not induce antigen-independent calcium flux in TKO cells upon induction of functionality of the BCR signalling cascade by tamoxifen.
Summary/Conclusions: Our data identify a clearly stereotyped receptor in DLBCL, LT. In contrast to CLL and ABC-DLBCL, BCR stereotypy was not associated with autonomous BCR signalling activity using a murine IgM backbone. The pathogenic potential of the novel constant region mutations for BCR activity in DLBCL, LT warrants further functional studies.

E1363

LOSS OF NR4A1 ACCELERATES MYC-DRIVEN LYMPHOMAGENESIS ACCOMPANIED BY OVEREXPRESSION OF GENES INVOLVED IN IMMUNOREGULATION

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Background: *NR4A1* (*Nur77*) belongs together with *NR4A2* (*Nurr1*) and *NR4A3* (*NOR-1*) to the Nur77 family of nuclear orphan receptors. As immediate early- or stress response genes their expression is diverse as it is the cellular outcome upon activation. Recently, there has been attributed a pivotal role to *NR4A1* and *NR4A3* as tumor suppressors in AML in humans and mice. In our comprehensive *NR4A* expression analysis in various lymphoma entities we demonstrated a significant reduction of *NR4A1* expression in aggressive lymphomas, which was associated with poor cancer-specific survival. Moreover, ectopic expression of *NR4A1* in aggressive lymphoma cells resulted in induction of apoptosis.
Aims: In order to better dissect the role of *Nr4a1* in lymphoid malignancies, we used a *Myc*-driven mouse model of lymphomagenesis and crossed the *EμMyc* mouse with the *Nr4a1*-/- mouse. Survival and tumor formation were monitored and RQ-PCR was performed on selected tumor specimens, whereby genes, found to be associated with *NR4A1* expression in the publicly available gene expression data set of DLBCLs generated by Lenz *et al.*, were taken. Moreover, the driver-function of *Nr4a1* in lymphomagenesis at the premalignant stage was investigated by using apoptotic assays and by carrying out transplantations of tumor cells into wt recipients.
Methods: Kaplan Meier analysis was performed for survival and tumor formation in *EμMyc Nr4a1*+/+ (n=134), *EμMyc Nr4a1*-/- (n=84) and *EμMyc Nr4a1*+/- (n=59), respectively. For RQ-PCR selected tumor specimens from wt and *EμMyc* mice with (n=14) and without (n=17) *Nr4a1* loss were used. For investigation of the role of *Nr4a1* at the premalignant stage, mice aged 4 weeks (n=4 per genotype) were sacrificed and AnnexinV staining and cleaved-caspase3 assay were performed on cells isolated from the spleen and bone marrow. *In vivo* tumor formation was induced by tumors derived from *EμMyc Nr4a1*+/+ (n=8) and *EμMyc Nr4a1*-/- (n=11) mice injected into the tail vein of wt mice. Kaplan Meier analysis was used for monitoring survival and tumor formation, and FACS analysis for analysis of bone marrow, spleen and tumor, respectively.
Results: *EμMyc Nr4a1*-/- mice showed decreased survival with a median of 92 days compared to *EμMyc Nr4a1* +/+ with median survival of 123 days ($p<0.001$) and tumors developed faster with a median of 45 days for *EμMyc Nr4a1*-/-, *vs* 107 days for *EμMyc Nr4a1* +/+; $p<0.001$. Both, survival (median=101 days; $p=0.037$) and tumor formation (median=66 days, $p=0.001$) gave intermediate values for *EμMyc Nr4a1* +/- mice. Furthermore, *EμMyc Nr4a1*+/+ exhibited induction of apoptosis in all investigated B cell subpopulations at the premalignant stage, whereas apoptosis was significantly diminished in *EμMyc Nr4a1*-/- mice. RQ-PCR showed that several genes involved in immunoregulation and Nf-κB target genes were upregulated in *EμMyc Nr4a1*-/- compared to *EμMyc Nr4a1*+/+. Last, tumor formation upon i.v. injection showed that tumors with *Nr4a1* loss engraft faster than tumors derived from mice without *Nr4a1* loss (25 days *vs* 38 days; $p=0.009$) and lead to a decreased number of inflammatory cells in the tumor.
Summary/Conclusions: Our results clearly demonstrate the influence of *Nr4a1* loss on tumor formation and consequently survival in a *Myc*-driven model of lymphomagenesis. Importantly, *Nr4a1* seems to impact cell death early in B cell development, even ahead of malignant transformation. Additionally, *Nr4a1* seems to be involved in driving immune responses towards an anti.-inflammatory, tolerogenic phenotype, thereby facilitating tumor growth and in altering the tumor environment. Collectively, these data underpin the tumor suppressive function of Nr4a1 in aggressive lymphomas.

E1364

DISSECTING THE PI3K PATHWAY IN A CYCLIN D1-DRIVEN MODEL OF MCL
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Background: Mantle cell lymphoma (MCL) presents as a highly disseminated B-Cell malignancy, accounting for about 6% of all non-Hodgkin lymphomas. Genetically, MCL is characterized by the t(11;14)(q13;q32) translocation, leading to the overexpression of the cell cycle regulator Cyclin D1. The disease is associated with short responses to current standard therapies and a great need for new therapeutic strategies. Interestingly, the PI3K/mTOR pathway has emerged as a promising therapeutic target in MCL, as cell lines and patients have shown substantial response rates to rapamycin and analogs.
Aims: The aim of this study is to functionally dissect the role of individual PI3K/mTOR pathway genes by performing a shRNA-based screen in genetically defined primary murine MCL tumor cells. Hereby, we want to identify synthetic lethal genes for Cyclin D1 and novel molecular dependencies in Cyclin D1-driven lymphomagenesis, thereby establishing novel potential therapeutic targets in MCL.
Methods: We have developed a new mouse model for MCL using Eμ-myc transgene mice that overexpress the MCL hallmark lesion Cyclin D1, as well as the reverse tet transactivator for inducible transgene expression. Using primary MCL tumor cell lines derived from this model as a platform, we performed shRNA loss-of-function screen entailing a two colored, antibiotic selectable and tet-inducible retroviral shRNA expression vector system. A shRNA library targeting more than 300 different PI3K related genes was introduced into primary murine MCL cells. After induction of shRNA expression by addition of Dox, shRNA representation in knockdown and control cells was deconvoluted by deep sequencing to identify differentially selected shRNAs.
Results: The shRNA screen identified more than 50 strongly (> than 4 fold) differentially regulated genes affecting MCL tumor growth and survival. We identified numerous targets within the PI3K pathway and the molecular dependency on this pathway was in line with the observed high sensitivity of these cells towards pharmacological mTOR inhibition. Individual shRNA knockdown experiments confirmed the newly identified candidate genes including components of the lipid second messenger system, such as diacylglycerol kinase isoform alpha (Dgka) and gamma (Dgkg). Knockdown of these lipid kinases by three or two different hairpins lead to decreased cell proliferation. Dgka knockdown was further validated on protein level by Western Blot analysis. Furthermore, two small molecule inhibitors targeting Dgka (R59022 and Ritanserin) significantly decreased cell viability.
Summary/Conclusions: Using an unbiased shRNA screen of more than 300 genes contained in the PI3K pathway, we were able to identify a range of proteins that significantly impaired cell proliferation and cell viability in Cyclin D1-driven lymphoma cells. Among the candidate genes were components of lipid second messenger pathways, such as class I diacylglycerol kinases alpha and gamma, which could be successfully validated in downstream analyses. Dependence on these kinases was further demonstrated using two different small molecule inhibitors, indicating an important role for the diacylglycerol second messenger system in MCL growth. Their mode of action on the PI3K pathway, especially in regard to Cyclin D1, will be further investigated in murine and human MCL cells. Furthermore, additional newly identified candidate genes will be further explored to characterize their role in Cyclin D1-driven lymphomagenesis, with the aim of identifying novel therapeutic targets in this difficult-to-treat disease.

E1365

MUTATIONAL PROFILING OF HODGKIN- AND REED-STERNBERG CELLS (HRSC) OF CLASSICAL HODGKIN LYMPHOMA (CHL) ENRICHED FROM ARCHIVAL FORMALIN-FIXED AND PARAFFIN-EMBEDDED TISSUE SAMPLES
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Background: cHL can be cured in the majority of cases. However, ~10–20% patients die of the lymphoma after relapse or progressive disease. There are unmet needs for understanding the mechanisms that cause cHL relapses, for development of new prognostic/predictive markers and effective targeted therapies. Comprehensive genetic characterization and advance in understanding the molecular pathology of cHL are indispensable to meet those needs. However, genetic information on cHL is still scarce mainly due to difficulties of isolating malignant HRSC, whose overall frequencies in the affected tissues range from 0.1-5%. Formalin-fixed paraffin-embedded (FFPE) tissue archives are the most abundant source of clinically annotated tumor specimens. However, FFPE tissue usability is limited because of poor DNA quality and difficulty to enrich neoplastic cells. Therefore, new enrichment techniques are necessary to enable larger scale comprehensive genetic investigations of cHL.
Aims: Our aims were: 1) to develop a technique for HRSC enrichment from the archival formalin-fixed paraffin embedded tissue; 2) to reliably detect genetic aberrations in the genomes of enriched tumor samples and to use this information for development of new prognostic and predictive markers as well as for better understanding of the genetic background of cHL.
Methods: We have developed a new high-throughput method for marker-based enrichment of archival FFPE tissue-derived HRSC nuclei by fluorescence-assisted flow sorting (FACS). Genomic DNA extracted from sorted nuclei was used for identification of mutations in 68 genes that are frequently mutated in lymphomas by targeted high throughput sequencing (HTS). Chromosomal copy number aberrations were investigated by the Agilent SurePrint 180k microarray.
Results: Enzymatically extracted FFPE tissue-derived cell nuclei retain their antigenicity and can be reliably labelled with monoclonal antibodies against nuclear (MUM1, PAX5) and cytoplasmic/cell surface (CD30) markers. A mean neoplastic cell purity of 70% (range 40-95%) was achieved by sorting HRSC cells according to their double expression of MUM1 and CD30 in 11 cHL cases. Using sorted non-malignant cells as a germline control we detected somatic singe nucleotide mutations and indels in all investigated samples. Mutations of *STAT6*, *PIM1*, *SOCS1*, *KMT2D* occurred in at least 18% (2/11) of cases. Also, individual cases contained copy number aberrations such as gain of chr2 (*CREL* locus), focal deletions of chr4, chr7, chr16 and chr19 affecting genes such as *JAK3*, *CDKN2D*, *MAP2K3* and *NOTCH3*. Taken together our study demonstrates that DNA extracted from the enriched cell populations is suitable for wide-scale genetic profiling.
Summary/Conclusions: A novel rare-cell-enrichment technique is suitable for genetic cHL studies and opens the possibility for the wider use of archived

FFPE tissue, thus enabling more robust study designs to answer clinically relevant questions in the field.

E1366

LACK OF STAT1 PREDISPOSES TO A DIFFUSE LARGE B-CELL LYMPHOMA-LIKE DISEASE

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Background: The highly conserved JAK-STAT signaling pathway regulates proliferation, differentiation, apoptosis and immune responses. Activating mutations in STAT3 are considered to drive the development of diffuse large B-cell lymphomas (DLBCL). STAT1 is a critical counter player of STAT3. Of note, many STAT1 target genes are frequently altered or mutated in DLBCL patients, such as *SOCS-1*, *B2M*, *PDL1*, *CARD11, CIITA* and *BCL6*. We observed that the loss of STAT1 suffices to provoke spontaneous haematopoietic tumors in mice.
Aims: We aimed at investigating the underlying mechanisms of spontaneous hematopoietic tumor formation in STAT1-deficient mice.
Methods: We characterized the spontaneous haematopoietic tumors by FACS and morphological analysis. To identify the cell of origin for the disease, we performed bone marrow transplantation assays. We high-purity FACS-sorted individual cell populations of diseased STAT1-deficient mice and transplanted them into recipient mice. *Ex vivo* derived transformed tumor cells were characterized for lineage-specific surface marker expression and identified as B-cells. Malignant B-lymphoid STAT1-deficient cell lines were established and expression levels of typical lymphoid-specific tumor- suppressor and promoter genes were assessed by qPCR. In parallel, *Stat1*$^{-/-}$ cell lines were used for RNA-seq analysis to identify the signaling pathways driving disease. RNA-seq data were compared to publicly available RNA-seq data from different haematological malignancies.
Results: STAT1-deficient mice develop a myeloid hyperplasia that manifests with an incidence of 60% and is characterized by the absence of *Rigl*. Transplantation of bone marrow unmasked the development of a B-cell malignancy which can be transferred by CD19^{+} cells. The malignant B-cells arising in *Stat1*$^{-/-}$ mice can be maintained *in vitro* and display alterations in gene expressions that are typically found in human DLBCL such as *Irf4*, *Prdm1* and *p53.* RNA-seq analysis revealed features shared with human DLBCL: increased reads a loci of *SpiB*, *Mef2b*, *Card11* and *Cd274* (PDL1) and decreased expression of *Socs-1*, *Cdkn2a*, *B2m* and *Prdm1*. Low levels of STAT1 combined with low levels of p16^{INK4A} correlate with a reduced life expectancy in DLBCL patients.
Summary/Conclusions: Loss of STAT1 in Balb/C mice provokes a myeloid hyperplasia which masks a B-cell malignancy resembling human DLBCL. DLBCL patients with low levels of *STAT1* have a poorer prognosis if they lack the tumor suppressor p16^{INK4A}.

E1367

MOLECULAR HETEROGENEITY OF MANTLE CELL LYMPHOMA

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Background: Mantle cell lymphoma (MCL) is a B-cell non-Hodgkin lymphoma characterized by t(11;14)(q13;q32) leading to constitutive cyclin D1 overexpression and cell cycle deregulation. The survival is still poor, especially for patients resistant to frontline drugs. Although patients are brought in remission, relapses often occur with disseminated lymphoma, which is more difficult to treat. There is a need for a better understanding of the clonal heterogeneity of this disease and to identify new signaling pathways with genes which could be targeted by novel drugs or be used as biomarkers to predict response to treatment.
Aims: To address the genetic heterogeneity in MCL in paired patient samples at diagnosis and relapse.
Methods: Highly pure malignant B-cell populations were isolated using fluorescence activated cell sorting in four patients diagnosed with MCL. In addition T-cells were sorted from the same patients as paired non-malignant control samples. Exome sequencing was performed on both the malignant B-cell population and paired T-cells (13 samples in total). Mutations were detected in parallel with CLC Biomedical Workbench 2.5 (Qiagen) and MuTect 1.04 (Broad Institute) (coverage ≥ 20, population allele frequency<0.01) and evaluated against the COSMIC (Wellcome Trust Sanger Institute), dbSNP and PubMed database. Exemption from informed consent was approved by the National Ethical Committee.
Results: Our data highlighted in each patient persistent gene modifications between diagnosis and relapse. We confirmed gene mutations already well-known in B-cell malignancies (e.g. *TP53*, *NOTCH1* and *MYD88*). Interestingly, aberrations not previously described in the COSMIC database, were observed with high allele frequency both at diagnosis and at relapse. This included genes in B-cell signaling (e.g. transcriptional repressor SPEN associated to NOTCH pathway regulation and blockage of the precursor B-cell differentiation), inflammatory response (e.g. *IRG1*), genes found in invasive carcinoma (e.g. integrin β4 subunit) and B-cell malignancies (e.g. *PLA2G4D*). In addition to common mutations or hit in putative drivers, new gene modifications as well as loss of previous ones could be observed at relapse. For example, genes involved in embryonic development and cell fate (e.g. the transcription factor SOX1) and genes involved in inflammation (*CCL13*) were not previously correlated to MCL and were novel at relapse. This suggests that a modified malignant clone has evolved and progressed. No gene modification was observed to be shared by all four patients. However, aberrations in the same signaling pathways were identified across individuals. From allele frequency distributions detected with MuTect we could detect discrete clonal or competing subclonal involvement: A patient harbored one major discrete clone at diagnosis while at relapse two clearly separated clones were present, whereas another patient presented a diffuse clonal pattern at diagnosis and a more discrete biclonal pattern at relapse.
Summary/Conclusions: Our work shows examples of molecular progression from diagnosis to relapse in MCL and supports the heterogenic nature and genetic complexity of this disease. We confirm mutations in genes already known as involved in the malignant transformation of MCL and identify new ones involved in the B-cell signaling pathways. This adds valuable knowledge to the biological understanding of MCL which is pivotal in the era of precision medicine.

E1368

NOVEL TARGET GENES OF DEREGULATED MIRNAS IN DLBCL REVEALED BY ENDOGENOUS AGO2 PAR-CLIP

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Background: Aberrant expression of microRNAs (miRNAs) is a widespread phenomenon in cancer. However, the functional significance of such deregulation is poorly understood as the target genes of miRNAs (the *targetome*) are notoriously difficult to predict computationally and moreover differ according to cellular context. An alternative approach is to directly sample the targetome using immunoprecipitation (IP) techniques such as PAR-CLIP. The drawback however of such techniques is the need for exogenously produced tagged proteins. Diffuse large B-cell lymphoma (DLCBL) is the most common form of non-Hodgkin lymphoma, typically aberrantly expressing *miRNA-155* (Lawrie, 2007). This miRNA is a well known key regulator of lymphomagenesis, needed for T and B cell function (Rodriguez, 2007; Thai, 2007; Vigorito, 2007), and its over-expression in pre-B cells or haematopoietic stem cells leads to oncogenic transformation (Costinean, 2006; O´Connell, 2008). Given their relevance, DLBCL and *miR-155* were chosen as models for the present study.
Aims: We set ourselves to adapt PAR-CLIP technique to allow non-engineered cells to be used based on IP of endogenous levels of Ago2. In addition, we also aimed at testing the minimum number of input cells needed for miRNA target identification.
Methods: Two DLBCL cell lines (ABC-type RIVA, and GC-type SUD-HL10) were transfected with lentiviral vectors that encoded *miR-155.* In parallel, we transfected these cells with an inhibitor of *miR-155* or with a scrambled sequence, as experimental controls (for reducing the number of false positives). Cells were then stably selected with puromycin, and grown in the presence of 100 uM 4SU for 18 h. Different amounts of these cells (300M, 50M and 10M) were then irradiated to cross-link the RNA to RNA-binding proteins. PAR-CLIP was then performed on cell lysates using anti-Ago2 mAbs for IP. The original protocol (Hafner, 2010) was modified to eliminate radioactive labelling. The recovered RNA was used for library building using TruSeq Small RNA Sample Kit v1 and the sequencing performed on an Illumina HiScanSQ system. After deduplication and alignment, T-to-C variants (indicative of miRNA-dependent binding sites) were identified, and PAR-CLIP clusters called using wavClusteR (Comoglio, 2015).
Results: Endogenous Ago2 IP, followed by a radioactive-free modified PAR-CLIP protocol yielded sufficient RNA for building libraries for NGS irrespectively of cell input. Samples gave an average ~9.7 x10^{6} aligned reads/library. There were an average of 7,370 PAR-CLIP clusters mapping to coding genes (range 4,675 - 11,004, correlating with the number of input cells, r=0.82). In all exper-

imental conditions we found that a number of the captured genes corresponded to experimentally validated targets of *miR-155*. Crucially, ontogeny analysis of the PAR-CLIP-captured genes demonstrated an enrichment of genes involved in haematopoietic and/or lymphomagenesis pathways.
Summary/Conclusions: To fully understand the role of a particular miRNA in a specific malignancy, it is essential to identify its target genes in a relevant cellular context. Using a haematological malignancy model of high clinical interest we have developed an optimised method for interrogating the miRNA:mRNA interface (*targetome*) within a cellular system without the need of ectopically express tagged Ago2, keeping physiological levels of the core component of the RISC complex unaffected. Moreover, our optimized protocol allowed us to reduce the number of input cells, therefore opening the exciting possibility of interrogating the targetome of patient primary samples.

E1369

DARATUMUMAB, A NOVEL HUMAN CD38 MONOCLONAL ANTIBODY FOR THE TREATMENT OF B-CELL NON-HODGKIN LYMPHOMA

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Background: Daratumumab (DARA) is a first-in-class human monoclonal antibody that targets the CD38 epitope and is approved for the treatment of relapsed/refractory (R/R) multiple myeloma (MM) patients. DARA is currently being evaluated in phase II clinical trials as monotherapy in patients with R/R Mantle Cell Lymphoma (MCL), Follicular Lymphoma (FL) and Diffuse Large B-Cell Lymphoma (DLBCL). DARA induces tumor cell death through effector mediated mechanisms in MM, including Antibody-Dependent Cellular Cytotoxicity (ADCC), Complement-Dependent Cytotoxicity (CDC) (*de Weers M. J Immunol, 2011*) and Antibody-Dependent Cellular Phagocytosis (ADCP) (*Overdijk MB. MAbs, 2015*). In Chronic Lymphocytic Leukemia (CLL), DARA induces killing mainly via ADCC and ADCP (*Matas-Céspedes A. Clin Cancer Res, 2016*). Furthermore, immunomodulatory effects (*Krejcik J. Blood, 2016*) and modulation of the enzymatic activity of CD38 (*Lammerts van Bueren J. Blood, 2014*) have been described to contribute to its antitumor activity.
Aims: To evaluate the activity of DARA on MCL and FL cells as monotherapy and in combination with current therapies, both *in vitro* and *in vivo*.
Methods: ADCC, CDC and ADCP activities were assessed by calcein release or flow cytometry. Penetration of DARA was analyzed in a 3D model by Selective Plane Illumination Microscopy (SPIM). Molecules per cell were analyzed using Qifikit and flow cytometry. *In vivo* activity was assessed in prophylactic and therapeutic set ups using SCID mice subcutaneously (sc) or intravenously (iv) injected with $1x10^7$of MCL or FL cells. Mice were treated (human IgG control or DARA) with two different schedules: prophylactic (3 doses of 10mg/kg one dose per week) or therapeutic (20/10/10/10mg/kg, one dose per week). For the combination regimens in FL, sc injected SCID mice were treated following the therapeutic schedule in combination with Rituximab (20/10/10/10mg/kg, one dose per week) and/or CHOP (initial unique dose).
Results: DARA (0.0001-1µg/mL) induced ADCC in a dose-response manner on MCL (n=6) and FL (n=4) cell lines in the presence of PBMCs *in vitro*. Moreover, DARA induced significant levels of ADCP at 1µg/mL on MCL (n=6) and FL (n=4) cell lines in the presence of murine macrophages *in vitro*. However, DARA did not induce significant CDC in any of these models due to a high expression of the complement inhibitors CD46, CD55 and CD59, and insufficient number of CD38 molecules per cell. In a 3D model of FL, SPIM analysis revealed a maximum penetration of DARA at 1µg/mL after 48h of treatment.
We tested DARA activity *in vivo* in two different mouse models (sc and iv) of MCL and FL. In the prophylactic setting, DARA completely prevented the outgrowth and induced tumor regression of MCL (n=6) and FL (n=6) subcutaneous tumors. In the therapeutic setting, DARA significantly increased the overall survival (OS) of these mice and reduced organ infiltration of tumor cells both in the MCL (n=10) and in the FL (n=10) systemic xenograft models. In addition, the combination of DARA with Rituximab/CHOP regimen in FL, resulted in a synergistic reduction of tumor growth (n=7-10).
Summary/Conclusions: DARA shows encouraging cytotoxic activity in MCL and FL cells in the presence of external effectors *in vitro*. In addition, DARA exerts unique and substantial effects as single agent on MCL and FL tumor cell growth in different mouse models and contributes to potent therapeutic efficacy in combination with current approved therapies. These results warrant further studies of DARA in the clinical setting for these conditions.

E1370

ECTONUCLEOTIDASES CD39/CD73 ARE HIGHLY EXPRESSED ON ATLL CELLS AND RESPONSIBLE FOR GENERATING AMP/ADENOSINE

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Background: Adult T-cell leukemia/lymphoma (ATLL) is a mature T-cell neoplasm, linked to the human T-cell lymphotropic virus, HTLV-1. Patients with ATLL are often at the risk of opportunistic infections. It might be possible that this immunocompromised state could be induced by the function of ATLL cells having similar phenotypes with regulatory T cells (Tregs). However, difficulties of *in vitro* studies using primary tumor cells have hampered the progress of ATLL research, and it is still controversial whether ATLL tumor cells have the immunosuppressive characteristics.
Aims: In this study, we analyzed, the roles of molecules expressed in ATLL cells associated with immunosuppressive functions of Tregs.
Methods: The protocol of this study was approved by the Investigational Review Board of Osaka University Hospital. Peripheral blood mononuclear cells (PBMCs) were collected from 8 asymptomatic HTLV-1 carriers and 20 ATLL patients (3 with smoldering type, 5 with chronic type, and 12 with acute type) after getting informed consent. PBMCs from 3 ATLL patients were separated into $CD4^+CD7^-CADM1^+$ ATLL cells and adjacent $CD4^+$ $CD7^+CADM1^-$ normal T cells using Fluorescence-activated Cell Sorter(FACS), and total RNA sequencing experiments were conducted. And we also examined the expression patterns of CD39 and CD73 at in HTLV-1 carriers or each type of ATLL patients.
Results: We compared whole transcriptome of ATLL cells and normal $CD4^+$ cells. Bioinformative analyses showed that many genes associated with immunosuppressive functions of Tregs were elevated or downregulated in ATLL cells. Among these genes we focused on CD39, CD73 and CD26, because recently it has been reported that extracellular adenosine, which is catalyzed by CD39, expressed in human Tregs, and CD73, expressed in murine but not in human Tregs, has strong anti-inflammatory function and plays major role in Treg-mediated immunosuppression. Therefore, we investigated the expression of CD39 and CD73 in ATLL cell lines and primary tumor cells. We found that all of 4 ATLL cell lines expressed CD39, but not CD73 just as human effector Tregs. In contrast, the expression patterns of CD39 in 20 ATLL patients were various (Table) and interestingly, some ATLL tumor cells express CD73. Also in asymptomatic carriers, we could detect CD39 and/or CD73 positive on $CD7^-CADM1^+$abnormal fraction of $CD4^+$ cells. CD26, expressed in human naïve but not in effector Tregs, was negative in all cell lines and primary cells except for abnormal cells in one smoldering patient. Next, the role of CD39 and/or CD73 in ATLL cells was assessed. Extracellular ATP is converted into AMP by CD39. As expected, $CD39^+$ ATLL cells converted significantly more ATP than $CD39^-$ ATLL cells, which was comparable with normal effector Tregs. Conversely, mass spectrometry analysis of AMP/adenosine concentration indicated the activity of CD73 mediated AMP hydrolysis was very slow; less than 10% of 1mM AMP was converted to adenosine by $CD73^+$ ATL cells, indicating that the aberrant expression of CD73 could not efficiently increase adenosine synthesis.

Table 1.

	asymptomatic carriers	smoldering type	chronic type	acute/lymphoma type	total
$CD39^-CD73^-$	0	2	1	2	5
$CD39^-CD73^+$	3	0	1	0	4
$CD39^+CD73^-$	1	0	1	6	8
$CD39^+CD73^+$	4	1	2	4	11
total	8	3	5	12	28

Summary/Conclusions: In this study, we showed that about two thirds of ATLL samples were $CD39^+CD26^-$just as effector Tregs and have comparable level of ATPase activity as Tregs, which are expected to play some immunosuppressive function in ATL patients. Recently it is also reported that in exhausted $CD8^+$ T cells in cancer patients, CD39 is co-expressed with PD-1. CD39 expression in ATLL cells may also have some roles in immunosuppression and thus in the escape from anti-tumor immunity.

E1371

Abstract withdrawn.

E1372

ACTIVATION OF SYK TYROSINE KINASE PLAYS A ROLE IN RESISTANCE AGAINST THE SELECTIVE BTK INHIBITOR ONO/GS-4059 IN DIFFUSE LARGE B CELL LYMPHOMA (DLBCL)

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Background: The B-cell receptor (BCR) pathway is implicated in the survival

and proliferation of several B cell malignancies. BTK is a key regulator of this pathway. In a preliminary clinical study, the selective BTK inhibitor ONO/GS-4059 showed therapeutic activity in relapsed/refractory DLBCL of the Activated B-cell phenotype (ABC-DLBCL) (Walter et al Blood 127pp411-419,2016). However, median treatment duration in ABC-DLBCL was only 3 months due to progressive disease and development of resistance. Two acquired resistant mutations *BTK* C481S and *PLCγ2* R665W have been reported as dominant resistant mechanisms to BTK inhibition in CLL but resistance mechanisms in DLBCL have not been fully elucidated.

Aims: To determine resistance mechanisms in the ABC-DLBCL TMD8 cell line and determine new rational combinations to take into the clinic with ONO/GS-4059.

Methods: The BTK inhibitor-sensitive ABC-DLBC cell line TMD8 and cloned ONO/GS-4059 and Ibrutinib resistant TMD8 cell lines (TMD8RO and TMD8RI) were used for this study. TMD8RO has *PLCγ2* R665W whilst TMD8RI lacks both *BTK* C481S and *PLCγ2* R665W. Cell viability and apoptosis after compound treatment were assessed using Cell titer Glo assay and Annexin V/ PI staining. The effect of ONO/GS-4059 on intracellular signaling and expression of immunoreceptor were assessed by immunoblot and Flow cytometry. The mutational status of BTK and PLCγ2 in TMD8R was determined by Sanger sequencing.

Results: ONO/GS-4059 induced apoptosis in TMD8 at nanomolar concentration and over 72 hours induced classical apoptosis in >80% of cells. Although ONO/GS-4059 induced rapid reduction in ERK and AKT activation, activation of ERK and AKT rebounded within 24 hours in surviving cells. Interestingly, surface immunoglobulin M (sIgM) expression was increased more than three times in these cells leading to subsequent activation of SYK. The specific SYK inhibitor GS-9973 combined with ONO/GS-4059 inhibited the downstream ERK and AKT reactivation and induced synergistic apoptosis in TMD8. On the other hand, SYK hyper-activation as determined by phospholyration of SYK and its downstream target BLNK was also observed in the two BTK inhibitor resistant cell lines. Additionally, expression of CD5 and CD22, which negatively regulate BCR signalling, was decreased in these cells. The combination of ONO/GS-4059 and GS-9973 restored sensitivity to ONO/GS-4059 and induced synergistic apoptosis in both resistance cell lines.

Summary/Conclusions: These data show that SYK is highly activated through increased sIgM expression and/or downregulated CD5 and CD22 following BTK inhibitor treatment in ABC-DLBCL. These changes may contribute to not only the development but also the maintenance of resistance to BTK inhibitor. The combination of ONO/GS-4059 with SYK inhibitor is therefore a rational strategy for preventing and overcoming BTK inhibitor resistances.

E1373

STRO-001, A NOVEL ANTI-CD74 ANTIBODY DRUG CONJUGATE (ADC) FOR TREATMENT OF B-CELL NON-HODGKIN'S LYMPHOMA (NHL)

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Background: CD74 is a type II transmembrane glycoprotein involved in the formation and transport of MHC class II protein. CD74 is rapidly internalized and highly expressed in many B-cell malignancies with limited expression in normal tissues (Stein R. et al., CCR 2007). STRO-001 is a novel CD74-targeting ADC comprised of a p-azido-methyl-phenylalanine (pAMF)-containing anti-CD74 aglycosylated human IgG1 antibody (SP7219) conjugated to a non-cleavable dibenzocyclooctyne (DBCO)-maytansinoid linker-warhead. Highly efficient site-specific conjugation enabled by Sutro's cell-free antibody production and click chemistry produced a well-defined homogeneous ADC with a drug-antibody ratio (DAR) of 2. Due to its limited cell permeability, the major catabolite released by STRO-001 has 1000X lower cell killing activity on CD74 positive and negative cells compared to a reference cytotoxic maytansine. Since conjugation sites were selected based on highest stability both i*n vitro and in vivo*, thereby limiting loss of drug moiety from STRO-001 in circulation, this novel ADC has potential for improved PK, safety and activity profiles.

Aims: The aim of this study was to investigate the therapeutic potential of STRO-001 in non-Hodgkin's lymphoma (NHL) cell lines and xenografts. A dose-escalating exploratory toxicology study was also conducted in cynomolgus monkeys.

Methods: Biotinylated SP7219 was used for immunohistochemistry (IHC). DBCO-Alexa647-conjugated SP7219 and flow cytometry were used for detection and quantitation of CD74 expression on NHL cell lines and B-cells from normal human donors. STRO-001 was used to determine the EC_{50} and percent span of killing in NHL cell lines. The anti-tumor activity of STRO-001 in SCID mice bearing NHL tumor cell xenografts was examined. STRO-001 was administered to cynomolgus monkeys in an exploratory dose-escalating study of repeat IV doses of 1, 3, 10 and 30mg/kg on days 1 and 15.

Results: Expression of CD74 in different lymphoma subtypes was evaluated by IHC on duplicate core (matched pair) biopsies. Medium to high CD74 expression in >70% of cells was observed in 86/100 diffuse large B-cell (DLBCL), 22/28 follicular lymphoma and 49/78 mantle cell lymphoma (MCL) samples. *In vitro* cytotoxicity assays show potent activity of STRO-001 in a diverse panel of B-cell tumor lines including 9 germinal center B-cell (GCB) diffuse large B-cell lymphoma (DLBCL), 3 activated B-cell (ABC) DLBCL, and 4 mantle cell lymphoma (MCL) cell lines with EC_{50} values ranging from 0.17-13 nM. STRO-001 has only modest effects on naïve B-cells, but exhibits more potent cell killing in activated human B-cells that have upregulated CD74 expression (similar CD74 expression as SU-DHL-6 cell line). CD74 cell surface expression is required for STRO-001 cytotoxic activity but expression level, as measured by antibody-binding capacity, does not correlate with *in vitro* potency (R^2=0.4154). STRO-001 exhibits dose-dependent tumor growth inhibition in rituximab-resistant SU-DHL-6 xenografts starting at 2.5mg/kg weekly x 3 doses. The standard of care combination of bendamustine/ rituximab (BR) + STRO-001 further improves tumor suppression in SU-DHL-6 xenografts compared to vehicle (p=0.002) or BR alone (p=0.02). Studies with a MCL xenograft model, Jeko-1, demonstrate potent anti-tumor activity compared to vehicle (p<0.0001) starting at a single STRO-001 dose of 3mg/kg, with a single 10mg/kg dose resulting in tumor regression for up to 64 days post treatment. STRO-001 treatment 14 days post tumor inoculation was used to evaluate disease progression in Mino, a slow growing disseminated MCL xenograft model. Vehicle-treated animals developed advanced disease with palpable tumors and distended abdomens, with median survival of 81.3 days. In contrast, Mino xenografts treated with STRO-001 at 3mg/kg or 10mg/kg exhibited improved survival, with most animals healthy and disease free at the time of sacrifice 135 days post inoculation. STRO-001 demonstrated B-cell depletion in cynomolgus monkeys, confirming the intended pharmacodynamic effect. Myelosuppression was observed at the highest dose but there was no evidence of off-target toxicity.

Table 1.

Table with SP7219 binding capacity (copy/cell) and STRO-001 cell killing EC_{50}

	Cell Line	ABC (Antibody Binding Capacity)	STRO-001 Cell Killing	
			EC_{50} (nM)	Span (%)
Activated B-cell-like Diffuse Large B-Cell Lymphoma	OCI-Ly3	77,435	0.46	96
	U2932	10,649	1.3	80
	RIVA (RI-1)	BD	3.3	77
	SU-DHL-2	BD	>100	
Germinal Center B-cell-like Diffuse Large B-Cell Lymphoma	WSU-DLCL2	51,090	0.17	97
	WSU-NHL	49,925	0.69	96
	SU-DHL-4	49,603	0.24	97
	Pfeiffer	47,123	0.54	88
	SU-DHL-6	23,235	0.56	96
	OCI-Ly1	21,529	0.69	96
	HT	20,788	0.34	68
	NUDUL-1	13,040	0.3	98
	OCI-Ly19	BD	1.3	38
Mantle Cell Lymphoma	Mino	28,117	0.68	97
	JVM-2	23,672	1.7	55
	JeKo-1	14,754	0.41	97
	Rec-1	8,247	13	45

BD=Below detection (detection threshold = 4131)

Summary/Conclusions: STRO-001 demonstrates potent *in vitro* cytotoxicity in NHL cell lines and anti-tumor activity in NHL xenograft models, including prolonged survival in the disseminated Mino MCL model. STRO-001 depletes B cells in a dose-dependent manner. Clinical studies of this novel ADC for treatment of B-cell malignancies are under development.

E1374

DETECTING MALIGNANT B-CELLS IN CEREBROSPINAL FLUID: DOES THE IDEAL METHOD EXIST?

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Background: Leptomeningeal dissemination (LD) is a relatively rare but often fatal complication of lymphomas, confirmed by the analysis of the cerebrospinal fluid (CSF). The diagnosis is suspected in case of neurological symptoms, parenchymal brain involvement detected with neuroimaging techniques and confirmed by the analysis of the cerebrospinal fluid (CSF). Cytomorphological examination (CM) is still considered as the "gold standard" but remains insufficiently sensitive.

Aims: The aim of our study was to assess the benefit of more sensitive techniques, *i.e.* immunophenotyping by flow cytometry (FCM) and clonality by PCR, for the detection of malignant B cells in the CSF of patients with suspected leptomeningeal dissemination.

Methods: This study was conducted on 326 CSF samples (236 patients) referred to our laboratory between January 2015 and December 2016. CM, FCM and PCR results were recorded and classified as positive (+), negative (-) or suspicious. Immunophenotyping was performed on a FACSCanto II (BD Biosciences®) using an 8-colour panel. A sample was FCM+ when a cluster of at least 20 events with neoplastic features was detected. PCR-based analysis of immunoglobulin heavy (IGH) and light (IGL) chain rearrangements were per-

formed following the BIOMED-2 design and protocol. All PCR experiments were done in duplicates, and cases were considered PCR+ when both duplicates showed the same clonal pattern, ruling out false positivity (pseudoclonal pattern) often seen in paucicellular samples.
Results: We confirm that FCM and PCR are more sensitive than CM. Indeed, every CM+ cases (n= 16) was also FCM+ and/or PCR+ while 13 cases were FCM/PCR+ but CM-. A total of 269 samples showed similar results by FCM and PCR with presence (n=22) or absence (n=247) of lymphomatous cells whereas 25 samples were classified as suspicious by at least one technique. Eleven samples were FCM+ but PCR-. False negative (FN) PCR results can be explained in part by extensive somatic mutation in IGH genes, preventing optimal annealing of primers and thus blocking efficient amplification. Other targets less prone to somatic mutations, such as IGL, should therefore be evaluated. Conversely, 21 samples were PCR+ but FCM-. Absence of FCM detection might have resulted from the presence of very large lymphomatous cells outside the scope of analysis. Also, rapid cell death is an issue with FCM (prevented by the use of stabilizing reagents) whereas molecular techniques do not systematically require intact cells. Most of the difficulties encountered with both methods are due to occult blood contamination and poor cellularity, leading to low-intensity clonal signals by PCR and inconsistent cluster of events with FCM. In addition discordant results between FCM and PCR might be explained by sampling heterogeneity. Considering those limitations, it seems highly advisable to choose the best suited method for the follow-up according to the results at diagnosis.
Summary/Conclusions: Our results suggest that a multimodal investigation using FCM and PCR is necessary for improved detection of leptomeningeal dissemination in B-cell malignancies. It seems premature to make clinical decisions based on a single technology. Both methods, which suffer limitations that need to be acknowledged, are complementary and should be performed at diagnosis. Specific limitations of each of them should be taken in consideration for follow-up studies.

E1375

THE SYK INHIBITOR R406 DRAMATICALLY INCREASES THE SENSITIVITY OF GCB AND ABC DLBCL CELL LINES TO THE BCL-2 INHIBITOR VENETOCLAX

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Background: The BCL-2 inhibitor venetoclax demonstrated significant single-agent activity in recent clinical trials of relapsed/refractory chronic lymphocytic leukemia (CLL). However, results in some other B cell malignancies characterized by BCL-2 overexpression have not been equally impressive. This particularly refers to diffuse large B cell lymphoma (DLBCL), where only 18% of patients responded to treatment with venetoclax in a recent phase I clinical trial (Davids MS et al, J Clin Oncol. 2017).
Aims: Investigate whether the SYK inhibitor R406 can increase sensitivity of DLBCL cells to venetoclax.
Methods: The following cell lines were used: Ly4, Ly1, Ly7, Ly18, DHL4, Toledo and BJAB (all GCB DLBCL) and U2932, DHL2, Ly3, Ly10, HBL1 and TMD8 (all ABC DLCBL). The percentage of apoptotic cells was determined by Annexin V/PI staining and flow cytometry analysis. Expression of BCL-2 family members was determined by immunoblotting or RQ-PCR analysis.
Results: In a recent study, we showed that MCL-1 increases the resistance of anti-IgM stimulated CLL cells to venetoclax, and that SYK inhibitors can effectively overcome this resistance by blocking B cell receptor (BCR)-mediated MCL-1 upregulation (Bojarczuk K *et al.* Blood. 2016). Since constitutive activation of the BCR pathway has been described in both ABC and GCB DLBCL (Davis RE et al, Nature 2010; Chen L et al, Cancer Cell. 2013), we investigated whether treatment with the SYK inhibitor R406 can sensitize DLBCL cells to venetoclax. Single-agent venetoclax had only modest activity against most DLBCL cell lines at concentrations ranging up to 0.25 μM (Figure 1). Substantial apoptosis induction (>20%) was observed in only 2 GCB (Ly1 and Ly18) and 2 ABC (U2932 and Ly10) cell lines. R406 as single agent had almost no effect on tumor cell viability, with only one cell line showing >20% apoptosis induction (HBL1). However, addition of R406 to venetoclax resulted in a dramatic increase in the percentage of apoptotic cells in six of the investigated cell lines (Ly18, DHL4, U2932, Ly10, HBL1 and TMD8). A synergistic effect was also observed with Ly1 using a lower concentration of venetoclax, whereas no effect or only an additive effect was observed in the remaining cell lines (Ly4, Ly7, Toledo, BJAB, DHL2 and Ly3). Among these, only Toledo expressed similar levels of BCL-2 as the R406 + venetoclax sensitive cell lines, whereas the levels of BCL-2 in the other cell lines were extremely low or undetectable. To understand the mechanisms how R406 increases the sensitivity of DLBCL cells to venetoclax, we evaluated changes in the expression of MCL-1 and other antiapoptotic BCL-2 family proteins that have been associated with venetoclax resistance. Five of the seven R406 + venetoclax sensitive cell lines (Ly1, DHL4, U2932, HBL1 and TMD8) showed a 20-45% reduction in MCL-1 levels following 24 hours culture with 2μM R406, whereas no changes were observed in Ly18 and Ly10. However, a substantial reduction in A1 levels was observed in Ly18 and U2932 cells, whereas no substantial changes in A1 and BCL-xL expression were detected in any of the other investigated cell lines. Finally, we also investigated the effects of R406 on expression of HRK, which is a propapoptotic BCL-2 family member that was recently shown to be induced by SYK inhibition in a subset of GCB DLBCLs (Chen L et al, Cancer Cell. 2013). A substantial increase in HRK expression (140-640%) was observed in 5 of the 7 R406 + venetoclax sensitive cell lines (Ly1, Ly18, DHL4, U2932 and TMD8).

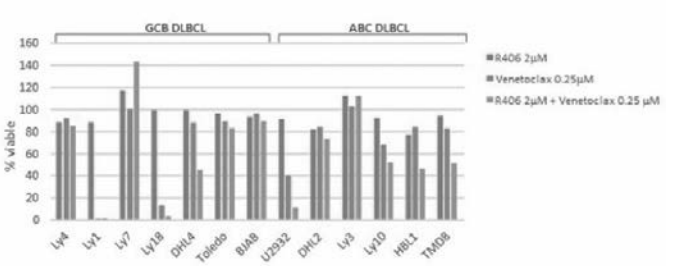

Figure 1. Viability of DLBCL cells following 24 hours *in vitro* treatment with R406, Venetoclax or both drugs combined. Values represent relative viability with respect to untreated cells.

Figure 1.

Summary/Conclusions: These data show that the SYK inhibitor R406 can significantly increase the sensitivity to venetoclax in the vast majority of BCL-2 positive DLBCL cell lines. The mechanisms of action require further investigation, but are likely to involve downregulation of MCL-1 and upregulation of HRK in a substantial proportion of cases.

E1376

VB EXPRESSION ASSESSMENT AND CLONALITY DETECTION IN T-CELL PROLYMPHOCYTIC LEUKEMIA (T-PLL) BY FLOW CYTOMETRY (FCM) AND NEXT GENERATION SEQUENCING (NGS): A COMPARISON OF BOTH METHODS

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Background: Vβ repertoire analysis can distinguish monoclonal from polyclonal (reactive) T-cell proliferations. The molecular quantification of clonal T-cell receptor (TR) gene rearrangements can also be used to record minimal residual disease (MRD) in T-cell malignancies. TR clonality can either be assessed by FCM employing Vβ antibody panels covering ~70% of the normal human TR Vβ repertoire or by molecular techniques like NGS with primers that amplify virtually all possible Vβ-Jβ rearrangements. T-PLL is the most common mature (post-thymic) T-cell leukemia. Clonal TR gene rearrangements are detected in virtually all T-PLL by FCM or PCR from peripheral blood (PB) or bone marrow samples.
Aims: To compare the results of parallel TRB-based clonality analyses by FCM and NGS in T-PLL.
Methods: We investigated diagnostic PB leukocytes of 73 T-PLL patients with median lymphocytes at 66% (range 13-93; harboring T-cells at 97% (55-100)). FCM of surface (not intracellular) Vβ expression was assessed by the IOTest Beta Mark kit (Beckman Coulter). Libraries for NGS were prepared using 100ng of DNA via a 2-step PCR and sequenced on the Illumina MiSeq (2x250bp, v2) with a median coverage of 17,908 reads (range 1,125–41,193)/sample. In the first PCR TRB rearrangements were amplified using TRB BIOMED-2 V- and J-region primers (van Dongen et al, Leukemia 2003). In the second PCR step, sequencing adaptors and sample-specific barcodes were added. Annotation of V, (D)- and J-regions of TRB sequences was done using ARResT/Interrogate (Bystry et al, Bioinformatics 2016).
Results: In all samples one or two dominant clonal TRB rearrangements were detected by NGS and represented in median by 83% of reads (range 15-90%). In 36/73 (49%) of these cases, also FCM demonstrated clonality. Interestingly, in 8/36 (22%) of cases the dominant Vβ by FCM differed from the molecular clonotype. In 5 of these cases the discrepancy was most likely accountable to a non-functional TRB clonotype detected by NGS corresponding to a bi-allelic TRB rearrangement with the second non-functional allele being preferentially identified by NGS. In 37/73 (51%) of cases no reactivity with one of the Vβ antibodies was seen. In 16 (43%) of these cases this could be attributed to expression of a TRB rearrangement for which the appropriate Vβ antibody was not present in the FCM panel. In another 12 (33%) of these cases a non-productive TRB rearrangement represented the dominant NGS clonotype. However, in further 9 cases (24%), the functional TRB clonotype (TRBV 5-6, 6-5, 25-1, 18, 20-1, 27) was not detected by FCM despite theoretical coverage. Of note, overal 10/73 T-PLL (14%) lacked surface TRα/β chain expression.
Summary/Conclusions: T-cell clonality is detected by TRB NGS in all T-PLL, whereas FCM-based Vβ repertoire analysis identifies a dominant single Vβ

domain expression in only 49%. A substantial proportion of such failures of FCM-based clonality detection can be best explained by lost surface TR expression and the limited coverage of the Vβ antibody panel. NGS-based clonality analysis can overcome these limitations, because it detects virtually all TRV VB-JB rearrangements. On the contrary, NGS is more sensitive and therefore enables the detection of minor subclones, which has great appeal for MRD analysis. Nevertheless, flow cytometric Vβ spectratyping is a faster, cheaper, and less labourious alternative. It has the additional advantage of detecting the actual TR Vβ chain expression and of visualizing individual T-cell subsets for quantification of Vβ cell populations.

E1377

IRF4 EXPRESSION IS ASSOCIATED WITH RESPONSE OF MANTLE CELL LYMPHOMA TO BRUTON'S TYROSINE KINASE INHIBITORS

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Background: Mantle cell lymphoma (MCL) responds poorly to conventional chemotherapy. Inhibitors of Bruton's tyrosine kinase (BTKi) have unexpectedly shown significant clinical effect; however despite this success, approximately one third of MCL patients have primary resistance to the drug, and patients who initially respond to treatment frequently acquire secondary resistance and aggressive relapse of the disease. Understanding how BTKi-resistance or sensitivity is mediated can identify new targets for therapy or predictive biomarkers of response. Using an *in vitro* model system we have identified the transcription factor IRF4 as a sensitive indicator for BTKi response in MCL cell lines and primary cells.
Aims: To identify molecules or pathways responsible for resistance to BTK inhibitor-drugs in mantle cell lymphoma using cell line models and primary cells.
Methods: Primary cells and validated MCL cell lines (REC-1, G519, JEKO-1, JVM2) were cultured either alone, or together with murine stromal cells (with or without CD40L transfection). The BTKi sensitive REC-1 cell line was continuously treated with BTKi to generate an acquired resistance model. Cultures were treated with BTKi drugs: ibrutinib or acalabrutinib in the presence or absence of B-cell receptor or CD40L stimulation, and their sensitivity or resistance to treatment was determined using flow cytometry to assess proliferation (Ki67), apoptosis (Annexin-V), or phosphorylation of BTK (pY223). Changes affecting downstream proteins were determined by protein expression or phosphorylation analysis (immunoblotting) and by mRNA expression (RT PCR). Following initial experiments the studies focussed on IRF4.
Results: Each MCL cell line showed basal phosphorylation of BTK (Y223) and its downstream effector molecule ERK1/2 (Y204/187); in each case phosphorylation was prevented by BTKi. Of the cell lines tested however, only REC-1 cells showed growth inhibition by BTKi (ibrutinib and acalabrutinib), demonstrating both dose-dependent apoptosis (p=0.01) and inhibition of proliferation. Further investigation showed that only the BTKi-sensitive REC-1 cell line downregulated IRF4 in response to BTKi; this downregulation was an early and specific response (mRNA downregulated after 4 hours, and protein expression after 8 hours). Furthermore in REC-1 cells with acquired partial resistance to BTKi, the downregulation of IRF4 was significantly less than in the parental cell line. Finally *in vitro* co-culture of REC-1 cells with CD40L prevented IRF4 downregulation and protected the cells from BTKi-induced apoptosis. Experiments with primary MCL cells reinforced these findings: *in vitro* CD40L induced proliferation, survival, prevented BTKi-induced IRF4 downregulation and protected the cells from BTKi-induced death. These findings were confirmed using *ex vivo* samples from treated patients (n=7) analysed before and during BTKi treatment: IRF4 was downregulated in 6 samples from patients shown to be clinically responding to BTKi and was not downregulated in 1 refractory case.
Summary/Conclusions: CD40L encountered in the cellular microenvironment supports the proliferation and survival of MCL cells, and protects them from the effects of BTK inhibition. This study has identified that BTKi induces downregulation of IRF4 in sensitive but not resistant MCL cells, and that downregulation is opposed by CD40L. This suggests that the expression of IRF4 following treatment with BTKi might be a biomarker for BTKi-sensitivity in MCL, and that proteins modulated by IRF4 may play an important role in MCL treatment response.

E1378

LOSS OF TPL2 KINASE ACCELERATES MYC-INDUCED LYMPHOMAGENESIS

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Background: While MYC t(8;14)(q24;q32) translocation was initially identified as a hallmark of Burkitt lymphoma, a number of other B-cell neoplasms are associated with MYC deregulation. These MYC-driven non-Hodgkin lymphomas have aggressive clinical behavior and respond poorly to treatment. However, MYC-dependent lymphomagenesis is believed to require additional oncogenic alterations, such as deregulation of genes that counteract the proapoptotic functions of MYC. TPL2 is a MAP3 kinase with an obligatory role in inflammatory signal transduction on the MEK/ERK axis but little is known about its involvement in B lymphocyte biology and lymphomagenesis.

Aims: The aim of this study is to define the impact of and the mechanism by which TPL2 kinase affects MYC-induced lymphomagenesis.
Methods: CD19+ positive B lymphocytes were isolated from peripheral blood of human healthy individuals and mouse B cells from spleens of WT (C57BL/6) and *Eμ-myc* transgenic mice engineered to overexpress c-*myc* in B cell progenitor cells under the control of the IgH chain enhancer. Mouse pre–B lymphocytes were isolated from bone marrow by flow cytometric cell sorting. Differentiation status of lymphomas was analysed by flow cytometry using B220, IgM and IgD antibodies. The TPL2 RNA and protein expression levels were assessed by qPCR and Western blot analysis, respectively. The extent of apoptosis was estimated by immunohistochemical evaluation of activated caspase-3 in paraffin embedded mouse lymphoma tissues and by flow cytometry using Annexin and 7AAD staining of *ex vivo* cultured lymphoma cells following cytokine deprivation.
Results: TPL2 RNA levels were found dramatically decreased in various human Burkitt lymphoma cell lines as well as in 7 primary Burkitt lymphoma biopsies compared to B lymphocytes of healthy individuals. In line with this finding, both pre-B and B lymphomas derived from *Eμ-myc* mice express very low levels of TPL2 both at RNA and protein level, compared to pre-B and splenic B lymphocytes isolated from WT mice. Interestingly, pre-B and B lymphocytes of healthy (premalignant) *Eμ-myc* mice express TPL2 in comparable levels to their WT counterparts, suggesting that the reduction of TPL2 expression in lymphomas is an additional oncogenic alteration. In this regard, genetic ablation of TPL2 in *Eμ-myc* mice (*Eμ-myc/tpl2*$^{-/-}$) significantly shortened their survival to 92 days from 140 days of Eμ-*myc/tpl2*$^{+/+}$ mice (p<0.005). Eμ-*myc/tpl2*$^{-/-}$ mice also displayed a trend to develop more pre-B cell lymphomas compared to Eμ-*myc/tpl2*$^{+/+}$ mice. This may be attributed to the decreased TPL2 expression in mouse pre-B lymphocytes, while it is upregulated in mature B lymphocytes. Finally, *Eμ-myc/tpl2*$^{-/-}$ lymphomas displayed reduced levels of apoptosis.

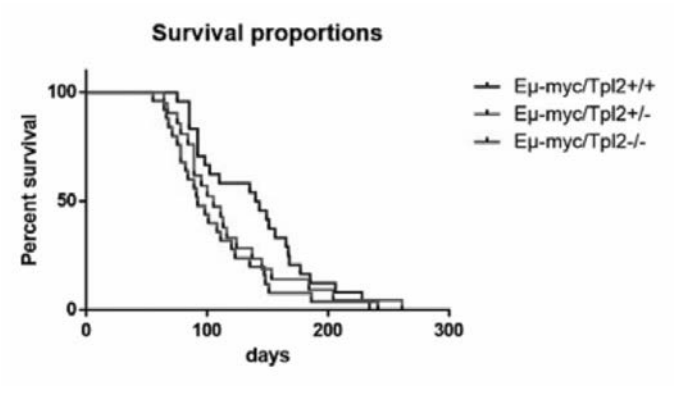

Figure 1.

Summary/Conclusions: This study reveals a novel pathway during myc-driven lymphomagenesis. We show that MYC deregulation imposes selective pressure in favor of clones with decreased expression of TPL2 kinase. This process seems to be advantageous for the malignant clone, since genetic ablation of TPL2 in the Eμ-myc mouse model accelerates MYC-induced lymphomagenesis likely by contributing to apoptosis resistance.

E1379

LIQUID BIOPSY: DECIPHERING A SIGNATURE OF CIRCULATING MICRORNAS AS NOVEL NON-INVASIVE BIOMARKERS IN DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Up to 40% of Diffuse Large B-Cell Lymphoma (DLBCL) patients still experience treatment failure or disease relapse after conventional chemoimmunotherapy. Therefore, the search of novel non invasive biomarkers able to early identify these patients is warrant in order to offer a different therapeutic approach. Recently, bodily fluids have emerged as an important source of infor-

mation in several diseases analyzable by liquid biopsies, representing minimally invasive methods for precision diagnostics and prognosis. Blood extracellular microRNAs (miRNAs) are under investigation as novel biomarkers. While tissue miRNAs in DLBCL patients have been extensively studied, only few reports, and limited to a small subset of miRNAs, evaluated the role of circulating/serum miRNA as potential prognostic factors.
Aims: To identify and validate a serum miRNA signature with prognostic value in a cohort of newly diagnosed DLBCL patients.
Methods: This is a on-going prospective non interventionist study on a cohort of newly diagnosed *de novo* DLBCL patients uniformly treated with six courses of R-CHOP (Rituximab, Cyclophosphamide, Vincristine, Doxorubicin and Prednisone). Serum samples of patients were collected at diagnosis and after the end of treatment. Treatment response was evaluated by standard Cheson criteria. The expression profile of selected circulating miRNAs described as associated with lymphoid malignancies by us (let-7c/miR-99a/miR-125b cluster) and by previously published studies (miR-22, miR-18a and miR-20a) was evaluated by quantitative real time polymerase-chain reaction (qRT-PCR) in serum samples collected at diagnosis of the first 18 patients enrolled into the study.
Results: Our results showed that the expression level of serum miR-22 as well as let-7c/ miR-99a/125b cluster was significantly higher at diagnosis, in patients unresponsive to R-CHOP treatment when compared with responsive patients. On the contrary, miR-18 and miR-20 levels appeared to be not significantly associated to treatment response. In addition, a global expression profile of circulating miRNAs was evaluated in serum samples derived from a smaller cohort of patients (n=4) after first-line chemo-immunotherapy. Interestingly, we found a striking difference in miRNA modulation upon treatment between unresponsive and responsive patients. In particular, we found 31 miRNAs significantly modulated after R-CHOP in the group of responsive patients, including miR-22. In contrast, this miRNA subset did not show remarkable expression changes in unresponsive patients. Moreover, we performed a study interrogating The Cancer Genome Atlas (TCGA) database about miRNA expression levels in samples of DLBCL patients. We found that the only available data are relative to the miRNA expression levels in tumor tissue samples of 47 out of 58 DLBCL patients. Kaplan Meier method and log-rank test revealed a signature of 13 miRNAs with potential prognostic value. Among these we found that miR-22, also emerged as modulated in our genome-wide analysis, was linked to risk of disease recurrence.
Summary/Conclusions: These preliminary data suggest that the serum miR-22 as well as miR-99a/let-7c/miR-125b miRNA cluster are of potential interest as non–invasive biomarkers to predict therapeutic response in DLBCL patients. Ongoing experiments in a wider cohort of patients are aimed to confirm these results and unveil potential miRNA signature with predictive value.

E1380

INTRACELLULAR CALCIUM AND METABOLISM HAVE CRITICAL ROLES IN DETERMINING ANTI-CD20 ANTIBODY EFFICACY IN DLBCL

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Background: Since the discovery and utilisation of the Type-I anti-CD20 antibody Rituximab, many have tried to enhance the efficacy of anti-CD20 antibodies in order to improve first-line treatment of B cell malignancies, leading to the evolution of Type II humanised anti-CD20 antibodies.To date, the complete biological role of CD20 and the mechanism of anti-CD20 antibody action remains unclear. However, CD20 has been shown to be involved in the store operated calcium (Ca2+) system. This complex has the ability to facilitate mitochondrial membrane permeabilisation, resulting in reduced mitochondrial function. Basal oxidative phosphorylation (OxPhos), ATP production, and maximal and spare respiratory capacity of cells can be calculated as a measure of mitochondrial function.
Aims: i) Assess and compare intracellular calcium concentration following treatment with anti-CD20 antibodies ii) Evaluate mitochondrial function of cells following treatment with anti-CD20 antibodies iii) Assess whether cytotoxicity of Type-I and Type-II anti-CD20 mAbs can be enhanced by exploiting cellular metabolism
Methods: We established a panel of four DLBCL cell lines (Karpas422, Pfeiffer, OCI-LY7 and SUDHL4). Following a 24-hour treatment with one of four anti-CD20 antibodies [one Type-I (Rituximab) and three Type-II anti-CD20 antibodies (BHH2, Obinotuzomab and Tositumomab)], intracellular calcium concentration was quantified and visualised using imaging flow cytometry. Next, we used the XF Seahorse Mito Stress Test to reveal bioenergetic profiles of the cell lines following a 24-hour treatment with the same antibodies. We used Metformin to inhibit oxidative phosphorylation (OxPhos) and then characterised the bioenergetic profile of our panel of cell lines again, this time to assess how combining each anti-CD20 antibody with an OxPhos inhibitor affected mitochondrial function. Metformin was also used to reduce the mitochondrial membrane potential (MMP) across our panel of cell lines. We confirmed MMP reduction by staining cells with JC-1, a chameleon dye used as an indicator of MMP and analysed samples using flow cytometry. Under the same conditions, we conducted clonogenic survival assays to see whether cytotoxicity of anti-CD20 antibodies could be enhanced by manipulating metabolism.
Results: Intracellular calcium concentration was decreased across our panel of cell lines following a 24-hour treatment with all Type-II anti-CD20 antibodies in our panel. This decrease was not observed following treatment with the Type-I anti-CD20 antibody Rituximab. Treatment with anti-CD20 antibodies resulted in a significant increase in the maximal respiratory capacity of our panel of cell lines; cells were able to produce more ATP in response to oxidative stress. Conversely, pharmacological inhibition of OxPhos impaired mitochondrial function, causing a significant reduction in basal OxPhos and in maximal respiratory capacity. Under this condition, cells were unable to increase ATP production in response to oxidative stress. We also show that treatment combining Metformin with either Type-I or Type-II anti-CD20 antibodies prevents the increase in maximal respiratory capacity observed with anti-CD20 antibody treatment alone. When analysing the clonogenic survival of cell lines, we have found that only the cytotoxicity of Type-II anti-CD20 antibodies is enhanced by simultaneously treating cell lines with Metformin.
Summary/Conclusions: Our data show for the first time that when cells are treated with Type-II anti-CD20 antibodies, intracellular calcium is decreased. Intracellular calcium remains unchanged following treatment with Rituximab. Next, we show anti-CD20 antibody treatment causes cells to increase maximal mitochondrial respiratory capacity to compensate for reduced basal mitochondrial function. We show that inhibition of OxPhos disables the cells from being able to compensate for the reduced mitochondrial function that is caused by CD20-antibody treatment. Importantly our data show that combining Metformin with Type-II CD20 antibodies leads to enhanced cytotoxicity, with a significant reduction in clonogenicity in our panel of DLBCL cell lines.

E1381

CYCLIN D1 ONCOGENIC OVEREXPRESSION LEADS TO A GLOBAL TRANSCRIPTIONAL DOWNREGULATION IN MALIGNANT LYMPHOID CELLS

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Background: Cyclin D1 is an oncogene frequently overexpressed in human cancers. In hematologicl neoplams, mantle cell lymphoma and multiple myeloma are clear exemples of deregulated cyclin D1 expression. It plays a dual function as cell cycle and transcriptional regulator, although the latter is widely unexplored.
Aims: In this study, we investigate the transcriptional role of cyclin D1 in lymphoid tumor cells. We aim to study initial cyclin D1 oncogenic overexpression in B cells as a model of the first steps in MCL oncogenesis.
Methods:
Chromatin immunoprecipitation (ChIP) followed sequencing was performed in four established MCL cell lines. RNA-Sequencing (RNA-Seq) and information from histone ChIP-Seq were correlated with genomic intervals displaying cyclin D1 binding. Transcriptional downregulation was studied through cytometric RNA total quantification in lymphoblastic cyclin D1-overxpressing models and RNA Pol II ChIP-Seq.
Results: Endogenous cyclin D1 showed widespread binding to active promoters and its overexpression was responsible for a global transcriptional downmodulation. Cyclin D1, instead of showing specific gene activation, seems to globally decrease cell transcription. Mantle cell lymphoma and multiple myeloma cell lines displayed an inverse relation with cyclin D1 quantity. This transcriptional effect was associated with an increased RNA polymerase II pausing in promoters due to cyclin D1 overexpression.
Summary/Conclusions: This mechanism expands the oncogenic cyclin D1 functions and places the transcriptional machinery as a potential therapeutic target in cyclin D1 overexpressing tumors.

E1382

MICROENVIRONMENTAL EXPRESSION OF IMMUNOREGULATORY MOLECULES AND CYTOKINES IN CLASSICAL HODGKIN LYMPHOMA PROGNOSIS

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Background: Over the past decade, new biologic insights have revealed a key role of tumor microenvironment in the pathogenesis of classical Hodgkin`s lymphoma (cHL). cHL infiltrating cells produce cytokines and growth factors that provide essential stimulatory signals for survival and proliferation of Hodgkin`s and Reed–Sternberg cells. Moreover, clinical behavior of cHL may be directly regulated by the cross-talk between tumor cells and infiltrating immune cells.

Aims: The aim of our study was to estimate the role of microenvironment expression of immunoregulatory molecules (PD-1 ligands, IDO) and cytokines (TGF-β, IL-13) in clinical outcome of cHL.
Methods: 74 patients (median age: 44, range: 17-71 years; males: 22, females: 52) were included in the study. 55.4% of patients were diagnosed with an early stages of HL, while 44.6% - with advanced stages. ABVD or BEACOPP (14/esc) were administered as a 1st-line therapy. 78.3% of patients achieved remission (CR/PR), while 8.1% had progression of disease during the therapy. We recorded 14.8% relapses in patients after the 1st line therapy during the follow-up period (median duration – 36 months; range 6–66 months). PD-L1, PD-L2, IDO, TGF-β, IL-13 mRNA expression levels were analyzed in fresh pre-treatment lymph nodes biopsies using qRT-PCR.
Results: Expression of PD-1 ligands was heterogeneous across the samples and did not depend on histological variant or stage of cHL. Only 12.1% of patients (9/74) were PD-L1 negative and all but one of those cases had a CR and a long-term remission. Patients with PD-L1 overexpression tended to have a higher rate of relapse, comparing to low/absent PD-L1 expression (p=0.1). We did not find any significant association between PD-L2 expression level and clinical outcome of cHL. Expression levels of IDO, TGF-β, IL-13 were evaluated in 38 cHL samples. 18.4% (7/38) patient were IDO positive and 81.6% (31/38) - IDO negative. The presence of IDO expression was associated with a higher risk of relapse in cHL patients (p=0.006). 85.7% (6/7) and 23.3% (7/30) of relapses were observed during the follow-up period in IDO+ and IDO- patients, respectively (p<0.05). The patients with double negative expression of PD-L1 and IDO were noted to have a favourable outcome of cHL. A 5-year event-free survival (EFS) rate was 80% for double negative PD-L1-/IDO- patients' *vs* 20% for double positive PD-L1+/IDO+ patients (p=0.008). IL-13 was expressed at various levels depending on the stage of cHL with the highest expression levels in advanced stages. A trend for a higher risk of relapse was observed for HL patients with increasing level of IL-13, (p=0.23). TGF-β expression level was not association with histological variant or stage of cHL, however multivariate analysis showed that $TGF\beta^{+}$ expression is a significant increase EFS in cHL patients with HRs of 6.7 [95% (CI) 1.3-2.1, p=0.04].
Summary/Conclusions: Our results suggest that tumor microenvironment plays an important role in clinical behavior of cHL. Hence, better understanding of molecular mechanisms of interacted between tumor and immune cells probably can provide us with a novel promising strategy for relapsed/refractory cHL treatment.

E1383

AN IN VIVO TRACEABLE AND MULTIPLEXING CRISPR/CAS9 GENOME EDITING SYSTEM

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Background: Gene gain of function and loss of function mutations, oncogene overexpression, gene amplification, chromosome deletion and epigenetic changes, may lead to lymphoma onset.The CRISPR-Cas9 genome editing system has become a feasible tool for exploring the functions of specific genes in different contexts.We want to use this technique to screen for lymphoma suppressor genes.
Aims: Construct an in vivo traceable and multiplexing CRISPR-Cas9 gene editing system, which is high efficient for studying *in vivo* functions of both individual genes or any given chromosome fragment.

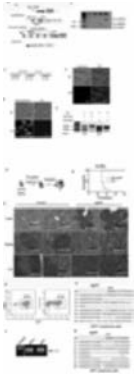

Figure 1.

Methods: Two retroviral vectors were constructed via molecular clone, one of which contains a locus for tandem U6-sgRNAs and inducible GFP reporter gene and the other contains Cas9 and tTA genes. This system's function of traceable and simultaneously mutate multiple gene efficiencies were validated in vitro. Eμ-myc HSPCs retrovirally transduced with sgp53 and Cas9 were transplanted into sublethally irradiated C57/BL6 mouse.
Results: Co-transduced cells can be tracked by the expression of GFP protein and multiple sgRNA can be efficiently introduced to the GFP-labeled cells for simultaneously mutating multiple genes or deleting a large chromosome fragment. Further we applied this system for both *in vitro* and *in vivo* genome editing. As an example, we show that *Trp53* mutation accelerated E*μ*-Myc driven lymphoma onset *in vivo.*
Summary/Conclusions: This traceable and multiplexing CRISPR/Cas9 system might be useful for various genome editing applications.

E1384

Abstract withdrawn.

E1385

HDAC6 INHIBITION SENSITIZES TUMOR CELLS TO ANTI-CD20 IMMUNOTHERAPY *IN VIVO*

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Background: Down-regulation of CD20, a molecular target for monoclonal antibodies, constitutes a clinically significant issue leading to decreased efficacy of anti-CD20-based therapeutic regimens. The epigenetic modulation of CD20 coding gene (*MS4A1*) has been proposed as a mechanism for the reduced therapeutic efficacy of anti-CD20 antibodies and confirmed previously with clinically available non-specific histone deacetylase pan-inhibitors (HDACis). However, the identification of particular HDAC isoforms involved in CD20 regulation seems to be of paramount importance. Since the use of pan-HDACi is associated with substantial side effects, especially difficult to manage in elderly and frail patients, the new specific HDAC6 inhibitors are currently being tested in multiple myeloma and non-Hodgkin lymphoma. They have already been shown to sensitize tumor cells to proteasome inhibitors and novel kinase inhibitors *e.g.* ibrutinib and demonstrated promising results in *in vitro* studies in chronic lymphocytic leukemia (CLL).
Aims: HDAC6 has been known for its regulatory role in protein degradation. We have previously reported that inhibition of proteasome activity can effectively increase CD20 levels in tumor cells. In our study we tested the hypothesis that selective HDAC6 inhibition sensitizes tumor cells to immunotherapy with anti-CD20 monoclonal antibodies (mAbs) by regulating CD20 levels.
Methods: We assessed the influence of HDAC6 inhibition in a panel of different subtypes of human lymphoma cell lines (Burkitt, DLBCL; both EBV+ and EBV- cells) on CD20 expression using flow cytometry and Western blotting. We confirmed our observations in primary samples from the patients with CLL, known to express low CD20 levels. Moreover, we performed cytotoxic assays using flow cytometry in order to assess complement-dependent cytotoxicity (CDC) as well as apoptosis. We used HDAC6-specific chemical inhibitors (tubacin, trichostatin A and clinically tested ricolinostat), as well as HDAC6 shRNA assay. We also performed animal studies using SCID mice injected with Burkitt CD20+ lymphoma cell line s.c. and treated with rituximab i.p. We used both the pharmacological (i.p. administration of ricolinostat) and genetic (cells stably transduced with HDAC6 shRNA) approach.
Results: The results of our studies demonstrate that HDAC6 inhibition significantly increases CD20 level and sensitizes tumor cells to rituximab- and ofatumumab-induced CDC, as well as to direct cytotoxicity of obinutuzumab. In *in vivo* settings HDAC6 inhibition potentiated the efficacy of rituximab by significantly reducing tumor size and prolonging the survival of the mice.
Summary/Conclusions: Our results clearly indicate that HDAC6 inhibition sensitizes tumor B-cells to anti-CD20 immunotherapy. Therefore, we propose HDAC6 inhibition with specific inhibitors as an effective strategy to be associated with the therapy with anti-CD20 mAbs. This strategy seems to be highly promising in CLL patients, often expressing very low CD20 level and do not fully benefiting from immunotherapy.

The work was supported by National Science Center 2013/09/N/NZ3/01407 (MB), 2015/16/T/NZ6/00034 (MB), 2014/13/N/NZ6/02081 (MS), 2015/18/E/NZ6/00702 (MW), 2013/11/B/NZ5/03240 (BP), Polish Ministry of Science and Higher Education grant IP2011 060271 (MW), DI2011 021241 (MB), DI 2014007344 (NM) and DI2013 006643 (AD), the Medical University of Warsaw grant 1M19/PM/112D/14/14 (MB) and Horizon 2020 Programme, project 692180-STREAM-H2020-TWINN-2015 (JG).

E1386

NKP46 EXPRESSION IS A DIAGNOSTIC AND PROGNOSTIC BIOMARKER IN PRIMARY GASTROINTESTINAL T-CELL LYMPHOPROLIFERATIONS. A CELAC NETWORK STUDY

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Background: Primary gastrointestinal (GI) T-cell lymphoproliferations (T-CL) are heterogeneous entities, which diagnoses are difficult to perform. T-CL include aggressive lymphoma such as enteropathy-associated T-cell lymphoma (EATL) as well as indolent monoclonal lymphoproliferations. Refractory coeliac disease type II (RCDII) is one of the indolent clonal T-CL that complicates coeliac disease (CD) and may evolve toward an overt EATL. The differential diagnosis of RCDII from CD and RCDI is difficult and essentially based on negative expression of sCD3 and CD8 and the presence of a clonal TCR rearrangement. Lymphocytes from RCDII are dependent for survival on IL-15, which reprograms T lymphocytes towards a cytotoxic NK phenotype.
Aims: We thus studied the expression of NKp46 on a representative panel of GI T-CL to assess its diagnosis and prognosis value.
Methods: Using formalin-fixed paraffin-embedded tissue biopsies, we assessed NKp46 expression by immunohistochemistry (IHC) and investigated its clinical and biologic significance on 177 intestinal, 11 lymph node and 7 other biopsies from 84 CD or RCD patients (RCDI, n=20; RCDII, n=40), 44 GI T-cell lymphoma patients (EATL, n=25; monomorphic epitheliotropic intestinal T-cell lymphoma_MEITL, n=4; indolent T-LPD, n=15), 11 healthy patients and 5 patients with a GI inflammatory environment as controls.
Results: By doing ROC analysis on number of cells expressing NKp46 on GI-TCL we identify that 25 intra-epithelial lymphocyte (IEL) per 100 epithelial cells (EC) clearly separates RCDII from CD and RCDI patients, with a good positive and negative predictive values (100 and 95% respectively). In healthy controls, CD or RCDI patients, NKp46 was only expressed on scattered IEL (median 3%, 0-15). Based on NKp46 expression the overall survival is poor if over 25% of IEL are positive for NKp46 (OS-5years 96.4% *vs* 72.8%, P=0.0004) (Figure 1A). Among patients with GI T-cell lymphoma, we show that NKp46 was expressed in most of aggressive lymphoma (EATL 80%, n=20/25 and MEITL 100%, n=4/4). On the other hand, NKp46 was never expressed on indolent T-LPD (n=0/15). The NKp46 expression was also associated with a poor prognosis in GI T-cell lymphoma patients (OS-5years 50.5% *vs* 5.4%, P=0.0011) (Figure 1B).

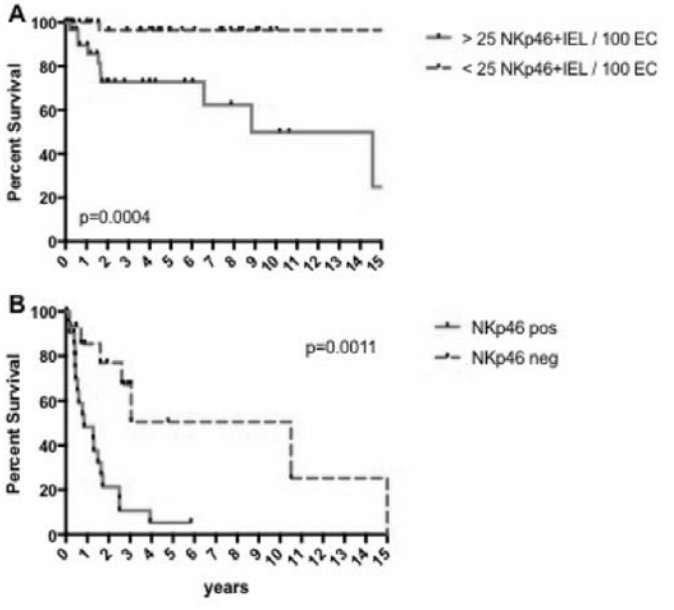

Figure 1.

Summary/Conclusions: The NKp46 expression in more than 25 IEL per 100 EC by IHC analysis can easily identify RCDII from CD and RCDI. Furthermore, the NKp46 expression is associated with aggressive forms of GI T-cell lymphoma. Finally, the NKp46 expression was strongly associated with shortened survival. Thus NKp46 provides a new biomarker for both diagnosis and prognosis in GI T-CL.

E1387

HIGH EXPRESSION LEVELS OF MIR23A CLUSTER IN DLBCL ANTAGONIZE INDUCTION OF APOPTOSIS

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Background: The microRNA cluster MIR23A, which encodes for the mature microRNAs miR-23a, miR-27a and miR-24, was shown to be deregulated in many different malignancies including subtypes of B cell non-Hodgkin lymphoma (B-NHL). Furthermore, high expression of miR-23a was correlated with poor overall survival in diffuse large B-cell lymphoma (DLBCL) patients (Wang *et al*., Med Oncol. 2014) indicating that miR-23a might act as an onco-miR (tumor promoting microRNA) in this entity. However, both targets and function of the MIR23A cluster in B-NHL remain unknown.
Aims: This study aims to elucidate the role of the MIR23A cluster as a potential onco-miR in DLBCL by identification of the lymphoma-specific targetomes of miR-23a and miR-27a and subsequent analyses of associated functions.
Methods: DLBCL model cell line U-2932 R1, which has a low basal expression level of MIR23A cluster, was used for the lentiviral-based generation of clones overexpressing miR-23a, miR-27a, or a scrambled control. Differentially expressed genes (DEG, fold-change >2, p-value <0.05) between samples were determined by mRNA sequencing (RNA-Seq). miR-23a and miR-27a targetomes were identified by immunoprecipitation of AGO2-bound mRNA (AGO2-RIP) followed by RNA-Seq. MicroRNA targets had to be enriched >2-fold with a p-value <0.05. Validations were performed by qPCR and immunoblotting. Gene set enrichment analyses (GSEA) and GO-term analyses were applied on identified targetomes and DEG to predict microRNA associated functions. Apoptosis was assessed by Annexin-V staining followed by FACS analyses as well as in immunoblots.
Results: Overexpression of miR-23a and miR-27a, respectively, in a DLBCL model cell line resulted in global alterations of gene expression (so-called indirect targets) with a substantial overlap of 104 of DEG affected by both microRNAs. Using AGO2-RIP, 26 novel direct targets of miR-23a, and 20 novel direct targets of miR-27a were identified. GSEA and GO-term analyses of direct and indirect targets indicated that the MIR23A cluster might regulate processes in apoptosis. Moreover, *BBC3* which encodes the pro-apoptotic protein PUMA was one of the identified direct targets of miR-27a. After etoposide induced apoptosis, miR-27a overexpressing DLBCL cells failed to induce PUMA on protein level. Importantly, functional analyses confirmed that miR-23a overexpression reduces and high levels of miR-27a significantly attenuate the ability of DLBCL cells to undergo apoptosis in response to DNA damage.
Summary/Conclusions: We demonstrate that high levels of miR-23a and miR-27a antagonize induction of apoptosis in a DLBCL model cell line. This might be one possible explanation why DLBCL patients with high miR-23a expression levels have a worse overall survival rate than patients with low levels. Thus, future studies should address the suitability of the MIR23A cluster as biomarker and potential target in DLBCL.

E1388

PLASMA CELLS ARISE FROM DIFFERENTIATION OF CLONAL LYMPHOCYTES AND SECRETE IGM IN WALDENSTRÖM MACROGLOBULINEMIA

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Background: Waldenström Macroglobulinemia (WM) is an indolent non-Hodgkin lymphoma characterized by bone marrow infiltration with malignant cells and hypersecretion of monoclonal immunoglobulin M (IgM). The malignant infiltrate comprises of two distinct cellular populations: the predominant lymphoplasmacytic cells (LPLs), and a smaller number of plasma cells (PCs).
Aims: In this study, we aimed to characterise the immunophenotype, molecular genetics and secretory function of PCs in 2 WM cell lines.
Methods: Using FACS, we identified LPLs as $CD45^{bright}/CD38^{dim}/CD138^{-}/CD19^{+}/CD20^{+}$cells and PCs as $CD45^{dim}/CD38^{+}/CD138^{+}$ cells from 2 cell lines - MWCL1 (Ansell lab) and BCWM.1 (Treon lab). We used standard PCR and Sanger sequencing to assess MYD88 (using MYD88 L265P specific primers) and Immunoglobulin heavy chain (IgHV) (using Biomed2 specific primers for FR3) status. Finally to determine which population was predominantly responsible for IgM hypersecretion, isolated PCs and LPLs from both cell lines were kept in culture for 72 hours and the culture media analysed by ELISA for IgM secretion.
Results: Using a conservative sorting strategy, we analysed 2 WM cell lines MWCL1 and BCWM.1, and found that MWCL1 had 5~6% PCs and 20-30% LPLs; while BCWM.1 had 4-5% PCs and 10-20% LPLs. Cells that were $CD38^{+}/CD138^{-}$ or $CD38^{-}/CD138^{+}$ were not included. Both cell lines expressed heterozygous MYD88-L265P mutation in both PC and LPL populations. We also observed the expression of the same auto-reactive IgHV sequences (VH3-15*01) in both PCs and LPLs from MWCL-1, suggesting similar clonal origin and a role for auto-antigens in WM cell survival. We noted VH3-23*01 in the LPL population of BCWM.1 but not in the PC compartment – the significance of this remains uncertain. Cell culture studies showed that PCs alone were primarily responsible for IgM production despite the relative lack of proliferation and eventual cell death in MWCL-1 (~65% plasma cells remained after 72

hours and produced 8.7 ~ 9.3 X 10^3 ng/ml of IgM). PCs isolated from BCWM.1 increased to 130% and produced 2.5 ~2.8 X 10^3 ng/ml of IgM. LPLs from both cell lines proliferated in culture (~ 130 ~ 140% in MWCL-1 and ~170 ~ 200% in BCWM.1 at 72 hours), gave rise to the more differentiated PCs (7.5 ~ 9.0% PCs at 72 hours in MWCL-1 and 1.2 ~ 1.4% PCs in BCWM.1), and secreted smaller amounts of IgM than PCs (3.5 ~ 5.0 X 10^3 ng/ml in MWCL-1 and 0.3 ~ 0.7 X10^3 ng/ml in BCWM.1.

Summary/Conclusions: Our analysis of the 2 WM cell lines provides evidence to support the common hypothesis that malignant PCs arise from the clonal malignant LPL population, and are primarily responsible for IgM secretion in WM.

E1389

LMP-1 MEDIATED UPREGULATION OF IL-2RA PROMOTES LYMPHOMAGENESIS AND CHEMOTHERAPY RESISTANCE IN NATURAL KILLER/T-CELL LYMPHOMA AND COULD BE A POTENTIAL THERAPY TARGET

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Background: Natural killer/T-cell lymphoma (NKTCL) is an Epstein–Barr virus (EBV)-associated, highly aggressive lymphoma. Treatment outcome remains sub-optimal, especially for advanced-stage or relapsed diseases. Our previous study demonstrated the prognostic value of IL-2Rα in NKTCL, but the role of IL-2Rα in the lymphomagenesis and chemotherapy resistance and its interactions with EBV in NKTCL remain to be investigated.
Aims: This study investigated the mechanism of IL-2Rα expression in NKTCL, and explored the role of IL-2Rα in lymphomagenesis and chemotherapy resistance as well as the potential role of anti-IL-2Rα treatment in NKTCL.
Methods: Expression of IL-2Rα was measured in NK-92 (LMP-1 weak expression) and SNK-6 (LMP-1 strong expression) cells by western blot, quantitative real-time PCR, enzyme-linked immunosorbent assay, and flow cytometry, respectively. LMP1-harboring lentiviral vectors were transfected into NK-92 cells to examine the correlation between LMP1 and IL-2Rα expression. Proteins in the downstream pathways of LMP1 signaling were measured in NK-92 cells transfected with LMP1-harboring or negative control vectors as well as in SNK-6 cells. Then IL-2Rα-harboring lentiviral vectors were transfected into both NK-92 cells and SNK-6 cells to examine the cell proliferation by CCK8, apoptosis by staining with Annexin V and detected by flow cytometry (FCM), cell cycle distribution by FCM analysis, and IC50 values exposed to three chemotherapy drugs (adriamycin, gemcitabine, and asparaginase) by MTT. Finally anti-IL-2Rα antibody was added to investigate its ability of reversal of drug resistance.

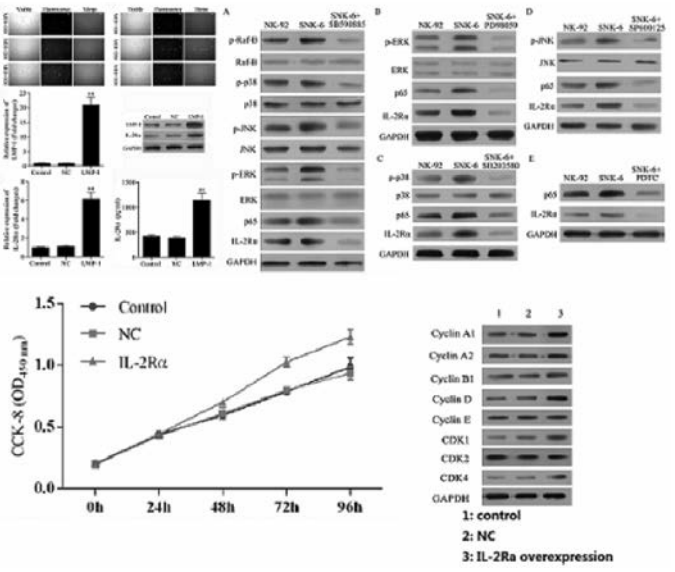

Figure 1.

Results: Expression of IL-2Rα was significantly higher in SNK-6 cells than in NK-92 cells, at both protein and mRNA levels. Expression of IL-2Rα was remarkably upregulated in NK-92 cells transfected with LMP1-harboring lentiviral vectors compared with those transfected with negative control vectors. Proteins in the MAPK/NF-κB pathway were upregulated in LMP1-expressing NK-92 cells compared with the negative control. Selective inhibitors of those proteins induced significant downregulation of IL-2Rα expression in LMP1-expressing NK-92 cells as well as in SNK-6 cells. When comparing with those transfected with negative control vectors, cell growth was significantly increased in both NK-92 and SNK-6 cells transfected with IL-2Rα-harboring lentiviral vectors, and the cell cycle assay displayed a significant decrease in the percentage of cells in the G0/1 phase ($p<0.05$) and an increase in the percentage of cells in the S phase ($p<0.05$), while apoptosis was not affected. Subsequent western blot tests demonstrated that cyclin A, B, D, and CDK1, 4 were involved in the regulation of cell cycle with overexpression of IL-2Rα. The IC50 values to all three chemotherapy drugs were significantly increased after overexpression of IL-2Rα ($p<0.05$), which can be fully reversed by addition of anti-IL-2Rα antibody.
Summary/Conclusions: IL-2Rα expression was upregulated in NKTCL by LMP-1-mediated activation of MAPK/NF-κB pathway. IL-2Rα can promote NKTCL cell proliferation partially through regulation of cell cycles and induce chemotherapy resistance, which can be reversed by anti-IL-2Rα antibody, indicating the potential role of IL-2Rα as a therapy target in NKTCL.

E1390

LENALIDOMIDE (LEN) DRIVES PROGRAMMED DEATH-1 (PD1) PATHWAY UPREGULATION IN A TUMOR MICROENVIRONMENT (TME) MODEL OF ACTIVATED LOW-GRADE LYMPHOMA CELLS

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Background: PD1 binding to its ligand PDL1 inhibits TCR/BCR signaling; impairs activation and effector functions of T- and B-cells; induces a state of T-cell exhaustion; and ultimately provokes tolerance towards cancer cells. PD1 is expressed on Hodgkin lymphoma (HL) and B-cell non-HLT-cells.The TME may play an essential role in maintaining PD1-induced immune exhaustion. LEN is an oral immunomodulator (IMID) with direct antineoplastic activity and immunologic effects, including stimulation of T-cell– and NK cell–mediated cytotoxicity in experimental models.Preclinical findings indicate that combination of IMIDs with immune checkpoints inhibitors may promote therapeutic synergy and long-term antitumor immunity to improve clinical outcome.
Aims: 1) To better characterize the PD1, PDL1 and the lesser-known PDL2, phenotype in peripheral neoplastic CD19+ lymphocytes and T-cell subsets in patients with low-grade B-cell lymphoma; 2)to evaluate the role of the TME in supporting the PD1 axis; and 3) to determine whether LEN influences PD1 or cognate ligand expression
Methods: Samples obtained from patients attending participating Hematology Units were used to determine PD1, PDL1, PDL2 phenotype (%±SEM) by Flow-cytometry (FC). Autologous activated T-cells (AAT) were obtained by *in vitro* co-culture of patient T-cells with anti-CD3/CD28 beads, rIL2 and with PBMCs. Cultures were monitored daily until sizeable clumping was observed and tested for PD1 and ligand expression. In selected experiments LEN(provided by Celgene) was added to cell cultures.
Results: Twelve cases of lymphoma were evaluated for PD1, PDL1 and PDL2 expression on malignant B- and T-cells by FC. The expression of PD1 and PDL2 was similarly expressed, while PDL1 was almost undetectable on B-cells. Levels of PD1 expression on CD3+ cells were variable across samples, however they were significantly higher than those expressed on malignant B-cells. Significantly higher PD1 expression and very low levels of ligands were detected inboth CD4+ and CD8+ cells.Co-culture of lymphoma cells with AAT cells showed consistent formation of B/T-cell clusters. Higher numbers of CD19+CD5-PDL2+ cells were detected than PDL1+ cells compared to baseline cells. PD1 expression also significantly increased in AAT co-culture on B-cells. PD1 expression

on CD3+ cells was unaffected by AAT, although the expression of both ligands increased significantly. Closer analysis of T-cell subsets showed that only in CD4+ cells, PD1 expression increased significantly following co-culture experiments.Preliminary data on lymphoma-AAT co-culture experiments (n=3) indicated that LEN (0.5/1 uM) did not negatively influence the formation of AAT clusters. After 48 h of co-culture, the expression of CD19+CD5-PDL1+ cells increased in 2/3 cases following LEN treatment while, PDL2 expression remained unchanged. PD1 expression gradually increased following exposure to LEN compared to untreated cells.CD3+ cells showed a significant increase in PD1 expression by LEN, while the expression of both ligands remained unaffected. Evaluation of activated T-cell subsets showed similar results, with the exception of stronger induction of PD1 and PDL1 expression by LEN in CD8+ cells.

Summary/Conclusions: Our data provide support for the potential involvement of the PD1-axis in lymphoma patients. Interestingly, LEN further induces the expression of PD1 in CD8+ and CD4+ cells and may contribute to reactivate PD1 signaling under treatment. The PD1 pathway may be potentially targeted to overcome both the intrinsic and LEN-induced exhaustion phenotype.

E1391

IDENTIFICATION AND DIAGNOSTIC APPLICATION OF GENOMIC NPM-ALK FUSION SEQUENCES IN ANAPLASTIC LARGE CELL LYMPHOMAS

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Background: ALK positive anaplastic large-cell lymphomas (ALCL) account for 10-15% of pediatric Non-Hodgkin lymphomas. Most of these patients carry the chromosomal translocation t(2;5)(p23;q35) resulting in the *NPM-ALK* fusion gene formation. The quantification of *NPM-ALK* fusion transcripts is a well-established tool for diagnostic purposes and risk stratification during the course of treatment.

Aims: Establishment of a PCR based assay to identify patient-specific genomic *NPM-ALK* fusion sequences for a DNA based monitoring of minimal residual disease in ALCL patients. Compared to RNA based methods the quantification of DNA is independent of the gene expression. Additionally, due to the higher stability of DNA, cell-free circulating tumor DNA (ctDNA) should be detectable in the patient´s plasma and may represent a tumor marker for "liquid biopsies" in ALCL.

Methods: Using a specifically designed multiplex long-range PCR assay, genomic *NPM-ALK* fusion sequences were identified in 45 ALCL patients. The genomic *NPM-ALK* breakpoints were analyzed concerning fine structure and breakpoint distribution pattern. Furthermore, the patient-specific genomic *NPM-ALK* fusion sequences were evaluated for their use as biomarkers in selected cases. For this purpose patient´s blood and plasma samples were quantified using a high sensitive digital droplet PCR assay.

Results: In more than 60% of cases the identified breakpoint was localized within repeat regions. The genomic breakpoints within the breakpoint cluster regions of the fusion genes were randomly distributed. Most of the *NPM-ALK* fusion sequences were characterized by the occurrence of small insertions or deletions indicating the involvement of the non-homologous end-joining (NHEJ) repair system for chromosomal translocation initiation.

Using a DNA based quantification assay in a subset of patients, the genomic *NPM-ALK* fusion sequences were detectable in circulating tumor cells in patient´s blood samples as well as cell-free tumor DNA in plasma samples.

Summary/Conclusions: The established multiplex long-range PCR assay is a useful diagnostic tool for the identification of genomic *NPM-ALK* fusion sequences. This individual tumor maker is independent of gene expression and can be used for therapy response monitoring and relapse detection.

E1392

ARSENIC TRIOXIDE TARGETS BCL6 FOR DEGRADATION AND INHIBITS THE PROLIFERATION OF BCL6-DEPENDENT DIFFUSE LARGE B-CELL LYMPHOMA

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Background: B-cell lymphoma 6 (BCL6) is a transcription repressor and is frequently over-expressed in diffuse large B-cell lymphoma (DLBCL). It suppresses the expression of its target genes ATR, TP53 and CDKN1A, leading to dysregulation of DNA repair and cell proliferation. It has been shown that BCL6 is an oncoprotein involved in the pathogenesis of DLBCL and represents a potential therapeutic target. Arsenic trioxide (ATO) targets various oncogenic proteins, including PML-RARA in acute promyelocytic leukaemia (APL), Tax in adult T-cell leukaemia/lymphoma (ATLL), cyclin D1 in mantle cell lymphoma (MCL), and NPM-ALK in anaplastic large cell lymphoma (ALCL), for degradation through the ubiquitin-proteasome pathway. ATO is now used for the management of APL, ATLL and MCL with proven clinical benefit.

Aims: To investigate if ATO targets BCL6 and inhibits the proliferation and growth of BCL6-dependent DLBCL

Methods: BCL6-dependency of a panel of DLBCL cell lines (*i.e.* OCI-Ly1, OCI-Ly7, SU-DHL-6, OCI-Ly18 and Pfeiffer) was determined based on their sensitivity to proliferation inhibitory activity of the BCL6 inhibitor 79-6 (Calbiochem). The effects of ATO and cisplatin as single agent or in combination on cell viability and apoptosis of DLBCL cells were examined with MTT assay and flow cytometric analysis. Expression of BCL6 and its target genes was examined with quantitative RT-PCR and western immunoblotting. The therapeutic efficacy of ATO treatment was also examined in a DLBCL (OCI-Ly7) xenograft mouse model.

Results: OCI-Ly1, OCI-Ly7 and SU-DHL-6 were highly sensitive to inhibitory activity of BCL6 inhibitor and were designated as BCL6-dependent. Treatment of DLBCL cells with ATO led to a decrease in BCL6 protein level and an upregulation of downstream targets of BCL6, including *PRDM1*, *CD44* and *CD69*. The effect of ATO on BCL6 protein were abrogated by treatment with proteasome inhibitormg132, suggesting that ATO targeted BCL6 for degradation through the ubiquitin-proteasome pathway. Interestingly, ATO also inhibited cell proliferation and induced apoptotic cell death of BCL6-dependent DLBCL cell lines, analogous to the effect of BCL6 inhibitor on these cells. In addition, there was a synergistic inhibitory and cytotoxic activity between ATO and cisplatin. Finally, ATO treatment suppressed the growth of DLBCL in a xenograft mouse model.

Summary/Conclusions: ATO targets BCL6 for proteasomal degradation and inhibits the proliferation and growth of BCL6-dependent DLBCL.

E1393

PROTEOMIC PHOSPHOSITE ANALYSIS IDENTIFIED CRUCIAL NIPA SERINE RESIDUES FOR NPM-ALK-MEDIATED TRANSFORMATION

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Background: Anaplastic large-cell lymphoma(ALCL) is an aggressive non-Hodgkin lymphoma that occurs mainly in children and younger adults. Patients typically show an advanced stage disease as well as an aggressive disease pattern with extralymphatic manifestations. At the molecular-genetic level, 60% of the patients with systemic ALCL exhibit a translocation t(2;5)(p23;q35), which leads to the expression of the NPM-ALK fusion protein. Under the control of the NPM promoter, ALK activation causes increased and autonomous cell proliferation. Nuclear interaction partner of ALK (NIPA) was first identified as a new interaction partner of the oncogene NPM-ALK in a yeast-2-hybrid screen which defines an E3-SCF ligase and is physiologically involved in cell cycle regulation at the transition from G2 phase to mitosis. It has already been shown in preliminary studies that co-expression of NIPA with the oncogenic tyrosine kinase NPM-ALK results in the constitutive phosphorylation of NIPA (Illert et al., 2012a). Until now, the specific signal transduction pathway, the crucial phosphorylation sites as well as the functional effect of the pathological NIPA phosphorylation in NPM-ALK-induced lymphomagenesis still remain unclear. Molecular insights into the activated pathways of the oncogenic tyrosine kinase NPM-ALK may help to identifiy new drugable targets for therapeutic implications.

Aims: In the present study, we investigated the molecular mechanisms as well as the functional impact of the NPM-ALK-induced NIPA phosphorylation.

Methods: For this purpose, biochemical methods with ALCL cells were used to examine functional effects of the constitutive NIPA phosphorylation. Moreover, we performed a "proteomic-phosphosite-analysis" to identify crucial NPM-ALK specific phosphorylation sites in NIPA. Based on these results, phospho-deficient NIPA mutants were generated to investigate the functional effect of this phosphorylation: MTT proliferation- and Softagar- Assays were performed after retroviral infection of Ba/F3 and primary Nipa-deficient MEF cells with NPM-ALK and the respective phospho-deficient NIPA to reveal transformation and growth ability.

Results: It has already been shown, that cell cycle dependent NIPA phosphorylation at critical serine residues 354, 359 and 395 leads to dissociation of the SCF-core complex. Interestingly, we were able to show in our study that constitutive phosphorylation of NIPA by NPM-ALK does not lead to changes in the SCF^{NIPA}-complex formation. Proteomic-Phosphosite-analyses identified 10 significantly upregulated (ratio >2; Log2Fold Change) NIPA phosphosites upon NPM-ALK expression. Interestingly, 80% of the identified Serine phosphoresidues lie within the NPM-ALK binding region of NIPA. This result was further substantiated by generation of a AAΔ310-402 mutant, where NIPA phosphorylation by NPM-ALK was totally abolished. To further prove biological significance of the identified residues, phospho-deficient mutants were established and transformation assays were performed. Here we were able to show drastically impaired cell proliferation in mutants with silenced serine/threonine residues 338, 344, 370, 381 and 387 upon NPM-ALK expression.

Summary/Conclusions: Taken together, we identified five phosphorylation sites in NIPA to be highly upregulated upon NPM-ALK expression. However,

NPM-ALK mediated NIPA-phosphorylation of those sites did neither change the SCF^{NIPA}-complex formation nor influence the NIPA localization at the nuclear pore complex, but silencing of these NIPA Serine/Threonine residues led to significantly reduced proliferation and altered transformation ability of Ba/F3 and primary MEF cells. Further analyses will shed some light into the mechanisms underlying these findings and evaluate NIPA as a possible new treatment option for ALCL.

E1394

APPLICATION OF CELL-OF-ORIGIN SUBTYPES DETERMINED BY DIGITAL GENE EXPRESSION IN HIV-RELATED DIFFUSE LARGE B CELL LYMPHOMAS

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Background: Diffuse large B cell lymphoma (DLBCL) can be divided according to cell of origin (COO) in germinal center B-cell–like (GCB) and activated B-cell–like (ABC) that have been shown different prognosis. Immunohistochemistry (IHC)-based algorithms and recently, Lymph2Cx assay, a digital test based on the expression of 20 genes, have been developed to facilitate COO assignment in clinical practice. Although the importance of COO is well established in DLBCL arising in immunocompetent individuals, its applicability on HIV-infected patients has been scarcely studied.
Aims: To study the characteristics and prognostic impact of COO subtypes in a series of HIV-related DLBCL using the Lymph2Cx assay and to compare the results with those obtained with an IHC-based algorithm.
Methods: A series of 55 patients with the diagnosis of HIV-related DLBCL (N=48), high-grade B-cell lymphoma (HGBL) with MYC and BCL2 and/or BCL6 rearrangements (N=3), or HGBL NOS (N=4) was studied. The following clinical parameters were collected from records: age, gender, ECOG, extranodal and bulky disease, B-symptoms, Ann-Arbor stage, LDH and beta2-microglobulin, HCV and HBV serology, history of opportunistic infection and of AIDS-defining illness, onset of combination antiretroviral therapy, CD4-counts and HIV loads. IHC and FISH studies were performed on tissue microarrays. RNA was extracted from FFPE samples with RecoverAll kit (Ambion, Carlsbad, California) and digital GEP was determined with the Lymph2Cx assay (NanoString Technologies, Seattle, WA). Cohen's kappa was calculated to measure the agreement between COO given by Hans algorithm and Lymph2Cx assay.

Table 1.

Characteristic	GCB N=35	ABC N=11	UNC N=9	P-value (GCB vs. ABC)
MYC rearrangement, N(%)	11/32 (34.4%)	0/10 (0%)	2/8 (25%)	0.030
BCL2 rearrangement, N(%)	2/32 (6.3%)	1/10 (10%)	1/8 (12.5%)	0.568
BCL6 rearrangement, N(%)	7/32 (21.9%)	3/10 (30%)	4/8 (50%)	0.444
Dual-hit, N(%)	1/32 (3.1%)	0/10 (0%)	2/8 (25%)	0.762
CD10, N(%)	16/34 (47.1%)	2/11 (18.2%)	1/8 (12.5%)	0.087
BCL6, N(%)	23/33 (69.7%)	4/11 (36.4%)	5/8 (62.5%)	0.055
MUM1, N(%)	16/33 (48.5%)	7/11 (63.6%)	6/8 (75%)	0.384
CD30, N(%)	4/33 (12.1%)	1/11 (9.1%)	3/8 (37.5%)	0.633
EBER, N(%)	5/33 (15.2%)	4/11 (36.4%)	2/8 (25%)	0.141
BCL2, N(%)	12/31 (38.7%)	6/11 (54.5%)	4/8 (50%)	0.287
MYC, N(%)	12/32 (37.5%)	2/11 (18.2%)	3/9 (33.3%)	0.213
Dual expressers, N(%)	6/31 (19.4%)	2/11 (18.2%)	1/8 (12.5%)	0.655

Abbreviations: ABC, activated B-cell–like GCB; GCB, germinal center B-cell–like; UNC, unclassified.

Results: The median follow-up of living patients was 8.5 years. IHC studies showed that 35.8% of the cases expressed CD10, 61.5% expressed BCL6, 55.8% expressed MUM1, and according to Hans algorithm 56.6% had a non-GC phenotype. CD30 was expressed in 15.4% of the cases and EBER was found in 21.2%. The expression of MYC was detected in 32.7% of the cases and BCL2 in 44%, and 18% were dual expressers. Rearrangements involving MYC, BCL2 and BCL6 were detected in 26%, 8% and 28%, respectively. The Lymph2Cx assay assigned a COO to all 55 studied cases, 63.6% were GCB subtype, 20% were ABC subtype, and 16.4% were unclassified. The only clinical feature significantly associated with a defined COO subtype was B-symptoms (ABC=81.8% *vs* GCB=28.6%, P=0.003) and HIV-load tended to be more frequently observed in ABC (90%) than in GCB (58.1%, P=0.066). Regarding IHC and FISH results, MYC rearrangements were only detected in GCB cases and expression of CD10 and BCL6 tended to be associated with GCB (Table 1). Hans algorithm and Lymph2Cx assay differently assigned COO subtypes (κ=-0.288, P=0.029) showing that 44.1% of the GCB cases had a non-GC phenotype according to Hans. Only patients treated with RCHOP were considered in survival analyses (N=47). COO subtypes had neither impact on OS nor PFS, independently of being determined with Hans or Lymph2Cx assay. Features associated with shorter OS and PFS were history of AIDS-defining illnesses, HCV-infection and dual MYC and BCL2 expression. Extranodal disease and increased MYC or BCL2 expression were also bad prognostic factors for PFS.
Summary/Conclusions: In HIV-related lymphomas, COO subtypes were discordantly assigned with Hans and Lymph2Cx assay and COO subtypes showed no impact on outcomes, independently of the method applied.

This work was supported by 2014 SGR225 (GRE) from CERCA Programme/ Generalitat de Catalunya, and by funds from Josep Carreras International Foundation, "la Caixa" Foundation and Celgene Spain.

E1395

CXCR4 AND CXCL12 ARE IMPLICATED IN BONE MARROW INFILTRATION PROCESS OF AGGRESSIVE B CELL LYMPHOMAS AND THEIR INHIBITION SUPPRESSES LYMPHOMA CELL GROWTH *IN VITRO*

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Background: The chemokine receptor CXCR4 together with its prime ligand CXCL12 plays a pivotal role in tumorigenesis of solid and haematological neoplasms. Our comprehensive study on the CXCR4 expression in aggressive lymphoma demonstrated that high CXCR4 expression was associated with poor clinical course of aggressive lymphoma patients.
Aims: Therefore, we aimed to comprehensively study the implication of the CXCR4 - CXCL12 axis in bone marrow infiltration process of aggressive lymphoma and to analyse the effects of CXCR4 antagonists on cell growth and migration of aggressive lymphoma cells *in vitro.*
Methods: To determine whether *CXCR4* and *CXCL12* expression have any effects on the bone marrow infiltration process of aggressive lymphomas, we performed gene expression analysis on bone marrow biopsies of our diffuse large B-cell lymphoma patient cohort. Therefore, we used 63 bone marrow specimens, whereby 52 bone marrow biopsies were taken at time of diagnosis. Additionally, we generated a novel CXCR4 antagonist -named WK1- by modification of the side chain of AMD070 - a commercially available CXCR4 antagonist. We treated various aggressive lymphoma cell lines (U2932 and RI-1 as ABC-DLBCL and BL2 and Raji as Burkitt lymphoma lines) with CXCR4 antagonists (AMD070 and its derivate WK-1 and the FDA approved CXCR4 antagonist AMD3100) and determined cell growth by using the EZ4U assay. Transwell migration using the Boyden chamber was used to estimate migration indices for AMD070 and WK-1.
Results: By correlating *CXCL12* expression levels of infiltrated bone marrow biopsies, we observed a significant positive correlation between *CXCL12* expression and the percentage of infiltration levels (Spearman-Rho=0.764; p=0.001). Furthermore, remission inthe bone marrow after standard immunochemotherapy was associated with a reduction of *CXCR4* expression (p=0.075). The cell growth of BL2 and RI-1 cell lines -exhibiting strong and moderated CXCR4 expression- was significantly inhibited by AMD070 and WK1. Whereas the cell growth of U2932 -exhibiting a weak CXCR4 expression- was just affected by WK1. AMD3100 did not show any effects on the lymphoma cell growth. The transmigration index to evaluate the chemotactic ability of lymphoma cells was reduced by AMD070 and WK1 treatment, however, the inhibitory effects of WK1 were lower compared to AMD070.
Summary/Conclusions: These data strongly suggest that *CXCR4* and its ligand *CXCL12* is implicated in the bone marrow infiltration process of diffuse large B-cell lymphomas. Additionally, our *in vitro* results indicate that treatment of lymphoma cells with CXCR4 antagonists might be a promising new therapeutic intervention to eliminate lymphoma cells.

E1396

EPSTEIN-BARR VIRUS LOAD IN PLASMA IS AN EARLY BIOMARKER OF HIV-RELATED LYMPHOMA

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Background: Epstein Barr virus (EBV) has been detected in the tumor cells of some non-Hodking lymphomas (NHL) and Hodgkin lymphomas (HL) and detectable EBV loads have been found in the plasma of immunocompetent patients with HL. In HIV-related lymphomas the importance of EBV load as potential lymphoma biomarkers has been scarcely studied.
Aims: We aimed to evaluate the usefulness of EBV load in plasma as lymphoma biomarker in HIV-infected patients.
Methods: One hundred and fifteen patients with NHL (HIV-infected =57 and HIV-uninfected=34) and HL (HIV-infected= 16 and HIV-uninfected= 8) were studied. EBV loads were determined in plasma by means of a commercial real-time PCR technique (EBV PCR kit, Qiagen GmbH, Hilden, Germany) at lymphoma diagnosis and in a group of HIV-infected patients also at one year before diagnosis (N=11) and at complete response (CR) (N=34). EBER expression was studied by *in situ* hybridization in tumor biopsies. The following clinical and biological parameters were collected from records: age, gender, date of lymphoma diagnosis, ECOG score, extranodal and bulky disease, B symptoms, Ann-Arbor stage, serum lactate dehydrogenase and beta2-microglobulin, International Prognostic Index (IPI), HCV and HBV serology, history of opportunistic infection and of AIDS-defining illness, onset of combination antiretroviral therapy, CD4-counts, HIV loads, type and date of response, relapse date, last follow up or death date. McNemar's test and Wilcoxon test were used to compare quantitative and qualitative variables, respectively. Survival analyses were performed using the Kaplan-Meier method. P-values of less than 0.05 were considered statistically significant.

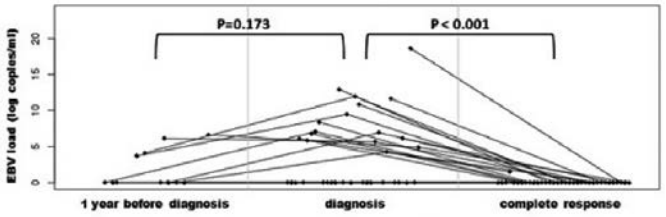

Figure 1. EBV loads of HIV-infected patients with lymphoma at three clinical points. Lines connect EBV determinations of the same patient.

Figure 1.

Results: At diagnosis, EBV loads were detectable in more HIV-infected patients than HIV-uninfected (48% *vs* 14%, P=0.002) and in more HL cases than NHL (70% *vs* 26.3%, P=0.006). In HIV-infected patients, detectable EBV load was associated with EBER expression, 66.6% of the patients with detectable EBV loads had EBER-positive tumors and 92% of the patients with undetectable EBV loads had EBER-negative tumors (P=0.003). All the remaining clinical and biological features were not associated with detectable EBV load in plasma. In HIV-uninfected patients, associations between EBV load and EBER expression (P=0.006) and EBV load and HBV infection (P=0.017) were observed. From 16 out of 34 (47%) HIV-infected patients with detectable EBV loads at lymphoma diagnosis, 15 had undetectable EBV loads at CR (P<0.001) (Figure 1). The exception was one patient with HL whose EBV load substantially decreased at CR but was still detectable. Moreover, 4 out of 7 HIV-infected patients with detectable EBV loads at diagnosis had detectable loads one year before diagnosis, and no patient with negative EBV loads at diagnosis had detectable loads before it, pointing EBV load can be used as an early biomarker of lymphoma. EBV loads at diagnosis had neither impact on overall survival nor progression-free survival.
Summary/Conclusions: EBV-load in plasma can be used as early biomarker of lymphoma in HIV-infected patients since EBV-loads can be detected up to 1 year before lymphoma diagnosis and are virtually undetectable at lymphoma CR.

This work was supported by 2014 SGR225 (GRE) from CERCA Programme/ Generalitat de Catalunya, and by funds from Josep Carreras International Foundation,"la Caixa" Foundation and Celgene Spain.

E1397

CLONOTYPE AND MUTATIONAL PATTERN IN TCRGD LARGE GRANULAR LYMPHOCYTE LEUKEMIA

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Background: T-cell large granular lymphocyte leukemia (T-LGLL) is a rare heterogeneous T-cell neoplasia whose leukemic cells usually express the αβ T-cell receptor (TCR); only a small subset of cases expresses the γδ TCR denoting the TCRγδ LGLL. Currently, among the different LGL diseases, TCRγδ LGLL remains less studied and several clinical and laboratory data already described in TCRαβ-LGLL have not yet been explored in TCRγδ-LGLL.
Aims: The aims of this work were 1) to characterize TCRγδ-LGLL defining *STAT* mutational pattern and CDR3 repertoire diversity/clonal composition (clonotype) and 2) to evaluate correlations among LGL phenotype, mutations, TCR rearrangement and clinical presentations.
Methods: In this work 11 patients affected by TCRγδ-LGLL were included. Sanger sequencing was used for mutational analysis on hot-spot regions in the two genes more frequently mutated in LGL disorders, *STAT3* and *STAT5b*. Immunophenotype of LGL clone was defined by flow cytometer analysis. CDR3 repertoire and frequency distribution of TCR gamma gene rearrangements were obtained by Next-Generation Sequencing (NGS).
Results: Our results showed that TCRγδ LGLL had a high incidence of *STAT* mutations, 9 out of 11 patients carrying *STAT3* or *STAT5b* mutations in a mutually exclusive pattern. At variance from CD8+ TCR αβ LGLL and CD4+ TCR αβ LGLL, the first being mostly characterized by *STAT3* mutations and the latter by *STAT5b*, TCRγδ LGLL patients were characterized by both the mutations. Thus, TCRγδ LGLL showed features shared by CD8 and CD4 TCRαβ-LGLL. Consistently, TCRγδ LGLL showed the same correlation between immunophenotype and kind of mutation observed in TCRαβ-LGLL: γδLGL patients with CD16+/CD56- LGL immunophenotype were characterized by *STAT3* mutations (as in CD8+ T-LGLL), while γδLGL patients with CD56+ LGL immunophenotype by *STAT5b* mutations (as in CD4+ T-LGLL). Moreover, we observed that patients with γδLGLs positive for Vδ2 showed usually indolent course, while Vδ1 was linked to a more symptomatic disease (4 out of 5 symptomatic patients were Vδ1+), whereas no correlation was found between mutations and clinical course. By NGS of TCR gamma gene, we observed that all patients were clonal but two, showing a polyclonal pattern borderline with clonality percentage defined by sequencing kit criteria. Interestingly, these two last patients were the only two patients without *STAT* mutations. As far as the remaining cases are concerned, among *STAT3* mutated patients (n=4), 3 were polyclonal and one biclonal, while *STAT5b* mutated patients (n=5) were more frequently monoclonal (4/5 monoclonal and 1/5 biclonal). In terms of clonal rearrangements, Vg3-Jg1/2, Vg9-JgP and Vg8Jg1/2 were the combination usages most frequently detected. Concerning the clonotype repertoire, CDR3 sequences of the immunodominant clones were present with low frequency in almost all the other γδ patients and two different CDR3 sequences were found shared, each one in two different patients at frequency >10% of the total rearrangements.
Summary/Conclusions: Our data indicate that TCRγδ LGLL can be considered at the intersection of the two types of TCRαβ-LGLL, sharing CD8 or CD4 TLGLL mutational features. As already described in TCRαβ-LGLL, also in γδ disease a decreased diversity of TCR repertoire was demonstrated. However, in these γδLGL patients *STAT* mutations do not correlate with a symptomatic clinical behavior while *STAT5b* mutations seems to be more frequently linked to monoclonal nature of the LGL lymphoproliferation. Rather, the marker Vδ1 appears to be correlated to symptomatic disease.

E1398

INCREASED EXPRESSION OF IRF8 IN TUMOR CELLS INHIBITS THE GENERATION OF TH17 CELLS AND PREDICTS UNFAVORABLE SURVIVAL OF DIFFUSE LARGE B CELL LYMPHOMA PATIENTS

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Background: The immunological pathogenesis of diffuse large B cell lymphoma (DLBCL) remains elusive. Searching for new prognostic markers of DLBCL is a crucial focal point for clinical scientists.
Aims: The aim of the present study was to examine the prognostic value of interferon regulatory factor 8 (IRF8) expression and its effect on the development of Th17 cells in the tumor microenvironment of DLBCL patients.
Methods: Flow cytometry, immunohistochemistry, and quantitative real-time PCR were used to detect the distribution of Th17 cells and related cytokines and IRF8 in tumor tissues from DLBCL patients. Two DLBCL cell lines (OCI-

LY10 and OCI-LY1) with IRF8 knockdown or overexpression and two human B lymphoblast cell lines were co-cultured with peripheral blood mononuclear cells (PBMCs) *in vitro* to determine the effect of IRF8 on the generation of Th17 cells. Quantitative real-time PCR and Western blotting were used to investigate the involvement of retinoic acid receptor-related orphan receptor gamma t (RORγt) in the effect of IRF8 on Th17 cell generation. The survival of 67 DLBCL patients was estimated using the Kaplan-Meier method and log-rank analysis.
Results: The percentage of Th17 cells was lower in DLBCL tumor tissues than in PBMCs and corresponding adjacent benign tissues. Relative expression of interleukin (IL)-17A was lower, whereas that of interferon (IFN)-γ was higher in tumor tissues than in benign tissues. Co-culture with DLBCL cell lines inhibited the generation of Th17 cells *in vitro*. IRF8 upregulation was detected in DLBCL tumor tissues, and it was associated with decreased DLBCL patient survival. Investigation of the underlying mechanism suggested that IRF8 upregulation inhibited Th17 cell generation by suppressing the effect of RORγt on CD4+ T cells.
Summary/Conclusions: Our findings suggest that IRF8 expression in the tumor microenvironment inhibited the generation of Th17 cells through its antagonistic effect on RORγt in the DLBCL tumor microenvironment, suggesting that it could be a prognostic factor for DLBCL.

E1399

GENOMIC PROFILING OF BCL2 AND MYC DOUBLE EXPRESSOR DIFFUSE LARGE B CELL LYMPHOMA

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Background: Diffuse large B cell lymphoma (DLBCL) is an aggressive disease featuring heterogeneous genetic, phenotypic and clinical characteristics. Recently, a negative prognostic impact of double expression of BCL2 and MYC (double expressor (DE) lymphoma) has been identified in several studies. SNP array (SNP-A) studies have already led to the identification of novel genomic aberrations in ABC and GCB subtypes of DLBCL whereas similar analysis has not been done in DE and non-DE DLBCL.
Aims: To characterize the landscape of genomic aberrations in DE and non-DE DLBCL groups using SNP-A and interphase fluorescence *in situ* hybridization (FISH).
Methods: Immunohistochemical and FISH analysis was performed on tissue microarray of formalin fixed paraffin embedded (FFPE) tumor tissue samples using Bcl2 (124, DakoCytomation) and MYC (Y69, Epitomics) antibodies and FISH MYC (Zytovision), Bcl2 (Abbott/Vysis), Bcl6 (Abbott/Vysis) break-appart probes and MYC/IgH (Zytovision) double-fusion probe. Infinium HD whole-genome genotyping assay with the HumanCytoSNP FFPE-12 BeadChip (Illumina Inc., San Diego, CA, USA) was performed for genomic analysis of the aberrations.
Results: A cohort of 91 primary DLBCL patients diagnosed between 2004 and 2012 was selected for the study. Immunohistochemical evaluation was informative in 84 cases, 25 cases (29.8%) were DE. On FISH analysis, 61 cases were informative for MYC, 56 cases for Bcl6, and 65 cases for Bcl2. 7 cases (11.4%) were positive for MYC translocation, 14 (25%) for Bcl6, and only 3 (4.6%) were positive for Bcl2. No cases of FISH MYC and bcl2 double positive DLBCL were identified. Genomic DNA from FFPE tumor tissue for SNP-A was available in 66 DLBCL cases. SNP-A analysis detected in total 329 genetic abnormalities not corresponding to known copy number polymorphisms (89% of all the patients, 59/66). These comprised 164 (50%) hemizygous and 2 (1%) homozygous deletions, 106 (32%) gains, 41 (12%) trisomies and 16 (5%) monosomies. The most common aberrations were 1p deletion, 1q gain, 6q deletion and 3q gain. Complex karyotype (>3 aberrations) was detected in 37/66 (56%) patients. Both DE and non-DE DLBCL groups had equal rate of aberrations per case (~5 aberr./case) and shared the most common aberrations – 1p deletion and 1q gain. In contrast, 11q deletion was more common in DE, while 6q and 17q deletions were more prevalent in the non-DE had group. Notably, we detected a higher proportion of hyperdiploidic karyotypes in non-DE group than in DE (16 *vs* 6 cases, respectively). Cases with MYC positive (FISH) and MYC gain (SNP-A) had the median number of two chromosomal aberrations with an exception of two MYC positive cases with complex karyotypes. These two cases shared the same 9q, 11q, 14q deletions and the monosomy of chromosome 19. Finally, of the 7 cases with normal SNP-A karyotype, BCL6 FISH-positive marker was detected in 3 patients.
Summary/Conclusions: SNP-A analysis highlights the genomic differences between the DE and non-DE DLBCL. Our finding of MYC positive (translocations and/or gains) association with low complexity karyotype status may suggest MYC to be an early initiating genetic event.

E1400

ARQ 531, A REVERSIBLE BTK INHIBITOR, DEMONSTRATES POTENT ANTI-TUMOR ACTIVITY IN ABC-DLBCL AND GCB-DLBCL

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Background: B-cell receptor (BCR) signaling has emerged as a critical pathway for B-cell lymphoma development. BTK, a key mediator of BCR signaling is a major target for ibrutinib. Ibrutinib has demonstrated efficacy in chronic lymphatic leukemia (CLL), mantle cell lymphoma and Waldenström macroglobulinemia. However, as anticipated by preclinical models, clinical objective response rates of only 37% in ABC and 5% in GCB diffuse large B cell lymphoma (DLBCL) were reported. ARQ 531 is a potent reversible inhibitor of BTK, highly effective in targeting BCR signaling. Kinase profiling indicated members of Tec, Src, Trk families as additional targets with especially potent inhibition of HCK and BLK kinases. ARQ 531 caused significant growth inhibition (GI_{50}=< 1μM) of hematological malignant cell lines and and showed greater efficacy than ibrutinib in a CLL mouse model.
Aims: We aim to assess biological and anti-tumor effects of ARQ 531 in *in vitro* and *in vivo* in DLBCL tumor models.
Methods: Biochemical inhibition and kinase profiling were assessed using recombinant proteins. The ARQ 531 binding kinetics on BTK were determined by Surface Plasmon Resonance assay. Anti-proliferative activity of ARQ 531 was tested in a MTS-based assay against a panel of hematological malignant cell lines. Pathway inhibition assessments, *in vivo* efficacy and *in vivo* target inhibition were performed in TMD8 (ABC-DLBCL) and SUDHL-4 (GCB-DLBCL) cell lines and xenografts. ADME and pharmacokinetic properties of ARQ 531 were also evaluated in rats, dogs and monkeys.
Results: ARQ 531 potently inhibited BTK (IC50=0.85 nM) and displayed long residence time (56 min). ARQ 531 exhibited strong anti-proliferative activity in TMD8 (GI_{50}=0.13 μM) and SUDHL-4 (GI_{50}=0.2 μM) cell lines. Ibrutinib, while potent on TMD8 cells (GI_{50}=0.002 μM), had a GI_{50} of 1.1 μM in SUDHL-4, a concentration not reached in human blood, consistent with published studies. Pathway analysis in TMD8 and SUDHL-4 cells showed that ARQ 531 potently inhibited both upstream activating signals (Src kinase family) and downstream signaling pathways such as AKT and ERK. Cell cycle analysis indicated that ARQ 531 inhibited cell growth through G1 phase arrest, similar to ibrutinib. In the TMD8 xenograft mouse model, ARQ 531 strongly inhibited BTK signaling, with better efficacy than reported with ibrutinib: tumor growth reduction was 97% after 12 days of dosing, with no re-growth observed for 17 days post dose interruption. In the ibrutinib-resistant SUDHL-4 mouse xenograft model, ARQ 531 potently suppressed tumor growth (>80% inhibition) compared to the control group.
Summary/Conclusions: ARQ 531 is a potent reversible inhibitor of BTK. Its distinct kinase selectivity can be used to target constitutive BCR signaling in DLBCL primarily resistant to ibrutinib, as demonstrated by the excellent efficacy in both ABC and GCB DLBCL xenograft models. These data support the clinical investigation of ARQ 531 in patients with hematological malignancies, expected to begin in mid-2017.

E1401

ROLE OF GENETIC POLYMORPHISMS ON R-CHOP EFFICACY IN DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS: AN INTERIM ANALYSIS OF A MULTICENTER PROSPECTIVE PHARMACOGENETIC STUDY

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Background: Standard chemotherapy represented by the R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone) regimen is successful in about 60% of patients (pts) with diffuse large B-cell lymphoma (DLBCL). Pts who do not benefit from this treatment, due to the development of tumor drug resistance, have a very poor prognosis. Currently, knowledge on reasons of treatment related failures in DLBCL are scanty and predictive biomarker of response are largely unknown.
Aims: We hypothesized that polymorphisms of gene involved in the pharmacokinetics and pharmacodynamics of drugs included in R-CHOP regimen may play a role in predicting the outcome in DLBCL pts. Thus, we designed a multicentre prospective pharmacogenetic trial aimed at identifying gene polymorphisms potentially predictive of drug efficacy/resistance in DLBCL pts treated with R-CHOP. We are reporting update data of an interim analysis on the first 80 enrolled pts.
Methods: The study includes chemonaive DLBCL pts (Ann Arbour I-IV stages) candidate to an R-CHOP standard treatment. The Ethical Committee of each participating centre approved the pharmacogenetic protocol, and all pts signed a written informed consent. In this interim analysis, the impact of single nucleotide polymorphisms (SNPs) on R-CHOP efficacy was evaluated by objective response (OR) rate, progression-free survival (PFS) and overall sur-

vival (OS).The efficacy of R-CHOP was evaluated according to Cheson criteria by performing standard hematochemical and instrumental (TC and FDFG-PET) tests and defining complete remission (CR), partial remission (PR), non response or progressive disease (PD). Genomic DNA was extracted from peripheral blood of 80 pts. SNPs analysis was performed by an Affimetrix array. To date, 21 SNPs from 19 candidate genes (*ABCB1, ABCC1, ABCC2, ABCG2, CYBA, CYP2C9, FCGR2A, GSTP1, IL2, MARCKS, MLH1, NCF4, NQO1, NQO2, RAC2, TNF, TOP2A, TP53, TUBB*) involved in pharmacokinetics and pharmacodynamics of R-CHOP (www.pharmgkb.org) selected and analysed in relation to R-CHOP efficacy. Univariate and multivariate logistic regression analyses were performed to evaluate associations between SNPs and clinical/pathological characteristics or survival parameters (PFS and OS).
Results: Median age was 63 years. There were 37 men and 43 women. 47.5% of pts were in stage I-II, 52.5% of pts in stage III-IV. 27.5% of pts had bulky disease, 43.8% of pts had involvement of extranodal site. 47.5% of pts had pathological LDH value. According to the revised IPI, 15% of pts were in the low risk group, 58.7% in the intermediate, and 26.3% in the high risk group. Overall, 468 courses of R-CHOP had been administered (mean: 5.85 courses, range: 4-6). 88,7% of pts had CR to R-CHOP whereas the remaining showed PR or SD (7.5%) or PD (3,8%). Multivariate analysis identified *FCGR2A* rs1801274 as a predictor of PFS (p=0.045). Pts with HR or RR genotypes showed shorter PFS than pts with HH genotype (HR: 2.437, CI95% 1.020-5,823). No statistically significant correlation was found between SNPs and OS.
Summary/Conclusions: Our preliminary data obtained in a limited number of pts, show an association between a SNP of the low affinity *FCGR2A* gene involved in the activity of rituximab and PFS. Further insights will derive from the completion of pts accrual to reach the planned number of cases at the end of our study.

This work was supported by a grant from the Associazione Giacomo Onlus, Castiglioncello (LI), Italy to E.M. and Cassa di Risparmio di Firenze, Firenze, Italy to S.N.

E1402

CDK4/6-INHIBITION BY ABEMACICLIB INDUCES POTENT EARLY G1-ARREST IN MCL CELL LINES AND SHOWS SEQUENCE-SPECIFIC INTERACTIONS WITH CYTARABINE AND IBRUTINIB

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Background: Mantle cell lymphoma (MCL) is characterized by t(11;14) resulting in a constitutive cyclin D1 overexpression. The cyclin D1-CDK4/6 complex inactivates Rb through phosphorylation, leading to G1/S-phase transition. Therefore, inhibition of CDK4/6 is an efficient and rational approach to overcome cell cycle dysregulation in MCL.
Aims: We evaluated the efficiency of the novel CDK4/6 inhibitor abemaciclib in various MCL cell lines and in primary MCL cells in combination with cytarabine (AraC) and ibrutinib.
Methods: MCL cell lines (Granta 519, JeKo-1, Maver-1, Mino) and primary MCL cells were exposed to abemaciclib alone and combined with AraC or ibrutinib. Cells were pretreated with abemaciclib and exposed to AraC or ibrutinib with or without consecutive wash-out of the CDK4/6 inhibitor. Proliferation and viability were measured by trypan blue staining and Cell Titer Glo assay. Combination Index (CI) to assess synergy or antagonism was calculated using the Fractional Product method by Webb (1963). Flow cytometry was applied for cell-cycle (PI-staining) and apoptosis analysis (Annexin V PE/7AAD-staining). Protein expression and phosphorylation status of various downstream proteins was analyzed by Western Blot analysis.
Results: Abemaciclib inhibited cell proliferation by induction of early G1-arrest. We observed an almost complete and reversible G1-arrest in all sensitive cell lines by FACS analysis (JeKo-1: *G1-phase +51,7%; S/G2-phase -51,7%* at 31,25 nM after 24 h; *G1-phase +35,4%; S/G2-phase -34,8% after 72 h*), whereas cell viability was not reduced. IC50-values of sensitive cell lines (JeKo-1, Maver-1, Mino) were <30 nM after 72 h. Western Blot analysis revealed reduced phosphorylation of Rb on serine 795 without changes in CDK 4 and cyclin D1 expression, in line with reversible cell cycle arrest. Wash-out of abemaciclib after 24 h resulted in synchronized S-phase entry in all sensitive cell lines (e.g. Mino: *G1-phase -20,4%; S-phase +30,5%*). Accordingly, sequential combination of abemaciclib followed by AraC showed strong synergy in Mino cells (*CI=0,22* for 31,25 nM abemaciclib / 3,33 µM AraC). In contrast, simultaneous exposure to abemaciclib had a protective effect against AraC treatment in all sensitive cell lines, due to an ongoing G1-arrest (Mino: *CI=-0,19* for 31,25 nM abemaciclib / 3,33 µM AraC). Sequential administration of abemaciclib and ibrutinib had synergistic or additive effects in sensitive cell lines (*CIs:* JeKo-1=*0,24*; Maver-1=*0,19*; Mino=*-0,03* for 31,25 nM abe / 2,5 µM ibru), whereas the simultaneous administration of both showed additive effects at most (*CIs:* JeKo-1=*0,02*; Maver-1=*-0,1*; Mino=*-0,09* for 31,25 nM abe and 2 µM ibru). In primary MCL cells abemaciclib had no impact on cell death or sensitization since no cell proliferation was observable and cells where resting in G1-phase.
Summary/Conclusions: The novel CDK4/6 inhibitor abemaciclib causes reversible G1 cell cycle arrest without loss of viability at low nanomolar doses. Rationale drug combinations exploiting the sequential effect may achieve major benefits. Pretreatment with abemaciclib might sensitize cells to ibrutinib, resulting in synergistic drug effects. In contrast, simultaneous application of Abemaciclib protects cells from AraC treatment whereas Abemaciclib-induced S-phase synchronization sensitizes MCL cell lines to AraC. Further analysis need to explore the interaction with other targeted approaches (inhibitors of the B-cell receptor pathway) to better understand the underlying molecular mechanisms.

E1403

CD8+ T-CELL CLONES PERSISTENT IN BONE MARROW AND PERIPHERIAL BLOOD DURING COURSE OF CD4+ ANGIOIMMUNOBLASTIC LYMPHOMA

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Background: Angioimmunoblastic T-cell lymphoma (AITL) – peripheral T-cell lymphoma, characterized by polymorphous infiltration of the lymph nodes, proliferation of high endothelial venules (HEV) and follicular dendritic cells (FDC). In addition to the lymph nodes, AITL affects spleen, liver, skin and bone marrow. The disease is almost always associated with Epstein-Barr virus (EBV), suggesting its role in the etiology of AITL. Neoplastic T cells in most cases are CD4+ and express pan T-cell antigens CD3, CD2, CD5, markers of normal follicular T-helper cells – CD10, CXCL13, PD-1. To confirm the diagnosis and assess disease dissemination combined morphological, immunohistochemical and molecular studies of affected tissues are being used. We have found that T-cell clones detected in the tissue of the lymph node (LN), often differ in T-cell receptor gene rearrangements from those detected in the bone marrow (BM), peripheral blood (PB) and other tissues. T-cell clonality testing itself may not distinguish between neoplastic or reactive lymphoproliferation in the BM and PB. Therefore, T-cell clonality of CD4+ and CD8+ populations of peripheral blood lymphocytes in patients with AITL had been tested during the course of disease.
Aims: To determine immunological characteristics of persisting in the PB and BM T-cell clones in AITL patients.

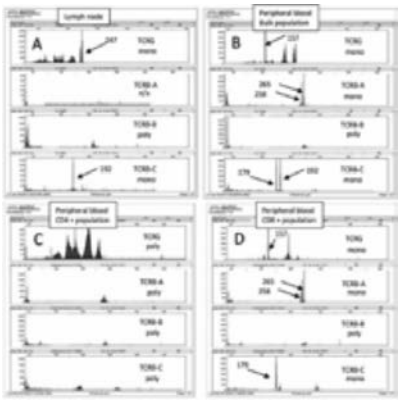

Figure 1.

Methods: The study included 26 patients (15 males and 11 females; age 36-92, median 67) with the diagnosis of AITL established on the basis of WHO 2008 diagnostic criteria. LN, BM and peripheral blood lymphocytes were tested for T-cell clonality according to BIOMED-2 protocol with subsequent fragments analysis on ABI PRISM 3130 (Applied Biosystems). The material was examined at the diagnosis and at various stages of patient's treatment. In 5 patients selection of CD8+ and CD4+ populations of PB lypmphocytes was performed with MidiMACS and MiniMACS Separators using CD4+, CD8+ MicroBeads (Miltenyi Biotec).
Results: Clonal TCRG and TCRB gene rearrangements in the LN of all AITL patients were found. In 6 of the 26 patients (23%) clonal products found in LN matched those from PB and BM. In 8 patients (30%) at least one of the clonal products isolated from the BM and/or PB mismatched the clonal products isolated from LN. In 12 patients (46%) clonal rearrangements found in the PB and BM were completely different from those identified for LN. Thus, at the diagnosis 20 patients (76%) had PB and BM T-cell clones distinct from LN T-cell clones. In 14 of 20 patients T-cell clones of PB and BM were tested repeatedly during the course of disease treatment. In 7 of 14 patients (50%) clonal products persisted for a long time and do not disappear upon reaching the remission of the disease. The observation period averaged 12 months (1 to 44 months). No correlation of T-cell clones persistence and the activity of EBV infection in the PB was found. Selection of CD4+ and CD8+ T-lymphocyte populations was performed for 4 patients in remission with persistent T-cell clones. In all cases,

clonal products, which were originally identified in the BM and PB were shown to belong to the CD8+ population of cells (Fig.). In one case BM and PB derived-CD4+ cells also shared a clonal product with LN cells tested at at the diagnosis. In one case CD4+ population selected from PB cells at the diagnosis carried clonal rearrangements, fully consistent with that of LN.
Summary/Conclusions: One may conclude that CD8+ T-cell clones identified in BM and PB, do not match those identified in LN for the majority of patients with AITL (76%). These clones can persist for a long time (the period of observation from 1 to 40 months), may not disappear in remission and probably have reactive nature. Therefore exclusive T-cell clonality in PB and/or BM should not be threated as minimal disease or relapse in AITL.

E1404

CD5 POSITIVE DIFFUSE LARGE B CELL LYMPHOMA SHOWED FREQUENT MYC EXPRESSION AND AGGRESSIVE CLINICAL BEHAVIOR
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Background: Aberrant expression of CD5 distinguishes a unique immunohistochemical subtype of diffuse large B cell lymphoma (DLBCL). This CD5+ DLBCLs, either *de novo* lesions or transformed from preceding low grade lymphomas, are characterized by unique molecular pathologic alterations and aggressive behavior. The incidence of CD5+ DLBCL was variably reported from 5 - 22% of all DLBCLs in western countries and Japan, however, no exact data available in Koreans.
Aims: This study aimed to investigate clinicopathologic features of CD5 + DLBCL among Koreans.
Methods: A total of 350 cases of DLBCL were reviewed 4 university hospitals from 2004 to 2012. Review of the histologic features along with immunohistochemical study for BCL1, BCL2, BCL6, CD5, CD10, CD23, CD30, IRF4/MUM1, MYC, Ki-67 and EBV *in situ* hybridization was performed. Florescent *in situ* hybridization (FISH) for MYC rearrangement and amplification was also performed. The results were compared with DLBCL-NOS (N=195).
Results: Thirty cases of CD5+ DLBCL were retrieved among 350 cases of DLBCL (8.6%), which showed predominance of female (20/30), elderly (mean age 64), and extranodal presentation (16/30). Richter transformation was suspicious in only one case, and EBV was negative in all. Most cases (22/30) belong to non-GCB subtype by Hans classifier. Rearrangement of MYC was found in 2 cases and amplification was found in one. Compared with DLBCL-NOS, CD5+ cases revealed significantly higher expression of MYC, BCL6, IRF4/MUM1 and Ki67 (all p<0.05). Double expression of both BCL2 and MYC was found in 9 of 30 cases (30%). Also, CD5+ DLBCL showed more frequent bone marrow involvement, advanced stages and high international prognostic index (all p<0.05). In univariable survival analysis, CD5+ DLBCL revealed significantly shorter progression free survival (median 8.2 months) compared with DLBCL-NOS (median 56.3 months) (p<0.05)
Summary/Conclusions: This is the first collective study of CD5+ DLBCL in Korea. The incidence, clinical presentation, and pathologic features including cell of origin coincide with previous reports from western population or Japanese. However, frequent high expression of MYC without chromosomal structural alteration was a unique finding in our study. Expression of CD5 should be routinely investigated in DLBCL to find this particularly aggressive subtype.

E1405

REACTIVE FLORID B-LINEAGE LYMPHOID PROLIFERATIONS IN HIV INFECTION MAY MIMIC LYMPHOMA
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Background: Approximately 7 million people are living with Human Immunodeficiency Virus (HIV) infection in South Africa (SA) (2015), which is associated with an increased risk of lymphoma. Although there is limited local information available, previously published data from the Johannesburg academic complex of hospitals (SA) showed an HIV prevalence of >90% in patients diagnosed with high grade B cell non Hodgkin lymphoma (NHL) who were tested for HIV (n= 568), during the period 2007-2009. The diagnosis of lymphoma with comorbid HIV infection is, however, challenging as lymphomas may present with atypical features and in unusual extranodal sites. In addition, there are reactive conditions which may mimic lymphoma (such as Tuberculosis (TB)). Within this setting reactive florid B-lineage lymphoid proliferations (RBLP) in the blood and bone marrow may raise a differential diagnosis of lymphoma.
Aims: This study aims to document the clinico-pathological features of florid RBLP in the setting of HIV infection in order to provide an approach to differentiating reactive and clonal processes.
Methods: A retrospective database search was performed of the laboratory information system (National Health Laboratory Service) that screened pathology reports for samples referred to the Departments of Molecular Medicine and Haematology and Anatomical Pathology at the Johannesburg Academic Complex during 2007-2011, supplemented with results of immunophenotypic analysis from 2007-2016. Demographic and clinico-pathological findings were collected for patients identified with florid RBLP who showed no definite evidence of monoclonality.
Results: During this period, 38 patients were diagnosed with florid RBLP with up to 70–80% of cells in blood or bone marrow comprising reactive B cells (including mature B, plasmablasts and plasma cells). All patients tested were HIV positive, with a median age of 28 years (range 6 months-79 years). There was a bimodal age pattern with a peak in children <1 year of age (34% of patients). This is different from the pattern noted in NHL in our centre, where lymphoma is virtually absent in children under a year of age. Common clinical presentations included cytopenias (85%); infection (70%) (commonly Cytomegalovirus (35%), TB (30%) and bacterial septicaemia (22%)); hepatosplenomegaly (42%); and lymphadenopathy (36%). Patients showed increases in serum total protein levels (reflecting hypergammaglobulinaemia), with increased inflammatory markers (C-reactive protein and erythrocyte sedimentation rate) and evidence of increased cell turnover (high uric acid, B2 microglobulin and lactate dehydrogenase levels). Extremely high HIV viral loads (VL) were documented (median 1 612 003copies/ml, range 12 000 - 10 000 000). Only one patient was virologically supressed. This is significantly different from lymphoma patients where median VL ranged from 16 000- 97 000 dependent on subtype. Median CD4 counts were also higher in this subgroup of patients when compared to patients with lymphoma (see table 1). Limited follow-up data was available, with only 8 patients documented to be attending an HIV clinic for long-term follow-up.

Table 1. Comparative data: HIV associated lymphoma and HIV associated RBLP.

	RBLP	Diffuse large B cell lymphoma	Burkitt lymphoma	Hodgkin lymphoma
Age years Median (range)	28[1] (0,5– 79) [n=38]	40[123] (5-88) [n=639]	33[2] (2-65) [n=149]	33[3] (4-84) [n=263]
Viral load copies/ml Median (Range)	1 612 003[123] (12 000 - 10 000 000) [n=20]	72 000[1] (27 – 4 800 000) [n=202]	97 000[2] (130 – 3 000 000) [n=60]	16 000[3] (38 – 2 500 000) [n=52]
CD4 count cells/uL Median (Range)	275 (14-2509) [n=16]	109[1] (3-960) [n=400]	176[1] (2-960) [n=129]	125 [n=52]

123p-value<0.001, Children excluded from CD4 count analysis.

Summary/Conclusions: In the setting of HIV, reactive conditions may mimic lymphoma and vigilance is needed in the confirmation of monoclonality. Patients with RBLP presented at a younger age when compared to their counterparts with lymphoma. They had extremely high VL with higher CD4 counts, suggesting this may be a feature of early HIV disease and the possibility of a seroconversion type illness should be considered.

E1406

MICROVESSEL DENSITY IN CD30 POSITIVE DIFFUSE LARGE B-CELL LYMPHOMAS
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Background: Diffuse large B-cell lymphoma (DLBCL) is the most common and one of the most heterogeneous lymphomas. Therefore, it is critical to further stratify cases of DLBCL into biologically similar and clinically meaningful subgroups, which will not only guide prognostic assessment and facilitate therapeutic decisions but also stimulate further research to understand the pathogenesis and develop novel potential treatments. Preliminary reports involving a number of different kinds of tumors have indicated that microvessel quantification may be useful in predicting disease outcome.
Aims: The aim of this study was to examine the relationship between microvessel density (MVD) as a parameter of tumor angiogenesis, and the immunophenotype in patients with diffuse large B-cell (DLBC) lymphomas.
Methods: We retrospectively identified cases of DLBCL diagnosed between January 2010 and January 2016 at our Institution. The following large B cell lymphoma subtypes were excluded from this analysis: post-transplant lymphoproliferative disorders with a DLBCL morphology, Primary Mediastinal large cell lymphoma and unclassifiable lymphomas with intermediate features between either DLBCL and Burkitt's lymphoma or DLBCL and Hodgkin's lymphoma. Immunohistochemistry was performed as part of the routine workup and CD30 was considered positive at ≥30% staining of neoplastic cells.

Microvessel quantification was performed by immunohistochemical staining, using monoclonal antibodies against platelet/endothelial cell adhesion molecule-CD31. A total of 82 cases of *de novo* DLBCL treated with R-CHOP were included in the training set for further analysis There were 45 men and 37 women, with a median age of 57 years (range, 16-84); 35 patients (43%) presented with B symptoms, and 49 (60%) had advanced Ann Arbor stages. Most of the patients had a good performance status (Eastern Cooperative Oncology Group score 0- 1, 87%), elevated serum lactate dehydrogenase level (61%), and low or low-intermediate International Prognostic Index (IPI) risk (IPI score 0-2, 63%). Involvement of multiple extranodal sites (>=2) was seen in 22% of cases, and bulky disease in 32% of cases.
Results: The median follow-up time was 47 months. Among the 82 cases in the training set, CD30 was positive in 24 cases (29%). No difference in response rate was observed between CD30 positive and CD30 negative patients. Patients with CD30+ DLBCL showed a significantly superior OS and PFS compared with CD30– patients. The 5-year OS was 79% in patients with CD30+ *vs* 59% in CD30– (P<0.05); 5-year PFS was 82% in patients with CD30+ *vs* 63% in CD30– (P<0.05). In patients with CD30 positive diffuse large B cell lymphomas we found a smaller number of vessels compared with patients CD30 negative (fig.1, p<0.05).

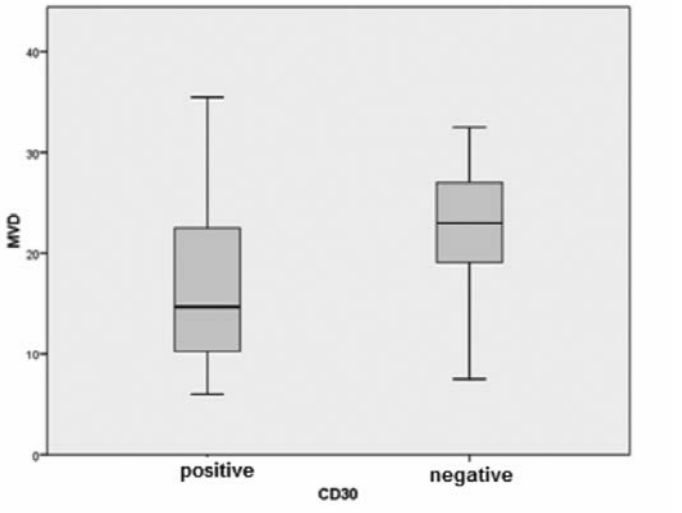

Figure 1.

Summary/Conclusions: CD30 is expressed in approximately 29% of all DLBCL and defines a novel subgroup of diffuse large B-cell lymphoma with a more favorable prognosis. Microvessel density expression is lower in CD30 positive DLBCL. The advent of brentuximab vedotin and its well-established effectiveness in other types of relapsed lymphomas has introduced the possibility of its application in this subset of patients.

E1407

ANTIGEN SELECTION PROMOTES CLONAL CYTOTOXIC T-CELL RESPONSES: HIGH-THROUGHPUT IMMUNOGENETIC EVIDENCE

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Background: Expansions of T-large granular lymphocytes (T-LGL) with a characteristic $CD3^+CD8^+CD57^+$ phenotype may be either idiopathic or develop in the clinical context of several conditions *e.g.* autoimmunity, viral infections, post-transplant and in hematologic malignancies. Whether this heterogeneity reflects a dynamic process of cytotoxic T-cell responses against auto- and exo-antigens remains to be established. That said, earlier, low-throughput immunogenetic studies have implicated antigenic drive in the development of T-LGL lymphoproliferations. However, due to the inherent limitations of low-throughput analysis, definitive conclusions were not possible.
Aims: To obtain comprehensive insights into the role of antigen selection in the pathogenesis of T-LGL lymphoproliferations using next-generation sequencing (NGS) for in-depth immunoprofiling of the clonotypic T cell receptor beta chain (TRBV) genes.
Methods: Included in the study were (i) a father and a son with T-LGL leukemia, the first case of intra-family occurrence; a single blood sample from the father and 2 samples from the son spanning 5 years were analyzed; and, (ii) a patient with T-LGL leukemia of donor cell origin developing after allogeneic hematopoietic cell transplantation (allo-HCT) for Philadelphia-positive acute lymphoblastic leukemia: for this case, the donor blood was analyzed as were two blood samples, one at the first documentation of clonal T-LGL expansion (at 6 months post allo-HCT while investigating persistent neutropenia that developed after Rituximab treatment for EBV reactivation) and a second 3 years later; at both timepoints, the patient had 100% donor chimerism and tested negative for *BCR-ABL* transcripts. TRBV-TRBJ-TRBD rearrangements were amplified on gDNA and subjected to paired end NGS, covering the CDR3 twice/sequence. To increase the consistency of results, raw NGS reads were analyzed by a purpose-built bioinformatics algorithm, performing: (i) quality filtering, (ii) merging of filtered in paired reads and (iii) quality filter of stitched sequences. Filtered-in sequences were submitted to IMGT/HighV-QUEST, and metadata was processed by an in-house dedicated bioinformatics pipeline.
Results: Only productive TRBV-TRBD-TRBJ rearrangements were included in the analysis. Overall, 1,129,289 filtered-in sequences from 6 samples were evaluated (median 188,095 sequences/sample). Major findings in the familial cases included: (i) pronounced skewing of the TRBV repertoire; (ii) existence of more than one immunodominant clonotype; (iii) in the analysis of longitudinal samples from the son, persisting clonotypes albeit with fluctuating frequencies (clonal drift); and, (iv) shared ('public') clonotypes between father and son. In the T-LGL leukemia of donor origin, the immunodominant clonotype was detected amongst the polyclonal donor repertoire and subsequently expanded in the recipient, persisting over time and accompanied by a few other considerably expanded, albeit smaller, clonotypes.
Summary/Conclusions: The borders between polyclonal *versus* oligoclonal *versus* monoclonal T-LGL lymphoproliferations are not sharply demarcated, but rather the transition from a polyclonal cytotoxic response to the development of T-LGL leukemia is a gradual process. Repertoire restrictions, public clonotypes and clonal drift strongly indicate selection by restricted (perhaps also shared) antigens in T-LGL leukemia ontogeny and evolution.

E1408

MINIMAL RESIDUAL DISEASE (MRD) EVALUATION IN LYMPHOMAS WITHIN THE FIL (FONDAZIONE ITALIANA LINFOMI) MRD NETWORK: INTER-LABORATORY REPRODUCIBILITY ON BORDERLINE SAMPLES

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Background: In B-cell non-Hodgkin lymphomas, minimal residual disease (MRD) is a highly valuable tool for the direct assessment of the reduction of the disease burden. In 2009, the four laboratories of the Fondazione Italiana Linfomi (FIL) - FIL MRD network - started a collaborative effort to harmonize and standardize their methodologies, performing QC (Quality Control) rounds twice a year for follicular lymphoma (FL) and mantle cell lymphoma (MCL) MRD assessment.
Aims: We evaluated the molecular results of bone marrow (BM) samples analysis performed during the QC rounds, to determine how borderline samples (*i.e.* those with a low MRD level) challenge the inter-lab reproducibility and data interpretation.
Methods: Between February 2010 and November 2016, in the context of 14 QC rounds, the FIL MRD Network labs received 188 BM (114 FL and 74 MCL) samples; 167 were analyzed by both nested polymerase chain reaction (PCR) and real-time quantitative PCR (RQ-PCR). *BCL2/IGH* MBR rearrangement was analyzed by nested PCR (Gribben, 1993) and by RQ-PCR (Ladetto, 2000). Clonality assessment was performed using an IGHV multiplex consensus PCR (Van Dongen, 2003) and RQ-PCR was carried out as described (Ladetto, 2000; Donoval, 2000). All analyses were conducted and interpreted according to the "EuroMRD Consortium" guidelines (van der Velden, 2007).
Results: The sensitivity and the accuracy of each molecular analysis was tested, reaching a uniform sensitivity of 10^{-5} and a quantitative range for RQ-PCR of at least 10^{-4}. Ninety-three/114 FL BM samples showed a 81% concordance (75/93) being PCR+/RQ-PCR+ or PCR-/RQ-PCR- in all the 4 labs. The remaining 18/93 (19%) were alternatively positive and negative in the inter-lab evaluations, representing samples with very low MRD levels, thus defined as "borderline" (brd): 6/18 were PCR-brd/RQ-brd, 6/18 were PCR-brd/RQ-positive not quantifiable (PNQ) and 6/18 were PCR-/RQ-brd. Among the 74 MCL BM samples, the concordance rate was 86% (64/74; +/+ or -/-). Ten/74 resulted brd samples: 1 was PCR-negative/RQ-brd, 5 were PCR-brd/RQ-brd, 2 were PCR-brd/RQ-PNQ and 2 were PCR-brd/RQ-negative. Overall, considering the 167

samples analyzed by both methods, 83% (139/167) of these were classified as +/+ or -/- by all the FIL labs. The remaining 28/167 (17%) were the samples that showed discordant results in the inter-lab assessments: while in 17 cases the "borderline status" was defined alternatively by only one method, 11 resulted brd samples by both techniques (11/167, 6.6%) (Fig.). Given that the 167 samples were tested in three replicates across the 4 labs, a total of 12 replicates/sample were analyzed: 31 brd samples were thus identified, 13 of which brd by both approaches. Of 156 evaluations performed on the 13 brd, 69/156 (44%) resulted PCR-positive and 87/156 (56%) PCR-negative, 58/156 (37%) were RQ-PNQ and 98/156 (63%) RQ-negative.

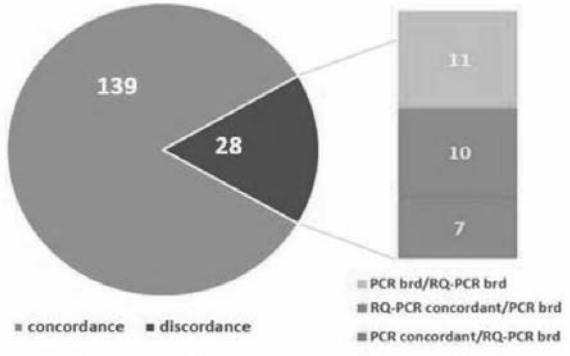

Figure. 167 BM samples analyzed by PCR and RQ-PCR. 139/167 samples were classified as positive or negative by all the FIL laboratories with both methods, while 28/167 showed discordant results in the inter-lab assessments. Among the 28 borderline (brd) samples, 21 resulted PCR-brd and 18 RQ-PCR-brd. In particular, 10 were concordant for RQ-PCR and brd for PCR, 7 resulted concordant for PCR and brd for RQ-PCR, 11 were classified as brd samples by both techniques.

Figure 1.

Summary/Conclusions: Despite the high inter-lab reproducibility in the MRD analysis that can be obtained and maintained by the QC round strategy, samples with the lowest MRD levels can still represent a challenge: 17% (28/167) of our series resulted brd, showing discordant results in inter-lab assessments; 39% of them (11/28) remained brd even applying both methods. The results did not change even increasing the number of replicates/sample. Thus, although representing a minority, brd samples are still problematic, especially when a clinically oriented interpretation is required. As the combined use of standard methods does not totally solve this problem, alternative, novel, methods such as digital PCR and NGS need to be tested in this context.

E1409

RHOA GLY17VAL MUTATION AND T-CELL CLONALITY ANALYSIS IN PATIENTS WITH ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA

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Background: Angioimmunoblastic T-cell lymphoma (AITL) is a rare subtype of T-cell lymphoma, characterized by generalized lymphadenopathy, hyperglobulinemia, and autoimmune manifestations. Interpretation of histological and immunohistochemical data can be difficult due to the small number of tumor CD4+ T cells, surrounded by abundant polymorphocellular infiltrate. AITL could often be misdiagnosed as reactive processes and other lymphomas, including Hodgkin's lymphoma. T cell clonality assessment plays an important role in AITL diagnosis. However, ambiguous clonality results may be obtained. Recently discovered somatic RHOA Gly17Val mutation is present in 53-71% of angioimmunoblastic T-cell lymphomas. We compared the efficacy of T-cell clonality testing and quantitative allele-specific PCR RHOA Gly17Val mutation assay in different tissues for AITL diagnosis.
Aims: To correlate the number of RHOA Gly17Val mutated cells in lymph nodes, blood, bone marrow and skin of AITL patients with corresponding T cell clonality results.
Methods: Lymph nodes (LN), skin biopsies, blood and bone marrow (BM) samples were studied for 40 patients with AITL. The male/female ratio was 25/15, median age was 65 years (36-92). To evaluate T-cell clonality rearranged TCRG and TCRB gene rearrangements were PCR-amplified according to BIOMED-2 standardized protocol and analyzed by capillary electrophoresis on ABI PRISM 3130 (Applied Biosystems). Sensitivity of T-cell clonality assay was limited to 10% of clonal T-cells of the total T-lymphocytes in the sample. Gly17Val mutation was analyzed by quantitative allele-specific (qAS) TaqMan Real-Time PCR assay. The detection level of this method was 1% of mutated cells in the total cell population.
Results: The clonal TCR gene rearrangements in LN were found in 37 of 40 patients (92%). RHOA (Gly17Val) mutation in LN was revealed in 60% (24 of 40) patients. T-cell clonality was detected in 26 of 28 primary samples of BM, but in 12 of 26 patients (46%) clonal TCR rearrangements were not matched in length with rearrangements detectable in LN. Number of cells with RHOA mutation was highest in the LN (in average 26.7% of the total cells), while in the bone marrow RHOA mutation was undetectable (in 7 patients), or detected in 10 patients in a small amount (in average 2% of the total cells). Combined histological investigation, T-cell clonality and RHOA (Gly17Val) testing showed BM lesion in 76% of patients (13 of 17) with at least one of the methods. Blood and bone marrow samples examined simultaneously showed slightly higher numbers of RHOA positive cells in the blood than in the BM in 5 of the 7 RHOA positive patients. Significant percentage of cells with a RHOA mutation (in average 25% of the total cells) was revealed in 5 of 6 skin samples from RHOA positive patients. We have found good correlation (Spearman's Rho-0,8198, p-level <0,00001) between T-cell clonality (matching with LN clonal peaks) and the number of RHOA positive cells in the AITL samples (n 51). Skin, blood and bone marrow samples with the T cell clonality peaks that differ from those found in the LN were also negative for the presence of cells with RHOA (Gly17Val) mutation.
Summary/Conclusions: RHOA (Gly17Val) point mutation is detected in LN by allele-specific PCR in 60% of patients with AITL. The percentage of tumor cells in BM is low (averaging less than 2% of the total cells). However, combined molecular and histological data suggest that BM may be involved in most patients. Extent of T cell clonality (matching with LN clonal peaks) correlates with the amount of cells having a RHOA mutation. T-cell clonality in BM, skin, spleen, etc. with rearrangements not matching those identified for the LN should be considered reactive and possibly associated with autoimmune process or antiviral response.

Other Non-malignant hematopoietic disorders

E1410

USEFULNESS OF CHITOTRIOSIDASE ACTIVITY, CCL18/PARC, 7-KETOCHOLESTEROL AND GLUCOSYLSPHINGOSINE CONCENTRATIONS FOR SCREENING OF LYSOSOMAL STORAGE DISORDERS

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Background: Gaucher (GD), Niemann-Pick Type A/B (NPA/B), Niemann-Pick Type C (NP-C) and the Lysosomal acid lipase deficiency (LALD) are lysosomal storage diseases (LSDs) difficult to diagnose due to the great heterogeneity of signs and symptoms, including haematological disorders, sometimes common to several pathologies, and the consequent alteration of biomarkers.
Aims: To assess the diagnostic utility of Chitotriosidase activity (ChT), CCL18/PARC, 7-ketocholesterol (7KC) and glucosylsphingosine (Lyso-Gb1) concentrations in previously mentioned LSDs.
Methods: ChT activity, CCL18/PARC and 7KC concentrations were measured in 146 plasma samples from subjects with suspected LSD (32 GD, 7 NPA/B, 90 NP-C and 17 LALD) received in our laboratory. In addition, a new biomarker, the Lyso-Gb1 concentration, was evaluated in 83/146 of previous mentioned subjects, 19 of them with confirmed LSD diagnosis. ChT was evaluated using a fluorogenic substrate, CCL18/PARC concentration by ELISA and 7KC and Lyso-Gb1 by liquid chromatography followed by tandem mass spectrometry.
Results: A total of 9/32 (28%) samples with suspected GD showed high ChT and/or high CCL18/PARC, 4/9 confirmed GD status; the rest were 1 NPA/B, 1 NP-C and two carriers of NP-C. Only 3/7 (43%) with suspected NPA/B and altered biomarkers were confirmed. Among the 23/90 (26%) with suspected NP-C and some elevated biomarker four were diagnosed of NP-C, and two carriers showed some biomarker higher than cutoff. Of the 8/17 (47%) referred to LALD suspicion with some elevated biomarker six were affected. All GD confirmed patients show high levels of Lyso-Gb1 whereas none of the other cases showed elevation for mentioned biomarker.
Summary/Conclusions: The screening of three biomarkers: ChT activity, CCL18/PARC and 7-ketocholesterol concentrations (the latter not applicable in GD) is a powerful tool to identify patients at high risk of suffering from LSDs which should undergo confirmatory diagnostic tests. In this line we would have reduced the number of cases needing confirmatory diagnostic test from 146 to 43 (29%) and 19/43 (44%) were positive for LSDs. Lyso-Gb1 concentration can allow the unambiguous identification of all the GD patients but is not useful for the other LSDs.

E1411

THE VALUE OF SOLUBLE IL-2R APLHA SUBUNIT MEASUREMENT IN CSF OF CHILDREN WITH HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS (HLH): PRELIMINARY OBSERVATIONS

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Background: Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening clinical syndrome due to a severe hyperinflammatory response. HLH is typically divided into a primary, genetic form and a secondary, acquired form. It is characterized by a very wide spectrum of clinical findings. Central nervous system affection "CNS disease" has been frequently described at presentation of HLH, during course of disease, or as isolated CNS-HLH that could preceed other systemic clinical manifestations by months to years.
Aims: To study the value of CSF soluble interleukin-2 receptor alpha subunit (sIL-2Rα) assay as a marker of CNS affection in children with HLH.
Methods: In this descriptive, observational study done at Alexandria University Children's Hospital, we analyzed the clinical data of a group of patients diagnosed as HLH. After informed consent was obtained, data was collected from patients who have undergone clinical examination, brain MRI, routine CSF analysis for evaluation of CNS-HLH, and sIL2Rα measurement in the CSF (Quantikine Human CD25/IL-2Rα Immunoassay, R&D Systems). Patients were considered as "CNS-HLH positive" when they had either neurological manifestations, abnormal findings on MRI or routine CSF analysis (elevated proteins &/or pleocytosis) and as "CNS-HLH negative" when they did not show any of these findings.
Results: We analyzed the data of 9 HLH patients; 4 females and 5 males. Their age ranged from 2 months to 13 years with a median of 5 months. Six patients had genetic diseases predisposing to HLH [Griscelli syndrome type II (GSII) & Chediack-Higashi syndrome (CHS)], and 3 other patients were diagnosed according to HLH-2004 diagnostic criteria and presumed to be of familial form (FHL). Out of the 9 patients, only 5 patients (55.6%) showed clinically evident neurological manifestations; 5 patients (55.6%) had elevated CSF proteins &/or pleocytosis, and 4 patients (44.4%) had an abnormal brain MRI. Overall 7 out of the 9 patients (77.8%) were "CNS-HLH positive" *versus* only 2 (22.2%) "CNS-HLH negative" according to classical criteria. Interestingly, the geometric mean of CSF sIL2Rα in CNS-HLH positive group was lower than in CNS-HLH negative group (734 *vs* 1952 pg/ml, p= 0.094). Moreover, CSF protein level and cell counts did not statistically correlate with CSF sIL2Rα level. Several patients showed interesting observations. Among the 3 patients with presumed FHL (6/7 HLH-2004 diagnostic criteria fulfilled, NK cell activity not tested), the one with the highest observed CSF sIL2Rα level (17329 pg/ml), a 2 months old infant, was "CNS-HLH negative", but had severe bilateral papilledema (discovered during workup for suspected autoinflammatory disorder). The second patient was a 3.5 year old boy, with history of a year of "CNS-HLH positive" findings and psychomotor regression, he had severe papilledema associated with high CSF sIL2Rα (3700 pg/ml). The third patient, an 11 months old "CNS-HLH negative" infant also had a relatively elevated CSF sIL2Rα of 220 pg/ml. On the other hand, 2 twin sisters evaluated at the age of 3 months for HLH secondary to CHS (positive family history, grey hair), had no systemic, neurological or radiological evidence of HLH, but had positive routine CSF analysis (elevated protein & pleocytosis) and CSF sIL2Rα (600 & 800 pg/ml).
Summary/Conclusions: We hypothesize that routine CSF sIL2Rα level assay could enhance earlier & better detection of CNS-HLH in children especially in primary and genetic forms. Moreover, the absence of statistically significant correlation between serum and CSF levels of sIL2Rα in our patients indicates that sIL2Rα is locally produced in the CSF secondary to cellular infiltration of the CNS and could be a valuable biological marker of disease activity. Larger prospective studies are warranted to confirm these results and determine diagnostic and prognostic value of CSF sIL2Rα levels, as well as its value for follow up of CNS disease.

E1412

GAUCHER DISEASE PATIENTS EXHIBIT A HIGH EXPRESSION OF LIPOCALINE (LCN2) AS POSSIBLE BIOMARKER OF RESIDUAL DISEASE ACTIVITY. EXPLORATORY STUDY AND CORRELATION WITH OTHER CYTOKINES

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Background: Gaucher Disease (GD) is characterized by a latent chronic inflammatory macrophage activation status expressed by an imbalance on pro-inflammatory cytokines, hyperferritinemia, hypergammaglobulinemia, altered calcium homeostasis and metabolic syndrome. Even patients under ERT do not fully revert this status and their risk to develop bone crisis, iron metabolism alterations, autoimmune disorders and neoplasm remain higher. This observation leaded to the creation of the minimal-residual-disease-activity concept. Monitorization of patients through chitotriosidase and CCL18/PARC has become essential however there are patients whom never normalize while others developed bone crisis/ complications after long-time under therapy and normal values. One of the key features for chronic inflammation is the anemia; this is characterized by hyperferritinemia a common feature at diagnosis of GD1 patients. Lipocaline (LCN2), a cytokine released by adipocytes, mononuclear cells and neutrophils with expression on endothelial cells, hepatocytes and other cells, has been involved into the monocyte polarization and perpetuation of the inflammatory state. Based on this, we have performed an exploratory study assessing LCN2 expression in GD1 patients.
Aims: To explore the Lipocaline (LCN2) expression as biomarker for disease activity in type 1 Gaucher Disease patients under different circumstances.
Methods: We have performed an exploratory study on 18 GD1 patients distributed in two cohorts. Cohort A was composed by 6 patients: 2 naïve (N) cases and 4 under miglustat (M) therapy; this patients were part of the clinical study QUELAFER and sere from baseline and after 4 months on chelation therapy were obtained. Cohort B included 12 patients on enzymatic replacement therapy (ERT), for this cohort sera samples were obtained for LCN2 determination and also a panel of cytokines (IL-10, IL-13, IL-4, IL-6, IL-7, Mip1a, Mip1b y TNFa), ferritin, hepcidin, chitotriosidase and CCL18/PARC were analyzed at beginning of ERT and after one year on it. Data were incorporated into a database for this porpoise including demographic and clinical available data. All patients have signed an inform consent for the use of their samples and ethical approved were obtained form institutional board of FEETEG foundation.
Results: Compared to reference controls provided by the manufacturer, all the patients showed increased levels of serum LCN2, the overall mean value for the initial sample was 171, 88 (66,72-261,72). As cohorts the differences among individuals were significant (Cohort A, p=0.02 and cohort B, p<0.01). Naïve

patients exhibit the higher values. In general 9 patients showed a reduction on LCN2 levels while 7 showed an increase and one the value was stable. All patients showed a reduction in ferritin and chitotriosidase, however a fully correlation with LCN2 expression were not founded. Globally there were no statistically differences, but as individual T-test showed a difference between both measures (p=0.027). A detailed description an analysis will be presented in case of acceptance.
Summary/Conclusions: Lipocaline expression is increased in GD1 patients in general, a correlation with other cytokines expression to establish the role of this biomarker is warranted.

E1413

COMPARISON OF TREATMENT AND OUTCOMES BETWEEN ACQUIRED PRIMARY AND SECONDARY THROMBOTIC THROMBOCYTOPENIC PURPURA
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Background: Thrombotic thrombocytopenic purpura (TTP) is a rare disease that is fatal if untreated. While the main treatment modality is plasmapheresis, immunosuppressants also play a crucial role in the treatment of TTP.
Aims: Our aim is to compare the clinical characteristics, treatment and outcomes of patients with acquired primary TTP to those with secondary TTP *i.e.* autoimmune and malignancy/ hematopoietic stem cell transplant (HSCT) related.
Methods: We reviewed all patients with TTP who received plasmapheresis at our institution from 1st Jan 2008 to 31st Jan 2017. Clinical and laboratory characteristics, treatment, response to treatment and complications were recorded. Complete remission (CR) was defined as platelet count normalisation, partial remission (PR) as platelet count doubling and >30 x 10^9/L and the rest as unresponsive/mortality (UM).
Results: Of 41 cases of TTP, 24% (n=10) was primary, 44% (n=18) was secondary to autoimmune diseases, 27% (n=11) was secondary to malignancy or HSCT, 5% (n=2) was related to DRESS syndrome and acute pancreatitis. The median age was 47(18-86) years and it was predominately female (81%). About two-thirds of the cases presented with neurological symptoms (66%), renal dysfunction (56%) and fever (59%). Only 12 patients (29%) had TTP pentad. Proportionate to incidence of secondary TTP, 85% required immunosuppressive therapy. CR was seen in 51% (n=21) and PR in 15% (n=6). Mortality rate at end of treatment was 41% (n=17) and at end of follow-up was 46% (n=19). Comparison of demographics, clinical presentation, treatment and outcomes between acquired primary TTP and secondary TTP are shown in table below.

Table 1.

		Primary TTP (n=10)	Autoimmune diseases related TTP (n=18)[£]		Malignancy/ HSCT related TTP (n=11)[#]	
Age (median, range)		57 (30–86)	41 (20–61)**		41 (18–84)	
Female (%)		80	83		82	
Neurological symptoms (%)		90	72		36**	
Renal dysfunction (%)		30	72*		46*	
Fever (%)		60	72		27	
Plasmapheresis session (median, range)		12.5 (8–34)	11 (4–128)		9 (2–45)*	
Rituximab (%)		30	72*		NA	
Vincristine (%)		20	17		NA	
Cytotoxic medications (%)		0	50**		NA	
Immunomodulators (%)		0	67**		NA	
Renal replacement therapy (%)		0	39**		46**	
Nosocomial infections (%)		30	78**		64	
Thrombosis (%)		40	22		9*	
Major Haemorrhage (%)		20	44		36	
Days of hospitalization (median, range)		21.5 (10–50)	48.5 (9–203)**		37 (7–100)	
D7	CR (%)	60	17	**	0	**
	PR (%)	20	22		55	
	UM (%)	20	61		46	
At completion of treatment	CR/PR (%)	80	61		55	
	Mortality (%)	30	50		46	
Relapse (%)		14	38		17	
Follow-up duration in months (median, range)		12.2 (0.3–111.3)	13.7 (0.2–128) 50		4.8 (0.3–86.6) 55	
Mortality at end of follow-up (%)		40	40 (5–193)		15 (1–144)	
Days to mortality (median, range)		15 (10–23)				

*Compared to primary TTP using chi-squared for categorial data and non-parametric Mann-Whitney U Test for continuous data. * P<0.1 **P<0.05.*

Summary/Conclusions: Compared to primary TTP, secondary TTP had an initial poorer response to plasmapheresis. Patients with autoimmune diseases required more immunosuppressive therapy and rituximab. Although the final response and mortality rates showed a trend towards poorer prognosis in secondary TTP, it was not statistically significant. Further studies are needed to improve the treatment of TTP, both primary and secondary.

E1414

EVANS SYNDROME IN CHILDHOOD: LONG TERM SINGLE CENTER EXPERIENCE
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Background: Evan syndrome (ES) is a rare entity in childhood, usually presenting with a course that is chronic and refractory to treatment.
Aims: To report on the clinical and laboratory characteristics of pediatric patients with ES diagnosed and long followed at a single center.
Methods: Data covering a 15 year period and concerning 14 ES patients were retrospectively studied. Clinical presentation, laboratory parameters, diseased severity, therapeutic approaches, number of relapses, presence of complications, time of follow-up and final outcome were reported. Disease was considered active when Hb <7g/dl and/or PLT <30,000/mm^3 and/or N<500/mm^3, in partial remission (PR) when 7-11g/dl and/or PLT 30,000 – 10,0000/mm^3 and/or N 500 – 1,000/mm^3, in short-term complete remission (SCR) when >11g/dl and PLT >100,000/mm^3 and N >1,000/mm^3 when still under or less than 12 months off treatment, and in long-term complete remission (LCR) when laboratory values as in SCR but free of treatment for over 12 months.
Results: Mean age at diagnosis was 5.4 years (18 months-12 years). Recent history of infection was reported in 3 (21.4%) and positive family history for autoimmune disease in 5 (35.7%) patients. At disease onset mean laboratory values and range were: Hb 10.5±3.38 gr/dl (4–14.5 gr/dl), WBC 6,233±3,593 /mm^3 (1,700–13,100/mm^3), N 3,282±3,166/mm^3 (100-9,442/mm^3), PLT 22,546±20,970/mm^3 (2,800-62,000/mm^3), LDH 372±302 U/L (226±1,162U/L), bilirubin 1.8±1.78mg/dl (0.3-4.8mg/dl). DAT was positive for IgG - IgG/C3d in 13 patients (92.9%) and for IgG/IgA in one patient (7.1%). As to immunology, 5 (35.7%) patients presented with mild IgG and/or IgM hypogammaglobulinaimia, 2 (14.3%) patients with decreased CD4/CD8 ratio, 3 (21.4%) with increased CD3+*TCRαβ*+CD4-/CD8- double negative T cells and 3 (21.4%) with persistently low C4. EMA were found positive in 2 (14.3%) and anti-TPO and/or anti-TG in 3 (21.4%) patients. In 10 (71.4%) cases simultaneous, whereas in 4 cases (28.6%) sequential cytopenia presence were reported. All patients presented with thrombocytopenia, all with positive DAT - but only 8 (57.1%) with Hb<11g/dl-, 7 (50%) with leucopenia and 6 (42.8%) with neutropenia. Severe presentation was reported at disease onset in 10 (71.4%) patients. All patients received IVIG (1-6g/kg) and high dose methyl-prednisolone (30mg/kg/H) – one or more times depending on clinical course. Prednisolone was administered in 10 (71.4%), cyclosporine in 8 (57.1%) and vincristine in 1 patient. Mean follow-up was 5.4 years (18 months - 13 years), during which 5 (35.7%) patients presented with one or more complications related to treatment: Cushing syndrome, osteopenia, hypertension, renal dysfunction and/or peripheral neuropathy. No severe infection or death was reported during the 15 year period. Disease relapses (1-3) were reported in 8/14 (57.1%) patients. With regards to outcome, 8/14 (57.1%) remained in LCR, 1 (7.1%) in SCR, 1 (7.1%) in PR, 3/14 (21.4%) in active disease, whereas 1 patient was lost to follow-up.
Summary/Conclusions: The rare entity of Evans syndrome in childhood seems to be associated with various immune manifestations and to carry complications related to treatment. Long term studies are needed to guide optimal management, which still remains challenging.

E1415

LOW DOSE RITUXIMAB IS A USEFUL ADDITION TO CORTICOSTEROIDS FOR NEWLY DIAGNOSED PATIENTS WITH WARM AUTOIMMUNE HEMOLYTIC ANEMIA
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Background: Warm autoimmune hemolytic anemia (wAIHA) is an infrequent autoimmune disorder with a high response rate to corticosteroids, albeit relapses are common. Low-dose rituximab has been used successfully in autoimmune cytopenias in an effort to increase response duration, while reducing adverse effects and costs associated with a traditional rituximab dose and prolonged prednisone exposure.
Aims: To evaluate the safety and efficacy of low-dose rituximab combined with corticosteroid treatment in newly diagnosed patients with wAIHA.
Methods: We performed a single-center, prospective, single-arm, open-label study in adult patients with newly diagnosed "primary" or idiopathic wAIHA from 2013-2016 using high-dose dexamethasone (40mg IV days 1-4) followed by prednisone 1mg/kg PO with slow tapering combined with low-dose rituximab (100mg total dose, days +1, +8, +15, +22). CR was defined as an increase in hemoglobin (Hb) ≥12 g/dL, PR was defined as Hb ≥10 g/dL or an increase of ≥2 g/dL. Response was evaluated at day +28, months +6 and +12. Informed consent was obtained from all participants.
Results: Eight patients were included, median age was 32 years (range 18-42), 6 were female. Median Hb at diagnosis was 5.8 g/dL (range 4-8.2 g/dL). All patients had response at day +28 (50% CR rate); median time to response was 12 days (range 3-17). During follow-up 7/8 achieved CR (median time to CR: 30 days, range 15-103), all of which were sustained at 6 months. Median follow-up was 24 months (range 8-40). One patient remained steroid-dependent and relapsed after 12 months, achieving a stable PR after re-treatment with low-dose rituximab. Furthermore, two patients had new-onset immune thrombocytopenia (IT; Fisher-Evans' syndrome), without hemolysis 6.5 and 8 months

after diagnosis. Two patients were diagnosed with systemic lupus erythematosus during follow-up, they remained in CR. Twelve-month CR rate was 80% (5 evaluable patients). One patient experienced grade 3 neutropenia two months after the last rituximab infusion that resolved without complications. Estimated relapse-free survival was 80% at 2 years (60% if IT is considered). No patient had a splenectomy performed.
Summary/Conclusions: This small study reports favorable outcomes for patients with newly diagnosed wAIHA treated with low-dose rituximab, and adds 8 patients with similar responses to the 7 cases previously published by the Italian group in 2012 and 2015. These results may be comparable to standard doses of rituximab, with a lower cost, and deserves further inquiry. The emergence of additional autoimmune phenomena (SLE, Evans' syndrome) is unpredictable and can be an obstacle for appropriate data analysis in prospective AIHA studies.

E1416

INFECTIOUS COMPLICATIONS IN PRIMARY AUTOIMMUNE NEUTROPENIA OF CHILDHOOD

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Background: Primary autoimmune neutropenia (PAN) of childhood is caused by the action of antibodies against membrane antigens of neutrophils leading to their peripheral destruction. Despite the low neutrophil counts, it is characterized by minor intercurrent infections with rare severe bacterial episodes, which can be a significant cause of morbidity.
Aims: The retrospective evaluation of the incidence and characteristics of infectious complications in children with PAN from one reference academic center in Greece.
Methods: The study included the clinical and laboratory findings of children with PAN, who were diagnosed in our department in the last eight years (2008-2016). All children had neutropenia lasting over 3 months with a positive test for neutrophil antibodies, using the granulocyte immunofluorescence test, the granulocyte agglutination test and the monoclonal antibody immobilization of granulocyte antigen test. Laboratory evaluation for nutritional deficiencies, infections, systemic autoimmune diseases or malignancies was negative. Clinical data related to the occurrence of bacterial infections and treatment, hospitalization and outcome were collected and analyzed.
Results: 48 children with PAN were enrolled; 28 were boys, the median age was 14.5 months (range 5-96) and median follow-up time was 20 months (range 4-93). 19 children (39.6%) all suffering from severe neutropenia (<0.5 x 10^9/L) had to be hospitalized 25 times for bacterial infections; 4 for pneumonia, 7 for acute otitis media, 1 for mastoiditis, 7 for urinary tract infections, 4 for bacterial infections of unspecified site,1 for perianal abscess and 1 for cellulitis, all with good outcome with proper antibiotic treatment. The average number of hospitalizations due to infections was 0,52/patient and the rate was 0.56/1000 patient-days. G-CSF was administered in 2 children due to severe infection, while 8 children received antibiotic chemoprophylaxis.
Summary/Conclusions: Although rare, infections are an important clinical issue in the management of children with severe PAN, sometimes requiring hospitalization. Early signs of infection should be promptly recognized and accordingly treated.

E1417

NEW EPO-RECEPTOR MUTATION IN A -17 YEAR OLD WOMAN WITH ERYTHROCYTOSIS

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Background: Erythrocytosis is defined when red cell, hematocrit (Hct) and hemoglobin (Hb), are elevated above normal limits. Causes of erythrocytosis can be primary and secondary. Secondary causes are divided into congenital and acquired. There is a group of patients with idiopathic erythrocytosis.
Aims: We present a case report of a novel EPO-Receptor mutation.
Methods: We present a case report of a 17-year-old woman with erythrocytosis. In the control blood test she had hemoglobin of 18.6g/dl and hematocrit of 62%. We contacted the patient and she attended hematology consultations for study and treatment with phlebotomy.The patient had no known drug allergies or toxic habits. She didn't have any known comorbidities or previous treatment. At evaluation she referred chronic headache without other symptoms. The physical examination was normal. At that time, three possible diagnose were suspected. Firstly, primary erythrocytosis, polycythemia vera (PV). In this disease, the bone marrow produces many red cells and the JAK2 V617F mutation has been demonstrated in the majority of patients. Exon 12 mutation has been described in patients with PV who did not have the JAK2 V617F mutation. The erythropoietin (EPO) level is undetectable as a compensatory mechanism. In our patient, JAK2 V617F mutation and exon12 mutation were negative and the EPO levels were undetectable (<1.5). The bone marrow aspirate and the bone marrow biopsy were normal. These results show that this patient doesn´t present PV, due to she only fulfilling one diagnosis criteria of PV. Secondly, acquired secondary erythrocytosis can be produced as a compensatory mechanism, including: cardiac or pulmonary disease, smoking, renal artery stenosis, sleep apnea/hypoventilation and malignant tumors. In the patient, pulmonary function test, abdominal ultrasound and kidney function were normal. Endogenous erythroid colonies were positive. Due to the test results, we ruled out the diagnosis of acquired secondary erythrocytosis. Finally, congenital secondary erythrocytosis. Genetic abnormalities have been identified in congenital causes of erythrocytosis. The congenital erythrocytosis are divided into two sets according to EPO levels. If the EPO levels are normal or increased, the patient could present high oxygen-affinity hemoglobin because of bisphosphoglycerate mutase deficiency or mutations in the genes in the oxygen sensing pathway. However, if the EPO levels are decreased, the patient could present an erythropoietin-receptor mutation. Our patient presented undetectable EPO levels and the EPO-receptor mutation was requested. The patient has been treated with phlebotomies and aspirin due to headache with good evolution. In this moment, she presents hematocrit levels of 46.8%.
Results: The test revealed an EPO-receptor mutation (c.1275_1290dup), which had never been described before.

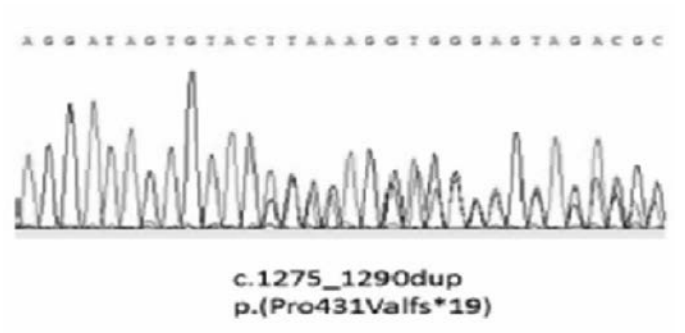

Figure 1.

Summary/Conclusions: The study of the patient with erythrocytosis must begin with a full medical history and confirmation of raised Hb and Hct. In the study of erythrocytosis, after ruling out primary and acquired causes we should always consider the possibility of congenital erithrocytosis, which often is underestimated. When EPO binds to its receptor a signaling cascade is activated, which cause red cells to be produced. This process is switched off when sufficient red cells have been produced by binding of SHP-1. EPO-receptor mutation results in failure of bind of SHP-1, causing uncontrolled production of red cells and erythrocytosis. We describe a new EPO-receptor (c.1275_1290dup) (figure1).

E1418

FAMILIAL HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS IN CHILDREN

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Background: Familial hemophagocytic lymphohistiocytosis (FHL) is an autosomal recessive disorder characterized with uncontrolled activation of T-helper lymphocytes and macrophages and over-release of inflammatory cytokines. The only curative treatment is hematopoetic stem cell transplantation (HSCT).
Aims: This study evaluates the clinical and laboratory data of children with FHL. Thirty five FEL cases followed and treated at our clinic between 2005 and 2017 were retrospectively evaluated in our study.
Methods: Information of patients were retrieved from patient files and from the records contained in the electronic information processing environment created after 2005. All patients vere treated with HLH-2004 protocol. HSCT was performed in nine patients.
Results: Twenty one of the cases were boys and fourteen were girls. The age at presentation for patients was two week-three years (mean 6.2 months). There was a history of consanguineous marriage in 26 of the families(74%). Fever, anemia, and hypertriglyceridemia were present in all HLH cases. Hepatomegaly was detected in 74%. Splenomegaly was more frequent in patients (85.7%). Hypofibrinogenemia was detected in all patients. All patients had neutropenia and thrombocytopenia. Hyperferritinemia was present in 94%% of patients. Only nine patients found to have hemophagocytosis in the cytological evaluation of the CSF(25.7%). Mutation analysis were performed in 18 patients and of these, 10 had PRF1, 5 had UNC13D, and 3 had STX11 gene mutation. All patients vere treated with HLH-2004 protocol. Of the 22 children who were placed in first remission. HSCT was performed in 9 patients (%25.7). The overall mortality rate was 57% (20 cases) in our series. Twenty children died opportunistic infection (n=10) or of disease progression (n=10).

Summary/Conclusions: In conclusion, FHL is a disease with high mortalite rates and the only curative treatment is HSCT. Donor search for HSCT must be started and HSCT should be performed after the remission.

E1419

ABNORMAL MONOCYTE POPULATIONS IN THE PERIPHERAL BLOOD OF PATIENTS WITH CHRONID IDIOPATHIC NEUTROPENIA

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Background: Chronic idiopathic neutropenia (CIN) is an acquired disorder of granulopoiesis characterized by an unexplained, prolonged reduction in the number of neutrophils and a generally benign and uncomplicated course. Neutropenia in CIN has been mainly attributed to increased apoptotic death of the granulocytic progenitor cells due to abnormal production of pro-inflammatory cytokines and pro-apoptotic mediators. Activated T-lymhocytes with a skewed oligoclonal/monoclonal profile and myelosuppressive properties have also a major role in the pathophysiology of CIN.
Aims: Monocyte subpopulations display a prominent role in innate immunity but also mediate pro-inflammatory responses and T-cell activation. The monocyte subsets have never been studied in CIN. The aim of the present study was to evaluate the monocyte subsets, namely the classical $CD14^{++}/CD16^{-}$, intermediate $CD14^{++}/CD16^{+}$ and non-classical $CD14^{+}/CD16^{++}$ cells as well as the monocytic $CD14^{+}/CD15^{-}/DR^{neg/low}/CD33^{+}/CD11b^{+}$ fraction of the myeloid derived suppressor cells (MDSC), in CIN patients.
Methods: We have studied 25 patients fulfilling the well-defined diagnostic criteria for CIN and 10 age and sex-matched healthy individuals. Three-colour flow cytometry was used to assess the peripheral blood monocytes subsets in the gate of CD14 positive cells and five-colour flow cytometry for the evaluation of the myeloid derived suppressor cells in the gate of cells with intermediate/high FSC/SSC properties.
Results: The mean number of neutrophils and monocytes in CIN patients was 1176±496/μl and 412±130/μl, respectively (range 200-1800/μl and 200-700/μl, respectively). The proportion of classical $CD14^{++}/CD16^{-}$ cells was significantly decreased in CIN patients (79.69%±7.60%) compared to the healthy individuals (87.90%±3.70%) (P=0.0009). In contrast, a significant increase was observed in the proportion of CD16 positive cells in CIN patients (16.81%±6.75%) compared to the controls (7.97%±3.16%) (P=0.0001). This increase was due to the higher proportion of the intermediate $CD14^{++}/CD16^{+}$ but not the non-classical $CD14^{+}/CD16^{++}$ monocyte subsets in CIN patients (12.74%±5.28% and 4.05%±2.51%, respectively) compared to controls (7.05%±2.47% and 2.73%±1.39%, respectively) (P=0.0014 and P=0.1383, respectively). Furthermore, the proportion of $CD14^{+}/CD15^{-}/DR^{neg/low}/CD33^{+}/CD11b^{+}$ MDSCs was significantly increased in the patients (6.18%±3.92%) compared to the healthy controls (3.31%±1.74%) (P=0.0412).
Summary/Conclusions: CIN patients display increased proportion of circulating intermediate $CD14^{++}/CD16^{+}$ monocytes that may have a role in the aberrant inflammatory responses commonly seen in these patients. The increased proportion of the $CD14^{+}/CD15^{-}/DR^{neg/low}/CD33^{+}/CD11b^{+}$ MDSC in CIN may simply reflect a compensatory reaction aiming to suppress the T-cell activation. Isolation of the above cell populations and transcriptome studies are currently in progress in our laboratory.

E1420

DIAGNOSTIC VALUE OF CELL BOUND AND CIRCULATING ANTI-NEUTROPHIL ANTIBODY DETECTION IN PEDIATRIC NEUTROPENIA

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Background: Chronic benign neutropenia (CBN) of infancy includes primary autoimmune (pAIN) and chronic idiopathic (CIN) neutropenia. A diagnosis of CIN is supported by the absence of demonstrable anti-neutrophil autoantibodies, which can be detected free in the serum and bound to neutrophils with the Indirect- and Direct-Granulocyte Immunofluorescence Test (I-GIFT, D-GIFT), respectively. Conclusive evidence is lacking on the diagnostic value of the D-GIFT, whose performance requires specific laboratory expertise, may be logistically difficult and hampered by very low neutrophil count in patient samples.
Aims: This study investigated whether the evaluation of D-GIFT improves the diagnostic accuracy of pediatric neutropenia.
Methods: I-GIFT and D-GIFT were performed by flow cytometry in 533 children including 174 (33%), 162 (30%), 81 (15%), 51 (10%) and 65 (12%) cases with pAIN, CIN, secondary autoimmune (sAIN), post-infection (PIN) and non-autoimmune (nAIN) neutropenia referred to this laboratory during 2002-2014, respectively.
Results: Using highly specific median fluorescence intensity cut-off values calculated by ROC curves, a positive D-GIFT was found in 49% of CIN patients, who showed similar clinical features as those included in the pAIN group. In 44 (27%) of 162 CIN patients I-GIFT was repeated 2-3 times in a year, resulting positive in 12 (27%) and 2 (5%) patients at the second and third screenings, respectively. Interestingly, 10 (71%) of the latter 14 patients showed a positive D-GIFT at the first serological screening.
Summary/Conclusions: D-GIFT evaluation improves the diagnostic accuracy of pediatric neutropenia. This can reduce the need for expensive and invasive investigations in CBN patients.

E1421

INAPPROPRIATE TREATMENT COULD MASK COBALAMIN DEFICIENCY: ROLE OF METHYLMALONIC ACID EVALUATION

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Background: Metabolic markers of cobalamin (Cbl) deficiency, such as methylmalonic acid (MMA) and homocysteine (Hcy) enable us to diagnose Cbl deficiency1,2. They differentiate Cbl deficient patients from those with low serum cobalamin levels (LB12), but without a real Cbl deficiency. Hcy evaluation is fully automated and available in many laboratories, whereas MMA determination is cumbersome and measured in few laboratories3. Routinely, Hcy is used to differentiate LB12 patients because a good concordance has been reported between Hcy and MMA4. However, in a study involving few cases, 26.5% patients with LB12 and normal Hcy showed high MMA levels4.
Aims: To evaluate the characteristics of patients with LB12 and normal Hcy and high MMA levels.
Methods: A prospective study was carried out in our University Hospital. Hcy levels were determined in LB12 (level <150pmol/l) patients with normal folate parameters for 18 consecutive months. MMA was assessed in those with normal Hcy. Serum B12, serum and red cell folate, and Hcy levels were evaluated using commercial automated methods. Hyperhomocysteinemia was defined by serum Hcy >17μmol/l. Serum MMA was assessed by using mass spectrophotometer and an increase in the MMA level was considered when MMA was >0.4nmol/l.
Results: A total of 237 patients with LB12 and normal Hcy were observed. In 27 (11.4%) MMA levels could not be determined. MMA levels were normal in 147 (70%). In 63 patients, MMA was increased (30%), including 25 cases (12%) with MMA levels >0.8nmol/l. In 48 out of 63 patients (76%), data on previous treatment were available. Of them, 40 (83.3%) patients had previously received inappropriate treatment (40% receiving folate) and 5 no previous Cbl treatment (10.5%). Only, 3 patients (6.25%) were treated with an adequate Cbl dosage.
Summary/Conclusions: MMA was increased in 30% of LB12 patients with normal Hcy, demonstrating cobalamin deficiency. Most of these patients (83%) were erroneously treated, including 40% receiving folate. As a consequence, in most of these cases this erroneous treatment decreased Hcy levels to normal values, but cobalamin deficiency was masked and could deteriorate, especially when folate treatment was used without and adequate cobalamin replacement.

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E1422

Abstract withdrawn.

E1423

RITUXIMAB IN AUTOIMMUNE HEMOLYTIC ANEMIA OF INFANCY

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Background: Autoimmune hemolytic anemia (AIHA) is not commonly seen during childhood, and is extremely rare in infancy. Absence of guidelines renders management of the disease difficult in children – and even more so in infants.
Aims: Aim of the report is to present a number of cases of infantile AIHA, refractory to conventional treatments, demonstrating response in administration of rituximab.
Methods: The report concerns four infants (3 baby girls and one baby boy) who presented with AIHA. Data regarding demographics, personal and family medical history, immunologic assessments, previous treatments and response to rituximab were studied.
Results: Age at diagnosis of AIHA was 4-6 months. In 3 cases (cases number 1, 2 and 3) personal and family history, as well as laboratory screening at diagnosis, did not reveal presence of any other hematologic, autoimmune or immunologic condition. In case number 4 AIHA followed the diagnosis of giant cell hepatitis. Hospitalization before rituximab administration ranged between 1 and 4 months, and included multiple transfusions, administrations of intravenous immunoglobulin (maximum dose 6g/kg), repeated doses of intravenous methyl-prednisolone (30mg/kg) followed by oral prednizolone (max 5mg/kg), all failing to achieve sustained response. Rituximab was administered at 375mg/m^2 in 4 weekly infusions. In 3 infants 5 monthly infusions followed. Stabilization of hemoglobin and improvement of hemolysis parameters were observed after the 3rd-4th weekly infusion in all infants. In 3 patients (no 1,2,3) CD19+ and CD20+ B cell assessment before and after rituximab administration was performed. Complete elimination (<1%) was observed in all patients after the 1st-2nd infusion. Despite B cells returning to normal 11 months after treatment, infant no 1 remained in clinical remission during follow-up (22 months post treatment). Infant no 2 remained in clinical remission for the 16 month post treatment follow-up, despite B cell normalization. Infant no 3 relapsed following B cell normalization, 11 months after rituximab administration. Infant no 4 did not undergo B cell measurements and relapsed one year after completing rituximab therapy. The 2 patients that relapsed were re-treated with 4 rituximab infusions: patient no 3 remained well for the 18 month follow-up, whereas patient no 4 remained well for 10 years – again relapsing and receiving her 3rd rituximab treatment with good response for the remaining 7 month follow-up. None of the patients presented with adverse reactions during the infusions or with severe infections as a result of immunosuppression. However, infant no 1 developed asymptomatic progressive IgG hypogammaglobulinemia 11 months after initial exposure to rituximab, eventually requiring IVIG administration.
Summary/Conclusions: Rituximab administration in refractory AIHA seems to be efficacious and safe in infants. However, close follow-up is warranted in order to ensure absence of long term complications, including the risk of post-treatment hypogammaglobulinemia, when the drug is administered at such young ages.

E1424

EARLY LESSONS FROM WHOLE-GENOME SEQUENCING IN THE CLINICAL DIAGNOSIS OF RARE INHERITED ANAEMIAS

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Background: Targeted re-sequencing has recently been adopted for the rapid diagnosis of anaemia patients whose disease is likely to have a genetic basis, however, currently results remain inconclusive in 30-60% of cases. Whole-genome sequencing (WGS), provides more uniform coverage than amplification-based panels and is allied to an unbiased approach offering the opportunity to explore both coding and non-coding regions. It is also possible to use WGS data to detect copy number variation with good resolution and sensitivity. Therefore WGS has the potential to offer an accurate molecular diagnosis in a proportion of unsolved anaemia cases and may therefore be a superior initial approach. Furthermore, WGS is likely to lead to the identification of novel genes involved in pathogenic and normal erythropoiesis.
Aims: We aimed to undertake WGS in a set of patients in whom targeted re-sequencing had not been able to identify a molecular cause for the inherited anaemia, in an attempt to increase the diagnostic yield of the molecular analysis of such patients and provide novel candidate genes as causative of anaemia.
Methods: We performed WGS of 20 individuals (2 singletons and 6 trios) at 30X coverage where all the probands have a rare anaemia of suspected genetic origin. Probands were pre-screened with a targeted panel containing ~50 candidate genes, none of which had harboured likely causative variants. Analysis of WGS data involved Stampy for read alignment, Platypus for variant calling and Ingenuity Variant Analysis (Qiagen) for variant annotation and filtering, followed by visual inspection and verification by Sanger sequencing.
Results: Known causative variants in a gene absent from the targeted panel were detected in two patients (25%), whereas candidate variants in novel genes not previously associated with anaemia were identified across the other six cases. Familial segregation and functional studies are underway to provide further evidence of causality for these novel variants, of which 60% are in genes with previous evidence of a role in erythropoiesis and 40% in genes with no known role in erythroid development.
Summary/Conclusions: These results illustrate the overlap in phenotypic abnormalities existing among these conditions and the importance of providing an accurate molecular diagnosis to enable correct diagnostic and clinical management of anaemia patients. We also demonstrate the benefit of using WGS over targeted resequencing given the difficulty of designing comprehensive gene panels and keeping them up-to-date as new candidate genes are identified.

E1425

CONGENITAL ERYTHROCYTOSIS: DISCOVER OF A NEW MUTATION

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Background: Congenital erythrocytosis (CE) is a rare hereditary disorder of red cell production, characterized by an absolute increase in red cell mass with elevated hematocrit and hemoglobin levels not accompanied by increased numbers of white cells and platelets. It can be caused by defects in the EPO-induced signaling pathway (primary), defects in the control of EPO synthesis by the oxygensensing pathway (secondary) or sinthesis of high oxygen affinity hemoglobins. EPO transcription is regulated by Hypoxia Inductable Factor (HIF). 3 prolyl hydroxylase domain proteins (PHD1, PHD2, PHD3), active in the presence of oxygen, are able to hydroxylate key prolines in oxygen depedent degradation domains of HIF alpha subunit and it is degradated by the proteosome. Mutations in those proteins are linked to CE.
Aims: Describe a new mutation in PHD2 gene associated to CE.
Methods: Clinical process consultation and search in Blood, European Hematology Association and Pubmed websites of keywords: "congenitalfamilial erythrocytosis" "phd2".
Results: We described a portuguese family followed by hematology service because of an isolated but sustained erythrocytosis, affecting 3 generations - grandfather, father (propositus) and son. Propositus referred headache and presented plecthoric face and hypertension. Analytically, it was confirmed erythrocytosis (haemoglobin>18g/dL and hematocrit>50%), without any other changes, except an indirect hyperbilirubinemia. Secondary causes of erythocytosis was excluded, with normal EPO and partial oxygen pressure. Bone biopsy only showed an erythroid hyperplasia, no JAK2 mutations identified, and normal hemoglobins electrophoresis, HBB and EPOR gene sequencing. We then proceeded to sequencing of gene included in EPO-induced signaling pathway and it was detected a new mutations in PHD2 gene (F366L), in heterozigoty. Despite it has never been described, other mutations in PHD2 were associated to slightly increased thrombotic potential so patients began antiplatelet aggregation therapy and phlebotmies. Additionally, they were diagnosed with Gilbert syndrome by a mutation in UGT1A1 gene promoter region [A(TA)7TAA].
Summary/Conclusions: An unknown mutation of PHD2 has been detected in 2 generation of a family with erythocytosis and it was co-segregated with the erythocytosis phenotype. That gene plays an important role in the regulation of EPO production and subsequently in erythropoiesis. Futhermore family studies have to be performed to better understand its pathogeny and management.

E1426

A RETROSPECTIVE STUDY OF THE THROMBOTIC MICROANGIOPATHIES DIAGNOSED IN THE LAST 17 YEARS IN ONE SINGLE CENTRE

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Background: Thrombotic microangiopathies (TMA) are characterized by the formation of platelet thrombi that obstructs vital organ microcirculation. The presence of the 5 classic parameters (haemolytic anemia, thrombocytopenia, fever, oliguria and neurological affection) is rare. ADAMTS13 determination allows a more accurate diagnosis than the presumption based on clinical and biochemical parameters.
Aims: To retrospectively analyze 44 TMA patients diagnosed in our centre in the last 17 years and characterize TTP, HUS and secondary TMA (sTMA) by revising their clinical data, correlate with ADAMTS13 level and identify predictors for survival and relapse.
Methods: TMA was defined as microangiopathic hemolytic anemia with thrombocytopenia under 150x10^9/L. All cases were classified as: 1. TTP (TMA with

ADAMTS13 <5% or TMA without baseline cause), 2. HUS (TMA with ADAMTS13 >5% and high creatinine level or positive E. Coli Shiga-Toxin or HUS related mutation), 3. sTMA (other TMA with a definite triggering cause). Clinical and laboratory parameters were analyzed in each group (TTP/HUS/sTMA) (ADAMTS13 ≤5% or >5%) by a univariate analysis using chi-square for categorical variables and ANOVA test for continuous variables. Kaplan Meier and multivariate Cox proportional hazards regression was used for survival and relapse.

Table 1.

Table 1. Univariate analysis for TTP, HUS, sTMA and ADAMTS 13 level. *Data is shown as mean and standard deviation (SD) or percentage of cases.*

	GLOBAL (N=44)	TTP (N=13)	HUS (N=8)	TMA (N=23)	P VALUE	ADAMTS13 >5% (N=20)	ADAMTS13 ≤5% (N=8)	P VALUE
LDH (u/l)	2483 (1923)	3612 (2658)	3936 (957)	2039 (1426)	0.04	2136 (1444)	3230 (1817)	*0.098*
Bilirubin (mg/dl)	1.49 (1.50)	2.53 (1.72)	0.75 (0.40)	1.38 (1.36)	0.008	1.09 (1.30)	2.06 (1.18)	*0.078*
Schistocytes (x100 erythrocytes)	3.6 (4.8)	6.7 (7.4)	2.6 (1.8)	2 (1.8)	0.015	2 (1.7)	7.7 (8.7)	0.009
Platelets (x10³/l)	53.32 (39.64)	15.54 (10.12)	77.75 (32.87)	66.17 (37.94)	0.0001	67.85 (34.88)	15.63 (11.82)	0.000
Creatinine (mg/dl)	3.66 (4.28)	1.03 (0.50)	7.02 (4.05)	3.96 (4.72)	0.005	4.71 (4.02)	1.06 (0.61)	0.018
Oliguria	50%	0	100%	60.9%	0.000	65%	0	0.002
Bleeding	43.2%	76.9%	12.5%	34.8%	0.008	20%	75%	0.011
Number of plasma exchanges	13 (10.13)	17 (8.86)	8 (4.20)	9 (11.24)	*0.057*	9.7 (11.66)	18.75 (7.86)	*0.055*
Hemodialysis	36.4%	0	100%	34.8%	0.000	50%	0	0.025
Splenectomy	11.4%	38.5%	0	0	0.001	0	50%	0.003
Overall survival	-	92.3%	100%	65%	0.23	89.4%	100%	0.37
Disease free survival	-	68.6%	80%	100%	0.02	92.3%	47.6%	0.04

Five-year overall survival and disease free probabilities (%). TTP thrombotic thrombocytopenic purpura, HUS hemolytic uremic syndrome, TMA secondary thrombotic microangiopathy, LDH lactate dehydrogenase.

Results: Patient distribution was: TTP 13, HUS 8, sTMA 23. ADAMTS13 was determined in 28 patients (low 8, high 20). Clinical and laboratory parameters of each group and univariate analysis are summarised in table 1. All patients received 1mg/kg/day steroids on admission and started plasma exchange. Patients in the TTP group showed increased levels of LDH, schistocytes, bilirubin and a low platelet count which was associated with bleeding. They also required a higher number of plasma exchanges to recover. Five patients relapsed, 4 with low ADAMTS13 level. 4 patients were splenectomized and received immunomodulators. One patient received only plasma exchanges when relapsed. One patient died immediately after diagnosis before receiving plasma exchange. HUS group patients had higher creatinine level which was associated with oliguria and dialysis requirement. Neurological symptoms were more frequent as well. Two patients progressed to renal failure and one was transplanted. Two other patients received eculizumab and 1 relapsed when treatment was interrupted during pregnancy. sTMA patients showed more cardiac events and fever. Main triggering causes were: 6 malignant hypertension, 5 systemic lupus erythematosus, 4 neoplasia, 3 pancreatitis, 2 pregnancy, 1 tuberculosis, 1 glomerulonephritis, 1 dermatomyositis. Six patients died (4 cancer related). In the multivariate analysis, high LDH level was significantly associated with relapse (p=0.012) while the number of schystocytes showed a trend to statistical significance (p=0.063).
Summary/Conclusions: ADAMTS13 determination is a useful tool in TMA differential diagnosis. A high LDH level, and also probably the number of schystocytes, could be valuable to predict relapse in TMA patients.

E1427

CHILDREN WITH CHRONIC-REFRACTORY AUTOIMMUNE CYTOPENIAS: A SINGLE CENTER EXPERIENCE

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Background: Autoimmune cytopenias are a group of heterogeneous disorders characterized by immune-mediated destruction of one or more hematopoietic lineage cells. They can be idiopathic or occur as a manifestation of other underlying disorders, such as autoimmune diseases, immunodeficiency, autoimmune lymphoproliferative syndrome, tumors, medications or infections.
Aims: The aim of this study was to evaluate the clinical course and significance of autoimmune cytopenias due to immunodeficiency or autoimmune diseases in children followed up at our hospital.
Methods: A total of 337 files of information belong to patients with chronic or refractory autoimmune cytopenias were evaluated retrospectively at our hematology department between February 1997 and September 2015. Ultimately, patients with immune deficiency or autoimmune diseases (23 patients) were included in this study. Data were analyzed using SPSS 15.0. The results are presented as the mean, SD, median, absolute number, or percentile.
Results: Twenty-three of the patients with chronic or refractory autoimmune cytopenias (6.8%) had an immune deficiency or an autoimmune disease. The median age of diagnosis was 3.1 years (between 6 months-16 years) and the ratio of male/female was 1.3. The median duration of following was 2.6 years (between 4 months and 18.5 years). A total of 13 patients (56.5%) had single-lineage cytopenias and 10 (46.5%) had multi-lineage cytopenias. Shows last diagnosis of the patients. In 22 of the patients, firstly cytopenias had detected than the primary diseases were diagnosed after median 2 months (between 0 and 77 months). Only one patient firstly had diagnosed as CVID, cytopenia has developed after years. All of the patients were treated with corticosteroids or intravenous immune globulin (IVIG) as first-line treatment. Ten patients needed second or further-line immunosuppressive therapies including rituximab, mycophenolate mofetil, cyclosporine A, azathioprine, and chloroquine. A total of 8 patients (34.7%) recovered from autoimmune cytopenias after the treatment of primer disease. That diseases were diagnosed as systemic lupus erythematosus in 4 patients, hypogammaglobulinemia in 3 patients, and celiac disease in 1 patient. Cytopenias have countinued in 14 of the patients. One patient with CVID died.
Summary/Conclusions: Cytopenia may be the first finding of an immunodeficiency or autoimmune disease and primary disease may be diagnosed in the clinical course. Early diagnosis is important because of beginning to the early treatment of underlying disease

E1428

INHERITED PROTHROMBOTIC RISK FACTORS IN TURKISH CHILDREN WITH HEREDITARY ANGIOEDEMA. SINGLE CENTER

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Background: Hereditary angioedema (HA) is characterized with recurrent mucocutaneous angioedema, abdominal pain, edema of larynx and extremities. HA is a life treatening, rare disease, it's genetic inheritance known as autosomal dominant. The incidence of the disease ranges from 1/10000 to 1/150000. 3 types of disease were described. Classic HA, is associated with C1 esterase inhibitor quantitative (type 1) or functional (type 2) defficiency. Type 3 HA is defined as form of HA which is seen in pregnant women and women use estrogen treatment. If plasma C1 inhibitor is deficient, complement, kinin-bradykinin, coagulation and fibrinolytic systems activate out of control and then vascular permeability increases and angioedema develops, tendency to thrombosis increases as well. Futhermore, it is known that acute treatment with C1 inhibitor concentrate and prophylactic use of danazol and antifibrinolytic drugs may also stimulate the thromboembolism. Therefore, prothrombotic risk factors are important in the patients with HA. Hence, we planned to search prothrombotic risk factors in patients with HA.
Aims: Hence, we planned to search prothrombotic risk factors in patients with HA.
Methods: Ten patients wih HA who were followed up at the Department of Pediatric Immunology and Allergy of the Erciyes University Medical Faculty were included in our study. The type and frequency of attack, use of prophylaxy and family story of HA were questioned. Factor V G1691A, prothrombin G20210A variant, methylenetetrahydrofolate reductase (MTHFR) and plasminogen activator inhibitor (PAI) mutations were investigated in all patients.
Results: Among the 10 patients of the study, five of the them were male (50%) and five were female (50%) and their ages mean was 151,90±48,21 months old (ranged from 75 to 210 months). No one had parental consanguinity. Nine patients (90%) had the family history of HA. Patients' affected family members were distributed by 5 sibling (50%), 3 mother and aunt (30%), 1 father (10%). One patient had no family story (10%). The mean serum value of C4 level in diagnosis was 4,71±1,62mg/dl (normal value:) mean value of C1 inhibitor level in diagnosis was 50,10±19,22mg/dl (normal value). It was learned that four patients (40%) had an attack of HA once every week, three patients had (30%) once per month, one patient (10%)had, once every 2-3 months. Two patients (%20) had no attack. Four patient had abdominal (40%), four patient had edema of hands, feet and face (40%). None of them received prophylactic treatment. One patient (10%) had heterozygous F V G1691A mutation, another one had also heterozygous protrombin G20210A mutation. The heterozygous MTHFR mutation were identified in seven patients (70%) and homozygous MTHFR mutation were found two patients (20%). Furthermore, four patients (40%) had heterozygous and one patient (%10) had homozygous PAI mutation.
Summary/Conclusions: C1 inhibitor, inhibits activated F XII,thrombin and plasmin. When the C1 inhibitor is deficient, dermal vascular thrombosis and systemic coagulation occur due to inhibition of activated FXII, thrombin and plasmin. Decrease level of PAI1 and PAI2, destructs plasmin activation which cause FXII activation and it effects prekallikrein and bradykinin cascade, therefore increase tendency of thrombosis and HA risk. In the literature, an adult patient who had heterozygous Factor V leiden mutation and purpura fulminans was reported. In our study, there is no clinical evidence supporting thrombosis, nevertheless it was observed that one of our patient with a homozygous PAI mutation had an attack more frequently. As a conclusion, in the patients, risk of thrombosis increases because of both HA and related treatment. Hence, prothrombotic risk factors should be investigate in patients with HA. In HA patients, known to protrombotic risk factors was crucial to estimate attack frequency-severity and treatment related thrombosis risk.

E1429

FLOW CYTOMETRIC ANALYSIS OF TISSUE SAMPLES IN 42 ADULT PATIENTS WITH MALIGNANCY-ASSOCIATED HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

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Background: Hemophagocytic lymphohistiocytosis (HLH) is a rare, potentially fatal hyperinflammatory syndrome, which in its most common, secondary form, can be induced by infection, malignancy or autoimmune disease. Diagnosis of HLH is made when at least five of eight clinical and laboratory HLH-2004 criteria are met. However, diagnostic criteria were established based on studies from pediatric patients, and it is debated if they can be applied to adults. Assessment of these criteria can be subjective (microscopic identification of hemophagocytes), time-consuming or not easily available (e.g. molecular analyses, functional tests of NK-cells).
Aims: The aim of the study was to evaluate phenotypic findings from flow cytometric (FC) analyses of bone marrow (BM) and other tissue samples from patients with hematological malignancies (hM) who developed HLH. The study was intended to investigate potential utility of a rapid phenotypic screening in diagnostics of suspected HLH.
Methods: Flow cytometric files for 42 patients with hM were retrieved from archive of the Department of Clinical Pathology and Cytology, Karolinska University Hospital. The patients were diagnosed and treated for hM-HLH at the Hematology Center of the same hospital, between 2009 and 2016. Tissue samples (bone marrow, peripheral blood, lymph nodes) were analyzed according to standard procedures, using monoclonal antibodies (BD, DAKO, Beckman Coulter, BioLegend). Cells were acquired using 4-color Canto A or 8-color Canto II cytometers (BD), and analyzed with BD FACSDIVA software. Neoplastic clones of myeloid or lymphoid character were excluded from reanalysis for the purpose of this study. Bone marrow samples were obtained from 31 patients shortly before and from 24 patients following HLH-diagnosis; in 13 patients paired BM samples were available.
Results: Patient characteristics are presented in table 1. Bone marrow B-cell lymphopenia was observed in 67% patients before and 74% after HLH diagnosis. Decreased amounts of NK-cells were noted in 48% persons at both time points. T-cell lymphopenia before HLH diagnosis was noted in 60% patients with myeloid malignancy but in only 25% cases of lymphoid malignancy, whereas in established HLH the respective figures were 27% and 46%. CD4/CD8 ratio was skewed-to-normal in both myeloid and lymphatic tumors before HLH was diagnosed. In cases of confirmed hyperinflammation, patients with myeloid tumors showed dominance of CD4+ cells but no such difference was noted in lymphoid disease. Loss of lineage specific markers of non-neoplastic T-cells was a constant feature in lymphoid malignancy, whereas aberrant expression of lymphatic markers (CD2, CD7, CD56) on myeloid cells was uniform in patients with myeloid tumors. Monocytosis was more often observed in myeloid as compared to lymphoid tumors at HLH onset (40% *vs* 31%), although it was of non-neoplastic character. However, monocytopenia was also noted in cases of established HLH, in 10% of myeloid malignancies and 15% of lymphatic malignancy cases.

Table 1.

Table 1. Patient characteristics.

Characteristics	Number of patients
Sex	
male	27 (64%)
female	15 (36%)
Age at HLH diagnosis [years]	
mean	64
median	57
range	26–85
Type of malignancy	
lymphoid	22 (52%)
myeloid	20 (48%)
Lymphoid malignancies	
T/NK-cell lymphoma/leukemia	7 (32%)
B-cell lymphoma	11 (50%)
Hodgkin lymphoma	3 (14%)
Multiple myeloma	1 (4%)
Myeloid malignancies	
AML (including APL)	15 (75%)
MDS	2 (10%)
MPN	2 (10%)
MDS/MPN	1 (5%)

Summary/Conclusions: In the presented cohort, quantitative shifts could be observed in BM samples around the time of HLH onset. However, different patterns were observed between patients affected by lymphoid or myeloid malignancies, which may represent disease-specific impact on BM microenvironment. Further study will be carried out to confirm findings in a large, possibly prospectively collected patient group. Control group of patients with respective malignancies but without HLH will be included.

Platelets disorders

E1430

BLEEDING IN PRIMARY IMMUNE THROMBOCYTOPENIA: WHO ARE MOST AT RISK?

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Background: Primary Immune Thrombocytopenia is rare disorder in which patients are at risk of bleeding due to autoimmune-mediated platelet destruction.
Aims: This study focused on describing the prevalence and types of bleeding events around the time of ITP diagnosis and after, as well as identify any factors that can potentially influence the risk of bleeding.
Methods: Data from the United Kingdom Immune Thrombocytopenia Registry were analysed for this study. The registry obtained its data from about 70 centres around the UK. Descriptive and logistic regression statistical techniques were used for this study.
Results: This analysis was based on 2365 (57.8% females) participants who are part of the Registry. The median age at diagnosis was 50 years (IQR 32, 66) and 77% of these patients were of European ethnicity. The commonest comorbid conditions was hypertension (23%). Median platelet count was 19 (IQR: 5, 53). Eighty percent had a platelet count below $30x10^9$/L around ITP diagnosis. The most common bleeding events were skin-related (46.5%) and to the oral cavity (14.4%). About 70% of the cohort experienced at least one bleeding event at some point after diagnosis. After ITP diagnosis the most common bleeds were again skin-related (34.3%) and oral cavity bleeding (14.8%). Epistaxis had risen from 11.6% before diagnosis to 17.7%. Bleeding at other sites did drop. However, the prevalence of intracranial haemorrhage rose from 0.9% pre-diagnosis to 1.2% after diagnosis. Prednisolone (79%) and IVIg (43%) remained the most used drugs followed by rituximab (26%) among those were treated. Romiplostim (15%) and Eltrombopag (9%) are used too but not anymore than mycophenolate (18%) and azathioprine (22%). Fourteen percent of the cohort had a splenectomy at some point. Age but not gender or ethnicity were found to be associated with having a bleeding event around the diagnosis of ITP. Young adults (18 to 30 years old) are less likely to experience a bleed than older adults (>70 years), who were most at risk. Platelet counts, expectedly, was associated with bleeding with those presenting with a platelet of $<30x10^9$/L were at higher risk. No comorbid illness or cotherapies were found to be associated with bleeding events.
Summary/Conclusions: The frequency of bleeding decreased for most sites but for some others a slight increase has been observed since ITP diagnosis. It is possible that bleeding events may have been recorded more accurately or observed more closely and over a longer period of time since diagnosis. However, control of bleeding was an issue after the diagnosis of ITP. Future analysis statifying its findings by time periods would be beneficial in describing if bleeding events were better controlled over the last few years, especially after the introduction of new therapeutic agents and the publication of the internal consensus report on the diagnosis and management of primary ITP.

E1431

A MULTICENTRE, SINGLE ARM, OPEN LABEL STUDY EVALUATING THE EFFICACY AND SAFETY OF ELTROMBOPAG IN PATIENTS WITH SEVERE PERSISTENT IMMUNE THROMBOCYTOPENIC PURPURA (ITP) WITHIN SIX MONTHS OF DIAGNOSIS

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Background: Patients with acute ITP who fail or are dependent on steroids or intravenous immunoglobulin (IvIg) are often committed to splenectomy or prolonged immunosuppression. Splenectomy is potentially curable but not without operative risk with many patients reluctant to undergo surgery, while the response to immunomodulation is often suboptimal with significant side effects. Although effective, to date, there is no published studies evaluating the benefit of eltrombopag among steroid dependent or resistant, non-splenectomised ITP patients diagnosed within 6 months.
Aims: To evaluate the efficacy and safety of eltrombopag in patients with severe "acute" and persistent ITP within 6 months of diagnosis.
Methods: A multicentre, single arm open label study involving 39 patients with severe ITP (a) with platelet count of $<30x10^9$/L despite a daily dose of prednisolone of 1mg/kg for at least 2 weeks from diagnosis OR (b) requiring prednisolone ≥10mg daily and/or recurrent doses of IvIg to maintain a platelet of $\geq 30x10^9$/L within 6 months of diagnosis. Prior splenectomy was not a requisite.

Patients with platelets <10x10^9/L will commence on eltromobopag 75mg daily while those with a count ≥10x10^9/L will commence on 50mg daily. A reduced dose is used for subjects of East Asian heritage. The dose of eltrombopag can be progressively increased by 25mg increment every 2 weeks to maximum of 150mg daily (patients of East Asian heritage should have a maximum eltrombopag dose of 100mg daily) if the platelet count remains ≤30x109/L or there is clinically significant bleeding every 2 weeks. The steroid can be progressively weaned to zero over the subsequent 6 weeks if clinically appropriate. The primary endpoint was overall response rate (ORR) at week 12, defined as the proportion of patients achieving complete response (CR; platelet >100x10^9/L), partial response (PR; platelet >50 x10^9/L) or minor response (MR; platelet ≥30x10^9/L with ≥50% reduction in the dose intensity of concomitant ITP therapy compared with screening). The protocol specified a 1-sided 5% level binomial test of the null hypothesis that ORR at week 12 ≤30% and reporting of a 90% two-sided confidence interval (CI).
Results: Of the 39 patients enrolled, 46% were women, median (Q1, Q3) age was 52 (37, 68) years, median (Q1, Q3) time since ITP diagnosis was 2.2 (1.1, 5.4) months, and median (Q1, Q3) screening platelet count was 21(13, 34) x10^9/L. Prior treatments included steroids (95%), IVIG (58%), and immunosuppresion (28%). 35 patients (90%) completed 12 weeks of treatment, 4 (10%) discontinued eltrombopag prior to week 12 [3 required new ITP therapy; 1 developed pulmonary embolism (PE)]. The median (Q1, Q3) dose eltrombopag at week 12 was 50 (50, 100)mg daily. The median (Q1, Q3) dose of steroid at week 12, zero (0, 5)mg daily. At week 12, the ORR was 64% (p<0.0001; 90% CI: 51-77%); CR, PR, MR rates were 41%, 15% and 8% respectively and the median (Q1, Q3) platelet count among responders was 168 (98, 252)x10^9/L. At week 26, the ORR was 54% (90% CI 40-67%); CR, PR, MR rates were 28%, 21% and 5% respectively. Two patients had serious adverse events (SAEs) with two episodes of venous thromboembolism (one deep vein thrombosis at platelet 97x10^9/L; one pulmonary embolism at platelet 240x10^9/L). There were no other adverse events or deaths.
Summary/Conclusions: The majority of patients with ITP diagnosed for ≤6 months had a favourable overall response rate to eltrombopag and the drug was generally well tolerated. Longer-term follow up data (beyond 6 mos) will be presented at the meeting.

E1432

A NOVEL RUNX1 MUTATION IN FAMILY WITH FAMILIAL PLATELET DISORDER WITH PREDISPOSITION TO ACUTE MYELOGENOUS LEUKEMIA

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Background: Familial platelet disorder with predisposition to acute myelogenous leukemia (FPD/AML) is a clinically heterogeneous group of rare disorders with autosomal dominant inheritance. Germline mutations in *RUNX1* were identified as causative lesions in several families with FPD/AML. *RUNX1* plays a key role in megakaryocyte maturation and differentiation but also in ploidization and platelet formation. In FPD/AML, *RUNX1* mutations are very heterogeneous and often specific to individual pedigrees, most commonly involving exons 3-5 in the RHD domain near the N-terminus of *RUNX1*. The presence of *RUNX1* mutation in FPD/AML is associated with 30-50% lifetime risk of developing AML. Clonal hematopoiesis can be detected in >80% of asymptomatic FPD/AML individuals at the age of 50.
Aims: To analyze mutational status of *RUNX1* and clinical and laboratory markers of the disease in family with hereditary thrombocytopenia including 3 cases of MDS/AML.
Methods: Platelet aggregation was measured on 4-channel aggregometer APACT 4004, with platelet rich plasma (PRP) in response to adenosine diphosphate (ADP), collagen, epinephrine and agglutination in the presence of ristocetin. DNA and RNA were isolated from peripheral blood leukocytes using standard procedures. Stored DNA from 2 patients who succumbed to AML was used for analysis. Mutational status of *RUNX1* was determined using direct Sanger sequencing of PCR products on DNA level. Analysis of alternative transcript length was performed using PCR with cDNA template, followed by detection on agarose gel electrophoresis. Sequences of alternative transcripts were obtained using direct Sanger sequencing. In 3 patients with MDS and/or AML, standard cytogenetics with G-banding was done, chromosomal changes were confirmed by fluorescent *in situ* hybridization (FISH).
Results: 9 individuals (4 adults, 5 children) from 3 generations in one family with FPD/AML were analysed. Novel hereditary *RUNX1* mutation NM_001754.4:c.509-13C>A was found in 7/9 family members as a likely cause of FPD/AML. The mutation is located in intron between exons 2 and 3 and disrupts RNA splicing, resulting in formation of alternative transcript with aberrant length (11 nucleotides longer than its physiological variant). Frameshift and premature termination of amino acid sequence presumably cause impaired function of the truncated protein. All family members harboring the mutation had various degree of thrombocytopenia (range 60-128x10^9/l) and did not require treatment in the absence of clinical bleeding. In 6/7 patients with mutation, decreased aggregation with collagen and epinephrine was found. Six of 7 patients with *RUNX1* mutation have iron deficiency anemia of unknown origin. Two women died of AML at the age of 51 and 38 years - the mother had karyotype 47,XX,+9[4]/46,XX[16] and her daughter harbored two pathological clones 45,XX,-7[5]/46,XX,-7,+8[3] and one clone with normal karyotype. The second daughter is followed due to pancytopenia with myelodysplastic changes, particularly marked megakaryocytic dysplasia with normal cytogenetics but severe aggregation impairment.
Summary/Conclusions: We revealed novel causal *RUNX1* mutation in familial thrombocytopenia. Identification of *RUNX1* mutation facilitates proper diagnosis of FPD/AML and identification of heterozygous carriers can help detect the development of myeloid malignancy. In the patient with pancytopenia, possible impact of other somatic mutations acquired during leukemogenesis will be examined.

Support: IGA-LF-2017-007, NPU LO 1304

E1433

Abstract withdrawn.

E1434

PROFILING CIRCULATING MICRORNAS IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA (ITP) TO EXPLORE THE ROLE OF MICRORNAS AND POSSIBLE BIOLOGICAL PATHWAYS INVOLVED IN THE PATHOGENESIS OF ITP

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Background: MicroRNAs (miRNAs) are small noncoding RNAs involved in regulation of gene expression. Dysregulated expression of miRNAs has been associated with several autoimmune diseases. ITP is an autoimmune disease characterized by isolated thrombocytopenia and increased risk of bleeding. The development of autoantibodies against platelets and megakaryocytes results in increased platelet destruction and insufficient platelet production remains central to the pathophysiology of ITP. Platelets contain high levels of miRNAs and a substantial fraction of circulating miRNAs originates from platelets. Circulating miRNAs are stable and relatively easy to measure and considered as potential disease biomarkers. The role of miRNAs in the pathogenesis of ITP has not been well explored.
Aims: Determine the expression profile of circulating miRNAs in ITP patients in aim to identify miRNAs that can be used as disease biomarkers and to explore the potential biological pathways that might be involved in the pathogenesis of ITP.
Methods: Exiqon Serum/plasma Focus microRNA PCR panel was used to determine the expression profile of 179 miRNAs in plasma acquired from 8 ITP patients with low platelet count and who failed to respond to various treatment for ITP, and from 8 age- and sex-matched controls. In addition, next generation sequencing (NGS) for miRNAs was performed in 2 pooled plasma samples (pool 1 from 4 ITP patients, and pool 2 from 4 matched controls), on the Illumina NextSeq 500 system. Statistical analyses were performed with the GenEx software and SPSS. Pathway analysis was performed using DIANA-miRPath v3.0 to explore the probable pathways involved in the pathogenesis of ITP.
Results: Comparing the expression profiling from the PCR panel between ITP patients and matched controls, 81 circulating miRNAs were differentially expressed (p<0.05), of those 17 miRNAs had a high statistical significance (Bonferroni correction). Among those, miR-191-5p and miR-26a-5p were down-regulated and miR-486-5p and miR-222-3p were up-regulated in ITP compared to controls. Interestingly, 15 of the 17 differentially expressed miRNAs from PCR panel were also differentially expressed in NGS. Using the 17 differentially expressed miRNAs in the miRPath analysis, we uncovered some immune system related pathways, including MyD88-independent toll-like receptor signaling pathway and TRIF-dependent toll-like receptor signaling pathway, as enriched pathways in target genes of miRNAs differentially expressed between ITP patients and controls.
Summary/Conclusions: We identified a large number of miRNAs that were differentially expressed in ITP patients compared to controls that might be associated with the pathogenesis of ITP. Pathways analysis uncovered some possible biological pathways that might be involved in the pathogenesis of ITP. Further validation of these miRNAs in a larger patient cohort and preferably in comparison to patients with other causes of thrombocytopenia such as aplastic anemia to explore the role of these miRNAs in the pathogenesis of ITP. Future studies of these miRNAs in relation to initiation of treatment with defined clinical outcomes as treatment response/ remission after initiation of treatment will clarify their potential as biomarkers for treatment response.

E1435

NORDIC COUNTRY PATIENT REGISTRY FOR IMMUNE THROMBOCYTOPENIA (NCPRITP): A COHORT OF PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA IN DENMARK, SWEDEN, AND NORWAY

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Background: Immune thrombocytopenia (ITP) is a rare disease characterized by isolated low platelet counts and an increased tendency to bleed. As yet, there have been no large, multi-country, population-based cohorts established to describe its long-term clinical course and investigate the effectiveness and safety of related therapies.
Aims: To describe the establishment of the NCPRITP and the characteristics of patients enrolled.
Methods: Encompassing Denmark, Norway, and Sweden, the NCPRITP started as a population-based post-authorization safety study to assess the long-term safety of romiplostim in treating ITP. It includes patients with prevalent chronic ITP (cITP – ITP lasting >6 months) as of 04/01/2009 and incident cITP diagnosed from 04/01/2009-12/31/2014, confirmed through medical record review. Since the start of the registry, guidelines have changed to define cITP as ITP lasting >12 months. For consistency, incident cases of ITP for a duration of >6 months will continue to be accrued through 2019. Through linkage of data from the national health registries and medical record review, the registry has rich clinical information for all enrolled ITP patients, such as comorbidities (including scores according to the Charlson Comorbidity Index [CCI] – a validated tool developed to predict 1-year mortality), treatments, lab values (e.g., platelet counts), and complete follow-up for several clinical outcomes of interest (e.g., clinically significant bleeding, the need for rescue therapies, and thromboembolic/thrombotic events). Additionally, available bone marrow samples are restained and reexamined for reticulin and collagen content to assess Thiele´s myelofibrosis (MF) grading.
Results: The NCPRITP includes 3,749 patients with confirmed cITP (35% Danish, 51% Swedish, and 14% Norwegian), with a female preponderance (58%) and median age of 56 years at cITP diagnosis. Forty-one percent of the cohort was prevalent at study inclusion; 59% represent incident cITP patients. Median follow-up time thus far is 4.3 years. At study enrollment, 24% had a platelet count <50x10^9/L, 16% were splenectomized, and 41% had at least one previous ITP therapy (mainly oral glucocorticoid steroids). The majority (68%) of the cohort had no underlying conditions included in the CCI at study enrollment, but 8% had a CCI score of 3 or higher, indicating severe comorbidity. Of note, based on hospital diagnoses of specific comorbidities recorded within 5 years before study enrollment, 8% had a history of a solid tumor, 9% had a history of diabetes, and 18% had a history of hypertension. Currently, 718 bone marrow samples from 566 patients have been retrieved.
Summary/Conclusions: The NCPRITP provides an example of how, within the Nordic countries' uniform health care systems, registries can be established to study the clinical course of rare diseases such as ITP and the safety of drugs used to treat these patients.

E1436

EPIDEMIOLOGY OF PRIMARY IMMUNE THROMBOCYTOPENIA (ITP) IN ADULTS IN RUSSIAN FEDERATION (RESULTS OF REGISTRY OF NATIONAL HEMATOLOGIC ASSOCIATION)

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Background: Primary immune thrombocytopenia (ITP) is a rare disease. The incidence of ITP is not well estimated in Russia and worldwide. Due to WHO information it varies from 1,6 to 3,9/100 000 person-years in adults. The gender and age-associated results in Russia and abroad are discussed and differ in several investigations.
Aims: evaluation of the incidence and demographic characteristics of primary immune thrombocytopenia in adults in Russia.
Methods: The data source is the Registry of the patients with primary ITP in Russia (intermediate data during the 2 years period). 1063 adult patients: 254 males (24%) and 809 females (76%), age from 18 to 89 years (median 44 years) with ITP (ICD-10 code D69.3).
Results: 41 region of Russia participated in the Registry formation. In 2016 more than a half of patients (58%) in comparison with 2014 (201 persons) and 2015 (441 persons) entered the Registry. All patient's mean age at the moment of diagnostic was 47.2 years, in females - 47.6, in males – 45.9. The gender-age distribution was following: male: age <30=20% of cases, age 31-40=19%, age 41-50=12%, age 51-60=19%, age 61-70=22%, age >70=8%. Three regions of Russia (Republic of Crimea, Irkutsk and Tula Regions) were selected for assessment of the incidence of ITP because of fully performed registration process. The number of newly diagnosed cases in these regions was 31, 42 and 56 patients per year respectively. ITP incidence was 1.3/100 000 person-years in Republic of Crimea, 1.7/100 000 in Irkutsk Region and 3.7/100 000 in Tula Region. The gender-age distribution was following: male: age <40=37%, age 41-60=26%, age >60=37%; female: age <40=50%, age 41-60=25%, age >60=25%. Totally, the ITP incidence rate in females was three times higher than in male population. Among patients with chronic ITP (82% of the total number of patients) the disease duration distribution was following: up to 3 years - 50% of the total number of patients, 3-6 years – 18.7%, 7-10 years - 11.6%, >10 years - 19.7%. The mean disease duration was equal to 6.7 years.
Summary/Conclusions: Overall incidence of ITP in three selected regions of Russia is: 1.3/100 000 person-years in Republic of Crimea, 1.7/100 000 in Irkutsk Region and 3.7/100 000 in Tula Region. It is compatible to the incidence in other European countries. Variations in parameters could be due to geographic location or not fully performed registration process. Our data demonstrate the rise of incidence rate in males with age and its decrease in female population. The number of patients included in the study on the epidemiology of ITP increased 5 times during 2016 in comparison with 2014 and 2015. Geographical variations of incidence of ITP in different regions of Russia require further study.

E1437

ELTROMBOPAG (EPAG) FOR THE TREATMENT OF PATIENTS AGED ≥65 YEARS WITH CHRONIC IMMUNE THROMBOCYTOPENIA (CITP): SAFETY AND EFFICACY RESULTS FROM THE EXTEND STUDY

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Background: ITP is an acquired autoimmune disorder characterized by isolated platelet reduction, which is considered chronic when it persists for >12 months. Evidence suggests that age may influence both the hemorrhagic manifestations of ITP and also response and adverse events (AEs) associated with some therapies. Changes in drug metabolism can contribute to increased AE rates in patients (pts) ≥65 yrs compared with younger adults. The oral thrombopoietin-receptor agonist, EPAG, is approved for the treatment of previously treated (eg corticosteroids, immunoglobulins) cITP pts, but limited data are available in pts ≥65 yrs old. The EXTEND study was a global, open-label, extension study that evaluated long-term efficacy, safety and tolerability of EPAG in adults with cITP who had participated in prior EPAG studies.
Aims: To describe the efficacy, durability of response, and safety of EPAG use in pts with cITP aged ≥65 yrs.
Methods: All pts on EXTEND started EPAG at 50mg/day, titrated to 25–75mg/day or less often as required, based on individual platelet count responses: to achieve counts in the range ≥50–200×10^9/L. Maintenance dosing continued after minimization of concomitant ITP medication and optimization of EPAG dosing. Pts could remain on EPAG either for 2 yrs in countries where EPAG was commercially available, or for >2 yrs until EPAG became commercially available.
Results: At baseline (BL), 50/302 pts (17%) on EXTEND were ≥65 yrs old (mean ± SD 73±5 yrs). Among these 50 pts, 24% were splenectomized, 62% female and 74% had platelet counts <30×10^9/L. Twenty-four pts (48%) withdrew early from the study, most commonly because of AEs (n=8, 16%), other reasons (n=7, 14%) and lack of efficacy (n=5, 10%). Median exposure duration was 2.3 yrs (range, 2 days to 7.9 yrs) and mean daily dose was 49.9 (range, 11–75)mg/day. Overall, 43 (86%) pts achieved platelets ≥50×10^9/L without rescue therapy; 37 (74%) achieved platelets ≥50×10^9/L for ≥50% of assessments; 26 (52%) maintained platelet counts continuously ≥50×10^9/L for ≥22 weeks (Figure). Median time maintaining platelet counts >50×10^9/L and twice BL values,

while not receiving rescue treatment, was 78 (range, 0–350) weeks. Incidence of bleeding symptoms (WHO grades 1–4) decreased from BL (66%) to 1 yr (15%). AEs were reported in 47 (94%) pts, most frequently nasopharyngitis (n=13, 26%), constipation (n=12, 24%), fatigue (n=12, 24%), diarrhea, arthralgia, urinary tract infection, cataract and cough (all n=11, 22%). Serious AEs occurred in 24 (48%) pts, most frequently (>5%) cataracts (n=7, 14%), pneumonia (n=3, 6%) and urinary tract infection (n=3, 6%). The most frequent AEs with suspected relationship to study drug were cataracts (n=4, 8%), headache, fatigue, and increased ALT, AST and bilirubin (all n=3, 6%).

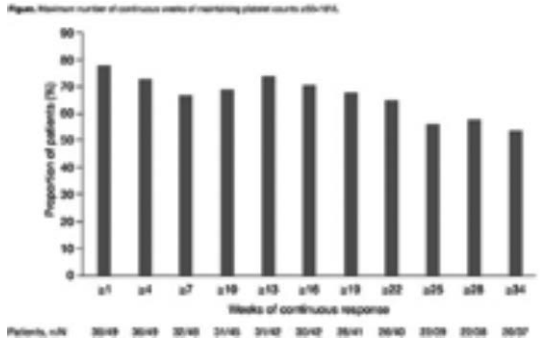

Figure 1.

Summary/Conclusions: The efficacy of EPAG in cITP pts ≥65 yrs was consistent with that seen with the overall EXTEND study population (Bussel *et al. Haematologica* 2016;101[s1];S517), with sustained platelet increases and reduced bleeding. EPAG was well tolerated; AE rates were similar to that reported in the overall EXTEND study population, but an apparent increase in cataracts was observed in pts ≥65 yrs old (cataract incidence was 7% and 22% in <65 and ≥65 age groups, respectively). Further outcomes in patients <65 yrs old will be presented. Results should be interpreted with caution as almost half of the pts withdrew from the study. EPAG is an effective treatment option for certain cITP pts ≥65 yrs; its use should incorporate baseline cataract screening and regular monitoring.

E1438

SAFETY AND EFFICACY OF THROMBOPOIETIN RECEPTOR AGONISTS IN PATIENTS WITH PREVIOUSLY TREATED CHRONIC IMMUNE THROMBOCYTOPENIA: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Background: The current American Society of Hematology guideline recommends the use of thrombopoietin receptor agonists, eltrombopag or romiplostim as one of the second-line therapies for chronic immune thrombocytopenia (ITP). The efficacy and safety of those drugs have been tested in several clinical trials. However, the safety profile was not consistent throughout trials and is not yet well understood.
Aims: We herein conducted a meta-analysis of randomized control trials to compare the safety and efficacy of thrombopoietin receptor agonists; eltrombopag and romiplostim *versus* placebo in patients with previously treated chronic ITP. Our primary outcome was drug-related adverse events greater than CTCAE grade 3.
Methods: We performed a literature search in MEDLINE, EMBASE, Cochrane library, and the American Society of Hematology website up to September, 2015 by two independent authors according to PRISMA guideline. We included only randomized clinical trials comparing eltrombopag or romiplostim *versus* placebo. Random-effects model was used to estimate pooled Odds Ratio (OR).
Results: A total of eight trials including 834 participants were included in the analysis. There was no significant difference of grade 3 or higher adverse events between placebo and treatment group (OR=1.01, CI 0.57-1.78). Thromboembolism (OR=0.59 CI 0.20-1.73), elevated ALT (OR=0.68 CI 0.26-1.74), headache (OR=1.26, CI 0.90-1.78), nausea (OR=0.82 CI 0.43-1.55), or fatigue (OR=1.13 CI 0.65-1.91) did not show a significant difference between groups, either. Clinical response, which is defined as platelets ≥50,000/μL at least once on treatment was significantly better in treatment group than in placebo group (OR=0.10 CI 0.07-0.15). Bleeding symptoms (WHO Grades 1-4) were significantly more frequent in the placebo group (OR=1.6, CI 1.14-2.24) during treatment.
Summary/Conclusions: Although several studies have suggested clinically significant treatment-related adverse events, such as thromboembolism, this meta-analysis showed that thrombopoietin receptor agonists are safe, well-tolerated, and effective in patients with previously treated chronic ITP.

E1439

CHILDHOOD IMMUNE THROMBOCYTOPENIA: A NATIONWIDE COHORT STUDY ON CONDITION MANAGEMENT AND OUTCOMES

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Background: Little is known about the management of pediatric ITP in real life, that is, routine clinical practice. Moreover, the predictive value of these factors upon disease outcome was explored individually and therefore the confounding effect of associated exposures remains unknown.
Aims: With this nationwide prospective cohort study, our objectives were to explore (1) the factors associated with treatment initiation (vs. watchful waiting) in children with primary immune thrombocytopenia (ITP) followed in routine clinical practice and (2) the predictors of chronicity at 12 months.
Methods: Between 2008 and 2013, 23 centers throughout France consecutively included 257 children aged 6 months to 18 years and diagnosed with primary ITP over a 5-year period. Data on ITP clinical features along with medical management were collected at baseline and 12 months. Multivariate logistic regressions were used to determine (1) and (2) as defined above, providing odds ratio (OR) with 95% confidence intervals (95%CI).
Results: 137 (53%) children were males, median age was 4.6 years, median platelet count was 7×109/L, and 214 (81%) patients initiated medication. Factors independently associated with treatment initiation included platelet counts <10x109/L (p<0.0001) and mucocutaneous bleeding symptoms at baseline (p<0.001). At 12 months, data were available in 211 (82%) children, of whom 160 (74%) had recovered. Predictors of chronicity included female gender (OR=2.2; 95% CI=1.0–4.8), age ≥10 years (OR=2.6; 95% CI=1.1–6.0) and platelet counts ≥10x10^9/L (OR=3.2; 95% CI=1.5–6.9).
Summary/Conclusions: In routine clinical practice, the decision to apply a watchful-waiting strategy seems to be driven by platelet counts even in the absence of bleeding symptoms, resulting in treatment being initiated in more than 80% of the children surveyed. Overall, younger children with ITP showed good prognosis, with lower platelet counts and, to a lesser extent, male gender predicting more favorable outcomes.

E1440

SIROLIMUS FOR THE TREATMENT OF CHILDREN WITH IMMUNE THROMBOCYTOPENIA AND EVANS SYNDROME. A SINGLE CENTRE EXPERIENCE

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Background: The treatment of chronic/relapsing immune thrombocytopenia purpura (ITP) is not well established due to the lack of evidence-based data, and is particularly challenging in children who are more at risk of severe side-effects secondary to prolonged steroid therapies. Sirolimus has been shown to be effective in patients with ITP secondary to ALPS[1]and in very few patients with primary disease or secondary to ALPS-like syndromes[2].
Aims: The aim of this study is to evaluate the outcome and toxicity of patients with ITP either primary or secondary to ALPS-like syndromes, with or without involvement of other cell lineages.
Methods: We retrospectively evaluated charts of patients followed in our Unit for ITP primary or secondary to ALPS-like syndromes. Patients with ALPS were excluded. ALPS-like was defined as the presence of at least one absolute or primary additional criterion for ALPS. Complete response (CR) and partial response (PR) were defined as a platelet count >/= 100x10^9/L and >30x10^9/L and at least 2 fold increase of the baseline count, respectively.
Results: 23 children aged 0-12 yrs (median 6) with primary ITP (7) or secondary to an ALPS-like disorder (16), were treated with Sirolimus. Seven patients (30%) with ALPS-like also had an Evans syndrome (ES), due to the association of leukopenia (1), or to the presence of trilinear cytopenia (6). Four patients with ALPS-like were found to have mutations on PI3KCD, CTLA4, TACI, and CARD 11 gene. All patients, but one treated in first-line, received Sirolimus as second (4), third (14) or fourth (4) line treatment, respectively. 18 patients had previously failed Micofenolatemofetile (MMF) therapy. Overall, 17/23 (74%) patients achieved a response that was complete and partial in 12 (52%) and 5 (22%) cases, respectively. Patients with ES responded in 6/7 (86%) cases. Children with mono-linear ITP achieved a response in 11/16 (68%) cases, in particular 4/7 (57%) and 7/9 (77%) patients with primitive or secondary disease, respectively. 12 out of 18 (66%) patients who failed MMF therapy responded to Sirolimus rescue. Three patients (13%) reported toxicity consisting of ovary cysts (2) and gastrointestinal issues (1) that required the interruption of the treatment in 2 cases.
Summary/Conclusions: To the best of our knowledge this is the largest cohort of patients with ITP or ES -other than ALPS -treated with Sirolimus, that showed to be safe and effective in most cases, including patients who previously failed

MMF treatment. Therefore, it can be considered as an alternative therapeutic option in the setting of ITP non only for patients with an underlying diagnosis of ALPS but also for the ones with primitive disease or with an ALPS-like disorder.

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E1441

ASSESSMENT OF ROMIPLOSTIM SELF-ADMINISTRATION BY PATIENTS WITH IMMUNE THROMBOCYTOPENIC PURPURA AND CAREGIVERS FOLLOWING RECEIPT OF HOME ADMINISTRATION TRAINING (HAT) MATERIALS: A CROSS-SECTIONAL STUDY

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Background: A HAT pack was designed as an additional risk minimization tool to support healthcare providers (HCPs) in selecting patients and training of patients/caregivers to mitigate medication error risk when self-administering romiplostim subcutaneously, a thrombopoietin-receptor agonist which is approved in the European Union (EU) to treat chronic immune thrombocytopenic purpura (ITP) refractory to other treatments.
Aims: To estimate the proportion of adult patients and caregivers who administered romiplostim correctly after HAT pack training.
Methods: This non-interventional, cross-sectional study enrolled 40 patients/caregivers and was conducted at 12 centres in Austria, Belgium, France, Germany, Greece, The Netherlands, Spain, and The United Kingdom, from 7 July 2014 to 20 November 2015. HCPs directly observed adults (≥18 years of age) with chronic ITP or caregivers new to administering romiplostim in the act of product administration at the first standard-of-care (SoC) 4-week visit after HAT pack training. Correct administration of romiplostim (primary endpoint) was defined as dose accuracy within 10% margin of error between prescribed and administered romiplostim doses, and correct romiplostim reconstitution and successful injection, and no HCP intervention during administration to correct patient/caregiver error. All analyses were descriptive and no formal hypothesis was tested.
Results: At the first SoC visit, 4 weeks (range: 2-8 weeks) after HAT pack training, 35 patients/caregivers (87.5%) administered romiplostim correctly. The dose accuracy was within 10% margin of error for all patients. HCP intervention was required in 5 instances: 1 patient did not ensure all romiplostim was dissolved, 1 patient and 1 caregiver needed verbal encouragement, 1 patient needed nursing intervention to read the correct dose from the vial due to poor eyesight, and 1 caregiver needed guidance with syringe and vial connection. Further follow-up data was available for only 2 of these 5 patients/caregivers; they both administered romiplostim correctly at a voluntary subsequent visit.
Summary/Conclusions: Given that this study was conducted on a convenience instead of random sample of patients, generalizability of the results may be limited. Direct observation can be susceptible to observation bias and to the Hawthorne effect with the patients/caregivers acting differently when observed. Nonetheless, the success of most patients and caregivers in correctly administering romiplostim after HAT pack training suggests that self-administration of romiplostim is a feasible option for suitable romiplostim-treated ITP patients.

E1442

FCΓIIA 131 H/R (A>G) RECEPTOR GENE POLYMORPHISM IN PATIENTS OF PRIMARY IMMUNE THROMBOCYTOPENIA (ITP)

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Background: Primary Immune Thrombocytopenia (ITP) is an autoimmune hematologic disorder characterized by isolated thrombocytopenia (<100,000/cmm) in the absence of other causes or disorders that may be associated with thrombocytopenia. The predominant mechanism is enhanced peripheral destruction of autoantibody coated platelets through binding of Fc portion of antibody with the Fcγ receptors on cells of reticuloendothelial system mainly monocytes/macrophages.
Aims: This study was aimed to investigate the association of polymorphisms in *FcγIIA 131 H/R* (A>G) gene with Primary Immune thrombocytopenia (ITP).
Methods: Genotyping for the *FcγIIA 131 H/R* (A>G) was performed using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) in 70 ITP patients and 70 healthy controls.
Results: The mean age of patients and control was 29.53± 13.86 yrs and 27.90± 8.89 yrs respectively. Male/Female ratio in patients and control was 1:2. Under additive model, the heterozygous genotype (AG) of the *FcγIIA 131 H/R* (A>G) polymorphism shows the significant association with ITP, (Odds Ratio 2.41 (95% CI, Lower - 1.19 Upper 4.90 *P*- value 0.0149) whereas the homozygous mutant genotype (GG) had no significant association with ITP (Odds Ratio 2.47 (95% CI, Lower - 0.63 Upper 9.72 with *P*- value 0.2979). Under dominant model, the Odds Ratio was 2.42 (95% CI, Lower - 0.34 Upper 0.94) with the significant *P*- value 0.0167. Mutant allele (G) frequency was 37.85% in patients and 25.71% in controls (Odds ratio 1.76 1.05-2.93 with the p-value 0.0397).
Summary/Conclusions: The study shows the association of heterozygous genotype (AG) of *FcγIIA 131 H/R* (A>G) with ITP. The dominant model also shows significant association with ITP. We conclude that mutant allele (G) in *FcγIIA 131 H/R* (A>G) gene polymorphism may have impact on susceptibility to ITP.

E1443

SHORT- AND LONG-TERM RESULTS OF FIRST LINE THERAPY WITH PULSED HIGH-DOSE DEXAMETHASONE IN ADULT IMMUNE THROMBOCYTOPENIA PATIENTS: A RETROSPECTIVE SINGLE-CENTER REPORT

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Background: Immune thrombocytopenia (ITP) is an autoimmune disorder mediated by clearance of antibody-opsonized platelets (plt) by spleen macrophages. Pulsed high-dose dexamethasone (HD-DXM) has proved to be effective in adult patients (pts) with primary ITP resulting in controlled studies in 89% short-term response and a relapse-free survival (RFS) of 58% at 50 months (mos) (Mazzucconi, Blood 2007).
Aims: To assess the short-term and sustained response rates of adult ITP pts receiving pulsed HD-DXM in everyday clinical practice.
Methods: Charts of pts with ITP - as defined by Rodeghiero, Blood 2009 - treated with HD-DXM were reviewed. DXM was administered according to the schedule of 40mg/day for 4 consecutive days to be repeated every 21 days for a maximum of 6 courses. A reduced-dose schedule of 20mg/day for 4 days was preferred for elderly/diabetic pts. Pts who had completed at least 3 courses were included in the analysis. Response to HD-DXM was classified according to IWP definitions (Provan, Blood 2010); therefore, steroid-dependent pts were considered as non-responders even if plt counts increased to safe levels during HD-DXM and were included only in the analysis of short-term response, but not evaluated for long-term response. Short-term response rate was determined at completion of the whole course of treatment. Relapse was defined as a plt count decrease ≤20x10^9/L after initial response achievement and RFS was defined as the time interval between last course administered and the date of relapse, censoring pts alive or dead without relapse. Follow-up was defined as the time between diagnosis and last available assessment. The probability of RFS was calculated using the Kaplan-Meier method.

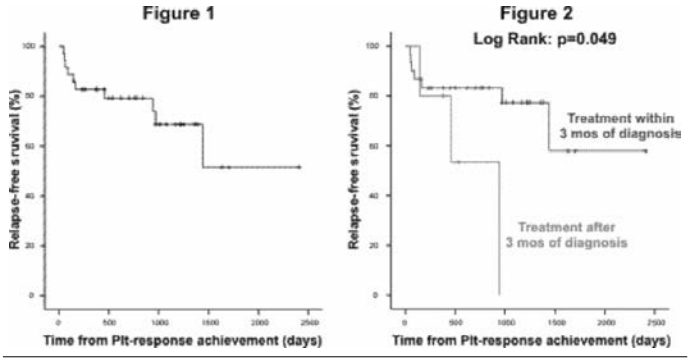

Figure 1.

Results: A total of 45 pts (M: 21) were eligible for analysis; median age at treatment was 60 yrs (range 18-87) and median time between diagnosis and treatment start was 3 days (range 0-4686). Pts received a median of 5,15 courses (range 3-6); 27/45 completed 6 courses: 21/45 received the full dose of 40mg/day (=960mg total dose) while 6/45 received the reduced dose of 20mg/day (=480mg total dose). Median total DXM dose was 800mg; IVIG along with 1st DXM course were required in 11/45 pts. In between courses, no bleeding complications were observed and no emergency therapies were required. Short-term response was achieved in 39/45 (87%): complete response (CR) in 28/45 (62%), response (R) in 7/45 (16%); 4/45 (9%) pts were classified as steroid-dependent ITP and excluded from subsequent analysis. Long-term response off therapy, lasting for a median time of 28 mos (range 5-80) without relapses was observed in 25/35 responding pts (71.5%; CR in 18/25, R in 7/25 at last follow-up) with a RFS of 51% at 50 mos (Fig. 1). Median plt count at last

follow-up was 192x10^9/L (range 54-336). Disease duration of less than 3 mos prior to therapy start was associated with better outcome (log rank p=0.049, Fig.2) with a median RFS not reached; median RFS for pts treated after 3 mos of diagnosis was 31 mos [OR: 3.8 (CI 95% 0.9-16.3), p=0.067]. No significant association between gender (p=0.67), age at treatment (more or less than 60 yrs) (p=0.85), DTX total dose (more or less than 480mg) (p=0.35) was found.
Summary/Conclusions: Pulsed HD-DXM is a well tolerated and highly effective first line treatment for ITP in every day clinical practice. The role of a reduced-dose schedule needs to be explored in a larger cohort of pts. Treatment of newly diagnosed ITP pts - *i.e.* within 3 mos of diagnosis (Rodeghiero Blood 2007) - seems to lead to longer RFS.

E1444

EFFECT OF OSELTAMIVIR TREATMENT ON PLATELET COUNTS

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Background: As platelets lose sialic acid during aging and circulation, they are cleared by the hepatic Ashwell-Morell receptor (AMR) (1). A recent study suggests that inhibition of sialidase by oseltamivir, a commonly administered anti-influenza medication that inhibits viral sialidase, could associate with an increase in platelet counts (2).
Aims: The aim of this study was to analyze the effect of oseltamivir treatment in platelet counts.
Methods: We performed a retrospective single-center study. From November 2009 until March 2015, a total of 168 patients from our Hematology Unit were prescribed oseltamivir due to clinical suspicion of influenza. A total of 120 patients were excluded because they had received myelotoxic chemotherapy within 30 days (n=82) or platelet count was not available before treatment (n=38). The direct immunofluorescent antigen test was carried out with nasopharyngeal aspirate specimens. Those specimens that were negative by the antigen detection assay underwent RT-PCR testing for influenza virus types A and B. Platelet count was available before and after treatment (median of 5 days) in 48 patients and in 44 patients also when the infection was cleared (median of 30 days).
Results: Patients were divided into those with proven influenza (n=34) and without influenza (n=14). Median age was 58.0 and 59.5 years; respectively. Treatment consisted of 75mg oseltamivir bid for 5 days, with the exception of 3 patients in the proven influenza group receiving 150mg bid for 10 days (allogeneic stem cell transplant recipients). We observed a significant increase in the mean platelet count after treatment with oseltamivir (170±95 x10^9/L *vs* 190±103 x10^9/L, p=0.04). As in the previous study (2), this effect was independent of whether influenza was diagnosed (Table 1). In addition, we did not discern significant fluctuation in platelet counts when treatment was immediately interrupted or after a 30-day time lapse (184±100 x10^9/L *vs* 182±91 x10^9/L).

Table 1.

Table 1. Platelet counts (x10^9/L) in patients with a clinical suspicion of influenza before and after oseltamivir treatment (median of 5 days). Results are given as mean and standard error.

Influenza positive (n=34)		Influenza negative (n=14)	
Before: 156 +/- 92	After: 174 +/- 99	Before: 203 +/- 94	After: 228 +/- 107
p=0,139		p=0,158	

Summary/Conclusions: Our study confirms the effect of oseltamivir on increasing platelet counts regardless of influenza infection. Although an increase in platelet counts related to the viral syndrome healing is not ruled out, the lack of long-term fluctuations after the end of treatment may indicate a late inhibition that contributes to reduction in platelet clearance via the hepatic receptor.

References

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E1445

CLINICAL UTILITY OF CARDIAC MRI IN IMMUNE MEDIATED THROMBOTIC THROMBOCYTOPENIC PURPURA

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Background: Immune Mediated Thrombotic Thrombocytopenic Purpura (TTP) is a life threatening thrombomicroangiopathy caused by acquired antibody mediated inhibition of ADAMTS13. Cardiac complications are a common cause of death and a raised troponin is a recognized risk factor for poor prognosis in TTP. There is scant evidence on the best investigations for patients suspected of being at risk of cardiac complications with no evidence on the clinical utility of cardiac magnetic resonance imaging (MRI) in acute TTP episodes.
Aims: A retrospective review evaluating the value of cardiac MRI scanning in TTP.
Methods: 24 patients underwent cardiac MRI scanning between September 2008 and November 2014 whilst being treated for an acute episode of immune mediated TTP. All patients had troponin-t measurement on admission and a transthoracic echocardiogram within 72 hours of presentation. All patients were treated for their TTP episode with plasmapheresis, steroids and Rituximab. Two cardiologists reported each MRI scan and only agreed, unequivocal findings were considered.
Results: The median age of patients was 49 (range 13-75), 71% of whom were women. Two patients had a diagnosis of hypercholesterolemia prior to TTP diagnosis but otherwise there was no previous cardiac history. 71% of patients had a raised troponin-t at presentation (normal <14ng/ml). Two patients developed bradycardia and one atrial fibrillation during their acute admission. One patient had symptoms of heart failure. Three patients had transient ST depression suggestive of ischemia on EKG monitoring and a further four had non-specific T-wave inversion. There were no incidences of cardiogenic shock or ST elevation myocardial infarction. 33% of patients (n=8) had abnormal cardiac MRI results. Late gadolinium enhancement (LGE) in the mid-apical regions, a finding suggestive of focal myocardial fibrosis and usually secondary to myocardial necrosis, was seen in all 8 patients, subendocardial LGE in six, transmural LGE in one and one patient had both subendocardial and transmural LGE. The latter patient was the only one with any abnormality seen on the preceding transthoracic echocardiogram. LGE seen in combination with regional wall motion abnormalities (RWMA) suggests irreversible myocardial dysfunction. No patient was found to have RWMA on transthoracic echocardiogram but this was seen in five patients on cardiac MRI imaging, all of whom also had LGE. The median troponin-t was not significantly higher in those with an abnormal cardiac MRI (normal MRI 100ng/ml, abnormal MRI 165ng/ml, p=0.9), nor was there a significant difference in median age (49 *vs* 49), symptom duration (abnormal MRI 7 days, normal 5 days, p=0.39) or presenting anti-ADAMTS13 antibody level (abnormal MRI 41%, normal MRI 40%, p=0.66).
Summary/Conclusions: Cardiac MRI scanning in TTP is a sensitive tool for detecting ischemic cardiac changes that would otherwise be missed by transthoracic echocardiogram. Mid-Apical late gadolinium enhancement appears to be a characteristic finding in TTP. These findings help increase the understanding of the pathophysiology behind the TTP disease process

E1446

THE FREQUENCY AND CLINICAL SIGNIFICANCE OF MEFV GENE MUTATIONS IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA

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Background: Immune thrombocytopenia (ITP) is an immune-mediated acquired disease characterized by transient or persistent decrease of the platelet counts. MEFV gene mutations are responsible for Familial Mediterranean Fever (FMF) a hereditary autoinflammatory disease characterized by recurrent febrile inflammatory attacks of serosal and synovial membranes. MEFV gene's protein product, pyrin or marenostrin, play an essential role in the regulation of inflammatory reactions. MEFV gene mutations are associated with a wide range of auto-inflammatory and autoimmune diseases. Recently, studies showed that MEFV mutations change the Th1, Th2 balance and increase the Th17 numbers. Th17 cells may have a key role in neutrophil activation and autoimmune diseases. ITP is an autoimmune-mediated condition that results from antibody-mediated destruction of platelets and impaired megakaryocyte platelet production. ITP is a complex disorder of immune dysregulation caused by anti-platelet antibodies produced by B cells. A recently proposed model for ITP involves antigen-presenting cells, B cells and T cells. Increased Th1/Th2 ratio and Th17 levels are implicated in the pathogenesis of ITP, as well.
Aims: This study addressed the prevalence of MEFV mutations and their effect on clinical features of ITP.
Methods: We studied the prevalence of exon 2 and 10 mutations (E148Q in exon 2, M694V, M694I, M680I, V726A, A744S and R761H in exon 10) in 81 adult ITP patients and 186 healthy controls. Patients were classified in two subgroups according to the presence of mutations. Demographic and clinical features were compared between groups to assess possible impacts of these mutations on clinical severity.
Results: Female to male ratio was 61/20=3,05 in the study group and 98/88=1,1 in the control group. The median age was 50 (21-79) in the ITP

group and 56 (24-76) in the control group. Overall MEFV mutation prevalence was%25.9 (21/81) in the study group and%24.7(46/186)in the control group, (p=0.963). MEFV mutation distribution prevalence was similar in both gender groups among ITP patients and their presence did not alter the age of disease onset, (p>0,05). Similarly, presence of mutations did not change the platelet count at diagnosis, the number of treatment courses, the rate of patients undergoing splenectomy and primary steroid resistance. Although statistically not significant, there was a trend towards a better overall response to steroids in patients carrying MEFV mutations,%94.7 *vs*%82.8,(p=0,28) respectively. The median time to loss of response to steroids was 60 (10-124) months in patients with mutations and 42 (19.2-64.8) months in patients without MEFV mutations, (p=0.64). The median time to splenectomy was 101 (42.5- 159.5) months in the MEFV mutation carriers and 51 (46-56) months in the non-carriers, (p=0.48). Time to loss of response to splenectomy was 38 (12-90.9) months in mutation carriers and 54 (14.9-93.1) months in non-carriers,(p=0.42).

Summary/Conclusions: To the best of our knowledge, our study is the first to address a possible effect of MEFV mutations on ITP. MEFV mutation carrier rates were similar in both ITP and control groups. Although MEFV carrier state had no affect on clinical features of ITP, mutation carriers tended to have a better overall response to steroid treatment, stayed longer in remission, had a longer time to splenectomy and relapsed earlier after splenectomy.

E1447

PD-1 AND CTLA-4 POLYMORPHISMS AFFECT THE SUSCEPTIBILITY AND CLINICAL FEATURES OF CHRONIC IMMUNE THROMBOCYTOPENIA

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Background: The programmed death-1 (PD-1) and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) play a central role in immune checkpoint pathways. The PD-1 negatively regulates self-reactive T and B cells in peripheral immune tolerance. The CTLA-4 antagonizes the binding of CD28 to its ligands including CD80 and CD86, and inhibits T cell activation. Previous studies have shown the lower expression of serum soluble PD-1 and CTLA-4 mRNA in patients with chronic immune thrombocytopenia (cITP) than healthy individuals. Single nucleotide polymorphisms (SNPs) of PD-1 and CTLA-4 have been reported to be associated with susceptibility of some autoimmune diseases; however, the possible association between these immune checkpoint SNPs and cITP risk remain controversial and obscure.

Aims: In order to explore the role of PD-1 and CTLA-4 in the pathogenesis of cITP, we investigated the impact of PD-1 and CTLA-4 SNPs on the susceptibility and clinical features of adult cITP.

Methods: We extracted the genomic DNA from 141 cITP patients and 223 healthy controls, and determined, 3 PD-1 SNPs (-606G/A, +7209C/T, A215V) and 4 CTLA-4 SNPs (-1722T/C, -1577G/A, +49A/G, +6230G/A) by using the polymerase chain reaction -restriction fragment length polymorphism (PCR-RFLP) method. The severity of bleeding tendency and thrombocytopenia was assessed according to the previously described criteria by Han JJ. The response criteria, 'corticosteroid dependence','severe cITP', and 'refractory cITP' were assessed according to the criteria of the ITP International Working Group. The characteristics and laboratory data of cITP patients with CTLA-4 and PD-1 polymorphisms were compared by using the Mann-Whitney U test for continuous variables and the chi-square test for categorical variables. This study was approved by the Institutional Review Board of Gunma University Hospital (Approval #160007).

Results: The minimum platelet count of all clinical course ranged from 0 to 98×10^9/L with a median count of 13×10^9/L. Eighty-six patients (61.0%) had bleeding tendency and 24 patients (17.0%) had severe thrombocytopenia (< 10×10^9/L). Eighty-six patients (61.0%) received the treatment with corticosteroid, and 38 patients (27.0%) were corticosteroid-dependent. As compared to healthy controls, the higher frequency of PD-1 +7209 TT genotype (low producer) was observed in cITP patients (12.8% *vs* 4.5%, p=0.004). There were no significant differences in CTLA-4 SNPs between cITP patients and healthy controls. In cITP patients, PD-1 +7209 TT genotypes (low producer) was significantly associated with high frequency of treated patients, treated patients with coticosteroid, and steroid-dependent patients compared with CC & CT genotype (high producer) (94.4% *vs* 71.5%, 94.4% *vs* 57.7% and 52.9% *vs* 23.6%; p=0.043, 0.003 and 0.018, respectively). On the other hand, CTLA-4 +49 AA genotype (high producer) was significantly associated with low bleeding tendency than AG & GG genotype (low producer) (36.4% *vs* 65.5%, p=0.010). CTLA-4 +6230 AA genotypes (high producer) was significantly associated with low bleeding tendency than AG & GG genotype (low producer) (27.3% *vs* 63.8%, p=0.017). CTLA-4 -1577 AA genotypes (high producer) was significantly associated with low frequency of bleeding tendency and steroid treatment than AG & GG genotype (low producer) (20.0% *vs* 64.1% and 30.0% *vs* 64.9%; p=0.014 and 0.041, respectively). CTLA-4 -1577 AA genotypes was significantly associated with higher minimum platelet count than AG & GG genotype (22.5 *vs* 14.0 $\times10^9$/L, p=0.048).

Summary/Conclusions: Our findings indicate that PD-1 gene polymorphisms contribute to the susceptibility of cITP, and PD-1 low producer genotype affects the severity of cITP. In addition, CTLA-4 high producer genotypes suggest the good clinical features and a little requirement of treatment in patients with cITP.

E1448

IS THE SPLENECTOMY OUTCOME PREDICTABLE IN PATIENTS WITH ITP?

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Background: Splenectomy may lead to a good response in 60-80% of adults with corticosteroid refractory immune thrombocytopenia (ITP). However, in the era of new drugs the proper selection of patients for splenectomy is essential to optimizing treatment outcomes. Accordingly, it is important to identify pre- or post-operative parameters that are able to predict the response to splenectomy.

Aims: To identify the pre- and postoperative parameters predictive of successful splenectomy in ITP.

Methods: We retrospectively analyzed 130 ITP patients (median age 43 years, range 19-74; 84/39 female/male; median time from diagnosis to splenectomy 19 months, range 2-132; median number of pre-splenectomy therapies 2, range 1-5; median follow-up from splenectomy 112 months, range 2-364) who underwent splenectomy between the years 1986 and 2015.Platelet kinetic study with Indium-111 was performed in 50 patients before splenectomy. Indications for splenectomy were: unresponsiveness to initial corticosteroid therapy, need for continuous glucocorticoid therapy to maintain safeplatelet counts and multiple relapses after discontinuing corticosteroids.Complete response (CR) and partial response (PR) were defined as platelet count (PC) $>100\times10^9$/L and 30×10^9/L one month after surgery, respectively. The patient was considered refractory if his PC remained $<30\times10^9$/L after splenectomy. Relapse was defined as a loss of CR or PR.

Results: CR and PR were achieved in 105/130 (79%) and 12/130 (7.5%) of the splenectomised patients, respectively. However, 13/130 (112.5%) patients were refractory. Twenty-nine of the 117 (24.8%) responsive patients relapsed. Predictors of good response after splenectomy identified by univariate analysis were: initial response to steroids (69.5% *vs* 22.7%, τ=0.358, p<0.0001), higher PC on the surgery day (90×10^9/L vs.37×10^9/L, ρ=0.353, p<0.0001), on the first (387×259/L *vs* 56×109/L, ρ=0.502, p<0.0001) and on the 7thdays after splenectomy (387×25^9/L vs.25×10^9/L, ρ=0.522, p<0.0001) and splenic platelet destruction (86% *vs* 0%, τ=0.358, p=0.0001). Using ROC analysis, cut-off prognostic values of PC were reevaluated: PC before splenectomy $>47x10^9$/L (AUC 0.864, sensitivity 63.6%, specificity 83.2%, 95% CI 0.785-0.943, p<0.0001), PC on the first day after splenectomy $>50x10^9$/L (AUC 0.956, sensitivity 44.4%, specificity 83.2%, 95% CI 0.912-0.999, p<0.0001) and PCon the 7thday after splenectomy $>300x10^9$/L (AUC 0.951, sensitivity 91.7%, specificity 45.6%, 95% CI 0.873-1.000, p<0.0001). By multivariate analysis, splenic platelet destruction, PC $>47x10^9$/L on the day of surgery (p=0.043) and PC $>300x10^9$/L on the 7thday after splenectomy (p=0.0013) were identified as predictive for favorable response. Patients who relapsed frequently were older >60 years (31% *vs* 23%,p=0.030) and had lower PC three months after splenectomy ($130x10^9$/L *vs* $278x10^9$/L, ρ=0.203, p<0.0001). However, ROC values could not be calculated.

Summary/Conclusions: Splenectomy is effective in approximately two thirds of patients with ITP. Our study suggests that splenectomy might be considered in the patients younger than 60 years, with splenic platelet destruction and PC $>50x10^9$/L on the splenectomy day.

E1449

FINAL RESULTS FROM AN OBSERVATIONAL STUDY (PLATEAU) OF ADULT PATIENTS TREATED WITH ROMIPLOSTIM FOR PRIMARY IMMUNE THROMBOCYTOPENIA (ITP) IN ROUTINE CLINICAL PRACTICE IN GERMANY

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Background: In the European Union, the thrombopoietin-receptor agonist romiplostim (Nplate®) is recommended since January 2016 for treatment of ITP in adult patients who are refractory to other treatments (*e.g.* corticoids, immunoglobulins).

Aims: The aim of this study was to assess the use of romiplostim in clinical practice in Germany.

Methods: This multicentre, prospective and retrospective observational study (data collected before and after initiation of romiplostim) enrolled ITP patients ≥18 years who received at least one dose of romiplostim in routine clinical practice, with an observation period of 2 years following romiplostim initiation. Endpoints included patient demographics, romiplostim use, platelet counts, adverse

drug reactions (ADRs), and other clinically relevant parameters. We report results from a full data set analysis.
Results: A total of 59 patients were enrolled (49.4% male; 54% aged 65 years or above) from 38 sites; 22 of them were excluded due to protocol violations (*e.g.* incomplete documentation, inclusion criteria not met). Of the 137 remaining patients (the full analysis set, FAS), 102 completed the 2-year observation period. Reasons for dropping out included loss to follow-up (10 patients), deaths (6 patients) and ADRs (3 patients). Median (Q1, Q3) time from ITP diagnosis to romiplostim initiation was 21.7 months (4-85) months in the FAS. 123 FAS patients received prior ITP therapies; most of them received corticosteroids (104 [75.9%]). 117 patients (85.4%) were non-splenectomized before romiplostim therapy, for reasons such as refusal of splenectomy, comorbidities, or age. Over the observation period, romiplostim was injected at a median (Q1, Q3) dose of 3.11 mcg/kg/bw (1.8 – 4.8; FAS) over a median (Q1-Q3) treatment period of 103 weeks (33-104). The median platelet count rose sharply from baseline (29.0 x 10^9/L) to two weeks of treatment (62.5 x 10^9/L). From week 3 to two years of follow-up, the median count remained in a range between 87.5 x 10^9/L and 145.5 x 10^9/L. Since the start of romiplostim therapy, 59 patients out of 137 (43.1%) received concomitant therapies, mostly corticosteroids (49 patients [35.8%]). The overall number of ADRs was 112 in the FAS, affecting 37 patients (27.0%). The most frequent ADRs were gastrointestinal (10.2%) and neurological (11.7%) ADRs, followed by constitutional symptoms (10.9%). Adverse drug reactions pertaining to blood/bone marrow affected 2.9% of patients (vascular/thrombotic events, bone marrow fibrosis), whereas bleeding as an ADR was seen in 0.7% of patients. The exposure-adjusted rate of bleeding events (grade 3 or 4) per 100 patient-years in the FAS was 7.2 before treatment initiation *vs*. 4.0 after starting the treatment. The rate of ITP-related hospitalization per 100 patient-years decreased from 23.3 before the start of therapy to 15.5 since the start of therapy.
Summary/Conclusions: This study of routine clinical practice in Germany showed that treatment with romiplostim in ITP patients resulted in a rapid increase in platelet counts to levels maintained between 50 and 250 x 10^9/L over time, regardless of the splenectomy status of the patients; most of them were non-splenectomized. The product was well tolerated and achieved a decrease in the rate of ITP-related hospitalization.

E1450

THE CLINICAL UTILITY OF NEUROPSYCHOLOGY TESTING IN IMMUNE MEDIATED THROMBOTIC THROMBOCYTOPENIC PURPURA
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Background: It is well recognized that neurological manifestations are common in thrombotic Thrombocytopenic Purpura (TTP) however research into the neuropsychological impact of the disease is lacking despite evidence suggesting patients who experience critical illnesses are at high risk for long-term cognitive impairment.
Aims: To review the clinical utility of neuropsychology testing in thrombocytic thrombocytopenic purpura.
Methods: Between 2010 and 2015, all patients within a single tertiary hematology center with a confirmed diagnosis of TTP were reviewed as outpatients after their acute episode. Those with persisting, non-physical neurological or psychological symptoms underwent cerebral MRI scanning and were referred for neuropsychological assessment. The Wechsler Adult Intelligence Scale III (WAIS-III) IQ test was used to assess factors including verbal IQ and performance IQs.
Results: 18 patients were included. 89% were female with a median age of 51 (16-67 years). 56% were Caucasian, 33% Afro-Caribbean and 11% of South Asian ethnic origin. 33% had experienced TIA or stroke-like symptoms during their acute TTP episode whilst 28% had no neurological symptoms during their initial presentation. The most common symptom leading to neuropsychology review was problems with concentration, experienced by 89% of patients. 44% had problems with memory, 39% felt depressed and 33% had anxiety issues. The median time from acute TTP episode to neuropsychology review was 29 months (range: 3-99 months). 25% had normal cerebral MRI scans, 50% had signs of sub-acute infarction on imaging and two patients scans showed both mature infarcts and microhaemorrhages. The median scores for both verbal and performance IQs were reduced compared to average (normal 100, range 90-110). The median verbal IQ was 87 (range: 65-122) and the median performance IQ 83 (range: 56-109). Taking all aspects of the WAIS-III review into consideration, one patient had a normal assessment. 50% (n=9) were found to have mild cognitive impairment, 33% (n=6) mild-moderate impairment and 11% (n=2) significant impairment. The two cases with significant impairment had a widespread pattern of dysfunction whilst in the other cases the most common pattern was of anterior/sub-cortical involvement (44%).
Summary/Conclusions: Persisting psychological symptoms after an acute TTP episode are highly suggestive of underlying cognitive impairment as a result of cerebral sub-acute infarction or microhaemorrhages.

E1451

FIVE NEW CASES OF HERMANSKY-PUDLAK SYNDROME: IDENTIFICATION OF NOVEL GENETIC VARIANTS IN HPS4 AND HPS3 ASSOCIATED TO RELEVANT CLINICAL COMPLICATIONS
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Background: Hermansky-Pudlak syndrome (HPS) is an inherited platelet disorder characterized by bleeding diathesis, oculocutaneous albinism and sometimes serious clinical complications. Heterogeneous clinical symptoms and a large numbers of possible genetic culprits (9 *HPS* genes, >118 exons) complicate unequivocal HPS diagnosis.
Aims: To assess the clinical and platelet phenotype in five patients with HPS suspicion and to identify their genetic defect by high-throughput sequencing (HTS).
Methods: We studied 5 patients from 3 families (2 Spanish, 1 Turkish) presenting with oculocutaneous albinism. Clinical records were reviewed and bleeding scored with ISTH-BAT. Platelet phenotyping (only Spanish patients) included: platelet aggregation, GPs expression and granule secretion, ^{14}C-serotonin uptake and whole mount electron microscopy. Patients DNAs were analyzed by HTS using a 71 gene panel.
Results: Clinical and laboratory findings in these patients are shown in Table 1. The Spanish patients (P1,P2,P5) showed impaired platelet aggregation to mild agonists and reduced platelet dense granules. In family 1 (F1), HTS identified a homozygous, potentially harmful, c.2054delC (p.Pro685Leu fs*17) variant in *HPS4.* One sister (P1) had Crohn´s disease and severe gastrointestinal (GI) bleeding. This variant had been reported in a 46yr Asian patient with pulmonary fibrosis (Bachli EB. Am J Med Genet 2004). *A* novel missense homozygous *HPS4* variant, c.272T>C (p.Leu91Pro), was found in two Turkish siblings (F2). One had severe GI bleeding requiring colectomy (P4) and the other developed pulmonary fibrosis. Patient 5, suffering from mild GI bleeding, bears a heterozygous novel variant in *HPS3* (c.2464C>T, p.Arg822X) and, most likely, an additional unrevealed mutation.

Table 1.

Family	Patients	Bleeding Symptoms	Other clinical features	ISTH-BAT	Light Transmission Aggregometry	Flow cytometry	14C-Serotonin Uptake	Electron microcopy	Molecular variants
1	P1: 13y, female P2:16y, female No Familial Consanguinity	P1+P2: Epistaxis, ecchymosis, menorrhagia P1: GI, and post-surgery	P1+P2: oculocutaneous (OC) albinism P1: Crohn's disease	P1: 11 P2: 3	Impaired aggregation with ADP (5mM), epinephrine (5mM) and ristocetin (1.2 mg/mL)	P1+P2: TRAP-induced CD63 release: 10% Plts+ vs. 65% in control	25% reduction in ^{14}C-Serotonin uptake	Absence of dense granules	*HPS4* c.2054del (p.Pro685Leu fs*17)
2	P3: 40y, female P4:48y, female Familial Consanguinity	P3+P4: easy bruising and epistaxis. P3: GI bleeding	P1+P2: OC albinism and nistagmus. P3: Colectomy P4. Bilateral pulmonary fibrosis	P3:7 P4:6	-	-	-	-	*HPS4* c.272T>C p.Leu91Pro
3	P5:25y, male No Familial Consanguinity	Epistaxis, ecchymosis and GI	OC albinism and strabismus Angiodysplasias	P5: 5	Mildly impaired aggregation epinephrine (5mM) and ristocetin (1.2 mg/mL)	TRAP-induced CD63 release: 1% Plts+ vs. 60% in control	75% reduction in ^{14}C-Serotonin uptake	Reduced number of dense granules	*HPS3* c.2464C>T (p.Arg822X)

Summary/Conclusions: HTS facilitates genetic confirmation of HPS diagnosis, and may help investigating phenotype-genotype relationships in HPS. The novel p.Leu91Pro variant in *HPS4* associates with severe clinical phenotype.
Funding: JMB: Gerencia Regional de Salud [GRS 1370/A/16]; JR: ISCIII & Feder (PI14/01956), CIberer CB15/00055, Sociedad Española de Trombosis y Hemostasia.

E1452

CHARACTERIZATION OF PLATELET ACTIVATION MARKERS IN EARLY ONSET PREECLAMPSIA
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Background: Preeclampsia is a serious pregnancy complication with potentially life-threatening consequences for both mother and baby, diagnosed when new onset hypertension and proteinuria develops after 20 weeks gestation. Early onset preeclampsia (EOP; onset <34 gestational weeks), is associated with higher maternal and fetal risks than late onset preeclampsia. At the extreme end of the severity spectrum, HELLP syndrome is characterised by

hemolysis, elevated liver enzymes, and low platelets. Previous studies have demonstrated enhanced platelet activation in pregnant women with pre-eclampsia, using cell surface markers and platelet microparticles[1]. Although severe pre-eclampsia is associated with increased inflammatory markers in vitro, levels of platelet activation do not necessarily correlate with severity of disease[2].
Aims: To assess the presence, and degree, of platelet activation in a cohort of patients with early onset preeclampsia (EOP)±HELLP syndrome, and to correlate this with evidence of *in vivo* coagulation activation using D-dimers.
Methods: Plasma samples from patients with EOP were accessed from a clinical biobank. Platelet activation markers were characterized using ELISA assays measuring platelet factor 4 (PF4), soluble glycoprotein VI (sGPVI) and neutrophil activating peptide-2 (NAP-2). Platelet microparticles ($CD42a^+$ microparticles) were measured by flow cytometry. Platelet activation biomarker levels were adjusted by platelet count and expressed as $/10^8$ platelets/ml). All data was analysed using GraphPad Prism 7. Parameters were reported as mean±SEM.
Results: Plasma samples from 19 individual patients were included. Patients with HELLP syndrome demonstrated significantly greater numbers of $CD42a^+$ microparticles when corrected for platelet count compared with those without HELLP syndrome ($598x10^3±203x10^3$ *versus* $297x10^3±37x10^3$, CD42a + microparticles/10^8 platelets/ml p=0.04). Similarly, patients with HELLP syndrome demonstrated increased levels of sGPVI than those without HELLP, corrected for platelet count (2.576±0.9667 *versus* 1.22±0.124 ng/ 10^8platelets/ml, p=0.0334). There was no difference in NAP-2 or PF4 levels between those with HELLP and those without HELLP, nor between severe and moderate pre-eclampsia patients. Severe pre-eclampsia patients in this cohort had a D-dimer level of 3.71±0.7742 µg/ml compared with non-severe patients 1.852±0.3501 µg/ml, p=0.0537. There was a significant correlation between sGPVI levels and D-dimer levels (Spearmann Rank correlation coefficient, r =0.532, p=0.04).
Summary/Conclusions: The results of this study demonstrate a positive correlation between severity of pre-eclampsia and platelet activation, as measured by levels of platelet-derived microparticles and platelet GPVI expression. A number of randomised controlled trials have evaluated the role of low-dose aspirin therapy as prevention for pre-eclampsia, and there is Grade 2B evidence for its use in those at risk of severe pre-eclampsia[3]. The evidence of enhanced platelet activation in our study provides rationale for the efficacy of aspirin in this setting, and the potential for novel antiplatelet agents to be studied for the same indication.

E1453

PRIMARY ITP IN ADULTS TREATED WITH ELTROMBOPAG: A RETROSPECTIVE STUDY USING DATA FROM THE UNITED KINGDOM ADULT IMMUNE THROMBOCYTOPENIA REGISTRY.

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Background: Primary ITP is an autoimmune disorder associated with a reduced peripheral blood platelet count. Although many patients are relatively asymptomatic, many suffer with bruising, mucosal bleeding and quality of life issues. The first line treatment has remained unchanged for decades and until recently, second-line therapy has been unsatisfactory, using empirical treatments. The recently approved thrombopoietin receptor agonists eltrombopag and romiplostim have transformed patient care and these agents are licensed second-line therapies in adults.
Aims: To describe the adult patients receiving eltrombopag using data from the UK Adult ITP Registry. In particular we were interested in understanding the mean dose used, number of prior therapies, median length of treatment with eltrombopag, median counts at baseline before treatment and at six months following treatment, and sustained response in patients who have received eltrombopag.
Methods: The UK Adult ITP Registry involved more than 70 UK collaborating centres, coordinated by The Royal London Hospital. In this study we analysed data from all patients receiving eltrombopag and analysed these using various statistical techniques.
Results: The total number of patients evaluable was 129. The median age at diagnosis was 49.4 years (26.9-66.4). There were 74 males (57.4%) and 55 females (42.6%). 29 patients (22.4%) had undergone prior splenectomy. The median age at eltrombopag initiation was 59.5 years (37.0-70.7 years). The median time from ITP diagnosis to eltrombopag initiation was 1.6 years (0.7-2.3 years). The majority of patients started eltrombopag between 2013 and 2016 (93%). 10 (8%) and 8 (6%) started eltrombopag within the first 6 months and between 6 to 12 months of ITP diagnosis, respectively. Most patients had received prior ITP therapies. Some 10 patients (7.8%) had received one prior ITP therapy and 99 patients (77%) had received three or more prior therapies before starting eltrombopag. The commonest prior therapies were corticosteroids in 110 patients (87%); IVIg 91 patients (72%); rituximab 68 patients (54%); romiplostim 47 patients (37%); and immunosuppressants 71 patients (56%). At baseline, prior to starting eltrombopag, the median platelet count was $21×10^9$/L (10-54) and the majority of patients (64.5%) had platelets less than $30×10^9$/L. The mean platelet count at 6 months was $206.2×10^9$/L and at 1 year was $288×10^9$/L. The median dose of eltrombopag used was 50mg/day. The median course length on eltrombopag was 14.7 (IQR: 4, 67) weeks. After initiation, 53 (41%) remained on eltrombopag as a monotherapy whereas 27 (21%) had other ITP treatment concurrently with eltrombopag. Forty nine (38%) changed treatment after eltrombopag, of which prednisolone (47%), IVIg (33%), mycophenolate (18%) and rituximab (14%) were the commonest and 10% underwent a splenectomy. Response to eltrombopag was assessed for 106 patients with adequate follow up time and platelet counts. 81 (76%) had a response, of which 54(51%) were above 100x109/L and 27 (25%) had a partial response (platelet counts between 30 to 100x109/L). Among those that had a response, 15 (14%) became unresponsive after some time whereas 2 (2%) patients were unresponsive soon after a brief episode of response. In short, 64 (60%) had a sustained response to eltrombopag (among patients who remained or came off eltrombopag).
Summary/Conclusions: The patient characteristics of those receiving eltrombopag appear to be typical of adult ITP. Only 10 patients (7.8%) received eltrombopag as a second line therapy. Three quarters had received 3 or more prior therapies before starting eltrombopag despite its licence as a second line therapy. As clinicians become more familiar with its use, a greater proportion of patients are likely to receive eltrombopag as a second line therapy.

E1454

EFFICACY OF TPO-MIMETICS IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA

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Background: Immune thrombocytopenic purpura (ITP) is an autoimmune disorder in which antibodies are produced to circulating platelets. The two currently available agents (Romiplostim and Eltrombopag) have similar efficacies and only slightly different safety profiles, being effective in restoring a safe platelet count in 70%-80% of cases with chronic ITP failing one or more lines of treatment, including splenectomy.
Aims: We evaluated the efficacy of TPO-RAs in patients with ITP.
Methods: From November 2008 and February 2017 65 patients (33 M; 32 F) were treated with a median follow-up of 29 months (1-96): 39 underwent therapy with Romiplostim and 26 to Eltrombopag. Median age was 69 years (range 39-94 years). In the group of patients treated with Romiplostim, 21 had already received more than 4 lines of therapy, while 18 received 1-2 lines of therapy. 13/26 patients who received Eltrombopag were at the 3nd line of therapy, 1 at the second, and the others were at least at the 4th line. The median platelet count was $21x10^9$/L (3-52) at the start of Romiplostim, with a median starting dose of 1 µg (1-2) and $17x10^9$/L (1-53) in patients treated with Eltrombopag, with a median starting dose of 50mg (25-50).
Results: Patients treated with Romiplostim we observed 22 complete responses and 10 responses, with a 82% response rate, while 7 patients were no responders. In our study 26 (66%) patients stopped Romiplostim after a median time of 16 months (1-93): 9 for stable response, 5 for no response, 3 for loss of response; 3 for adverse events (2 for bone marrow fibrosis, 1 for headache associated to visual disturbances and gastrointestinal disorders), 2 underwent splenectomy, and 3 patients interrupted the treatment for other causes (es. diagnosis of cancer). The median platelet count at suspension of Romiplostim was 91.5 $x10^9$/L (3-320). In patients treated with Eltrombopag 16 achieved a complete response, 5 a response, obtaining response in the 80% of cases; 5 were no responders. 14 (53%) patients stopped Eltrombopag after a median time of 1,5 months (1-12): 6 for adverse events (2 cases of major cardiovascular events, liver toxicity, skin rash, pharyngitis), 5 for no response, 1 for loss of response, 2 patients who achieved a CR interrupted Eltrombopag obtaining a sustained remission after discontinuation. The median platelet count at suspension was $97x10^9$/L (2-739). Patients who did not interrupted treatment are still receiving therapy with a median of 29 months (3-96). Several studies reported Romiplostim and Eltrombopag to be highly effective against chronic ITP, with average immediate responses exceeding 80% in our study. We observed that therapeutic response was influenced by the starting platelet count. In particular platelets count before therapy influenced the first response observed. In particular in patients treated with Romiplostim PLT pre-treatment directly correlated with the first response and the mantainance of response during treatment at month 1°, 2° 3° and 6. Patients with a median starting platelet count of $15×10^9$/L obtained a response (CR + R), while almost all patients who started therapy with PLT<$15×10^9$/L at baseline can obtain an initial response, but the majority is non-responder.
Summary/Conclusions: TPO-mimetics have proved efficacy in patient with ITP and their use can be applied in several conditions (bridge to splenectomy; sustained response; switch and discontinuation). Future study on large series of patients are needed to best correlate baseline platelets with hematological response. The two currently available agents (Romiplostim and Eltrombopag) have similar efficacies and only slightly different safety profiles, being effective in restoring a safe platelet count in 70%-80% of cases with chronic ITP failing one or more lines of treatment, including splenectomy.

E1455

PREVALENCE AND RISK FACTORS FOR THROMBOSIS IN ADULT ITP PATIENTS

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Background: Immune thrombocytopenia (ITP) is characterized by severe thrombocytopenia due to autoantibody- and cell-mediated peripheral platelet destruction and attenuated thrombopoiesis. Despite a higher risk for bleeding, thromboembolic events (TEE) have been observed.
Aims: We aimed to investigate the prevalence and type of TEE and the potential risk factors in adult ITP patients.
Methods: Retrospective cohort study, including all ITP patients followed in our clinic between 01/1990 and 05/2016. Information on gender, age, date of ITP diagnosis, platelets count, type and clinical form of ITP, type of ITP treatments and its response, severe bleeding and follow up time were collected. Furthermore we evaluated date of first appearance, number and type of thromboembolic events, cardiovascular risk factors, date and cause of death. We assessed and compared risk factors of ITP patients with and without TEE in univariate and multivariate analysis.
Results: Medical files of 480 patients registered as ITP were reviewed; 42 patients were excluded from the analysis (not fulfilling the ITP criteria according to Rodeghiero *et al.* Blood 2009). In total 438 patients were retained for analysis, 10% out of them (44 patients) presented ≥1 TEE after ITP diagnosis. Within these patients, in total 54 TEE occurred: 34 venous (61%), 19 arterial (34%) and 3 arterial and venous (5%) thrombotic events. The most frequent venous TEE were pulmonary embolism, deep vein thrombosis, and superficial vein thrombosis; arterial TEE were cerebrovascular insults, myocardial infarction and peripheral artery thrombosis. At time of TEE, 43% of patients were on treatment with corticosteroids, 14% with thrombopoietin receptor agonists (TPO-ra) and 18% were off-treatment. In the univariate analysis, older age at diagnosis (≥50 years, P=0.015), longer interval since ITP diagnosis (P=0.001), persistent or chronic ITP (versus acute, P=0.009), ≥2 treatment lines (P=0.0002), TPO-ra at time of thrombosis (P=0.027), non-response to first-line treatment (P=0.010), smoking (P=0.011), arterial hypertension (P=0.005), and obesity (P=0.041) revealed to be significant. The multivariate analysis model showed that older age at diagnosis (RR, 2.272; 95% CI, 1.167-4.426; P=0.016), ≥2 treatments (RR, 2.539; 95%CI, 1.305-4.941; P=0.006), persistent or chronic ITP (RR, 3.830; 95% CI, 1.111-13.196; P=0.033), and smoking (RR, 2.622; 95% CI, 1.250-5.499; P=0.011 were independent risk factors for TEE. When the variable "number of treatments" (<2 *versus* ≥2) was excluded from the multivariate model, having a splenectomy increased the risk for TEE. The cumulative incidence of TEE at year 1, 5, 10, 15 and 20 years since diagnosis of ITP was 6.2% (95% CI, 4.1-9.3), 11.9% (95% CI, 8.3-17.0), 15.8% (95% CI, 11.1-22.4), 24.2% (95% CI, 16.9-34.7) and 32.8% (95% CI, 22.8-47.3) respectively (Figure). Death occurred in 7/44 (16%) patients with TEE, and in 12/394 (3%) patients without TEE (P=<0001). Most frequent causes of death were infection (32%) and bleeding (21%).

Figure: Cumulative incidence of thromboembolic events with 95% confidence interval

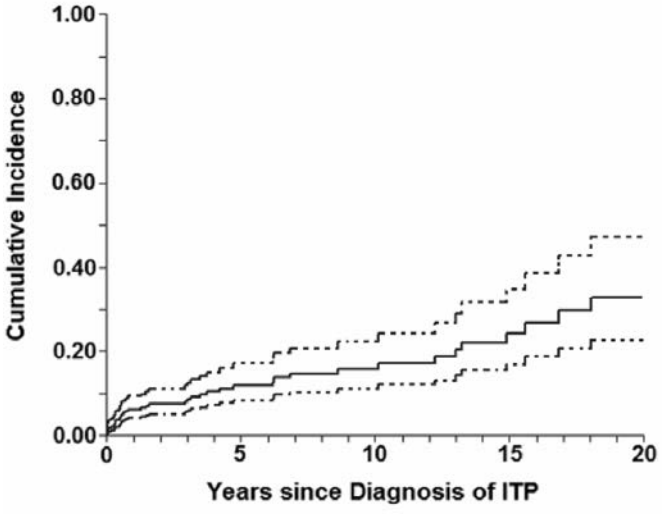

Figure 1.

Summary/Conclusions: Adult ITP patients are at risk for thromboembolic events. Patients older than 50 years, having a persistent/chronic form of the disease, requiring two or more lines to treat the ITP, previous splenectomy, and smokers were more likely to develop TEE. The knowledge about the risk of thromboembolic events in adults ITP patients could have an impact on management attitude for patients at risk.

E1456

OSELTAMIVIR FOR THE TREATMENT OF ITP PATIENTS NOT RESPONDING TO CONVENTIONAL TREATMENT: BIOLOGICAL CHARACTERIZATION AND CLINICAL RESPONSES

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Background: Oseltamivir phosphate, a drug that conventionally serves as an antiviral sialidase inhibitor classically prescribed for the treatment of patients with influenza, has shown to induce an increase in platelet counts in 2 patients with primary immune thrombocytopenia (ITP) (1,2). A previous study has suggested a mechanism of Fcγ receptors (FcγR)-independent platelet clearance in ITP patients with anti-Glycoprotein (GP) Ibα autoantibodies (3). However, little is known about the exact response mechanism of this drug in ITP.
Aims: To analyze the biological features and clinical responses following oseltamivir treatment in patients that are non-responders to conventional treatments.

Table 1.

Table 1. Patients' characteristics

	ITP with NO response to oseltamivir (N=2)		ITP with response to oseltamivir (N=2)	
Age(y) Median (range)	78.5 (78-79)		49.5 (32-67)	
Sex (male/female)	1/1		0/2	
Splenectomized	0%		50%	
Previous treatment:	- Steroids + IVIG - Steroids, Eltrombopag		-Steroids + IVIG -Steroids+IVIG+Romiplostim	
Platelet count (x10^9/L) before and after OT Mean ± standard error	Before	After	Before	After
	3	8	3	134
	16	13	0	101
RCA-1 expression in patient platelets before OT (ratio)	3.08 (2.88-3.28)		6.97 (5.65-8.29)	
Decrease in RCA-1 expression in patient platelets after OT (ratio)	1.40 (1.19-1.60)		5.61 (4.76-6.45)	
RCA-1 expression in control platelets, patient sera, before OT (ratio)	1.05 (0.86-1.24)		2.76 (2.40-3.12)	
Platelet autoantibody specificity	Anti-IIb/IIIa (100%)		Anti-Ibα (100%)	

RCA-1 (which indicate loss of sialic acids of terminal glycans) data are expressed as fold change from control samples. Abbreviations: IVIG, immunoglobulins; OT, oseltamivir treatment.

Methods: We performed a prospective study in 4 ITP patients who exhibited no response to standard therapies (steroid, IVIG and/or splenectomy) and showing relevant platelet desialylation levels. Patients were given off-label oseltamivir at the referring physician´s discretion. Desialylation of GP platelet surface was examined via flow cytometry (FC) analysis, with fluorescein-conjugated *Ricinus Communis* Agglutinin I (RCA-1), which binds galactose residues only if the terminal sialic acid has been removed. FC data are expressed as fold change compared to control samples. Additionally, patients' sera were incubated with normal human platelets to analyze the ability to induce desialylation of normal platelets. Analysis of plasma proteins was performed by Western blot (FXI, FXII) and HPLC (transferrin). Platelet autoantibody specificity was detected by a solid-phase modified antigen capture ELISA test (MACE).
Results: Patients' characteristics are summarized in Table 1. Two patients achieved complete platelet response (>100x10^9/L) after oseltamivir treatment. The oral dose was 75mg twice daily, for a variable duration (5 days in one case and 4 months in the other showing response criteria since the third week of from start) combined with low doses of other treatments (azathioprine or romiplostim). A sustained platelet response was observed after 4 weeks of the sial-

idase inhibitor discontinuation. Patients with no response after oseltamivir treatment (n=2) were given similar doses for 5 days. Patients with response had antibodies directed solely to GPIb and had greater platelet loss of sialic acids. Moreover, their sera induced significant desialylation of normal platelets. However, no desialylation in patients' plasma proteins was detected. Biological analysis after treatment discontinuation (median of 3 weeks), revealed a sustained sialylation level of platelet glycoproteins over time, particularly in patients with sustained platelet response.
Summary/Conclusions: Chronic ITP patient with anti-GPIbα autoantibodies who do not respond to conventional therapies and exhibit significant platelet desialylation may achieve a complete response to treatment with oseltamivir.

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Quality of life, palliative care, ethics and health economics

E1457

BORTEZOMIB THERAPY IS ASSOCIATED WITH SIGNIFICANT RESOURCE IMPLICATIONS FOR BOTH PATIENTS AND PROVIDERS: RESULTS OF A TIME-IN-MOTION STUDY
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Background: Bortezomib is a proteasome-inhibitor, which has improved outcomes in multiple myeloma (MM). Its use is approved within the UK NHS. Bortezomib is frequently administered as a subcutaneous injection in a hospital day treatment unit. Whilst the administration of a subcutaneous injection is brief, the process for the patient travelling to hospital, assessment and waiting for the delivery of the injection can take considerable time. From a patient perspective, significant amount of time spent without economic activity and travel costs add up during the course of therapy. From the health-care provider the process of safely administering bortezomib has significant resource implications beyond those of drug procurement.
Aims: We set up a time-in-motion study to evaluate the costs to health care provider and patients during bortezomib therapy to estimate the 'real-world' cost of delivering bortezomib therapy.
Methods: Retrospective data collection was undertaken, using electronic prescribing records for patients treated between July 2014 -August 2016. Travel distance and time was estimated using Google maps and costed using HMRC mileage (an approved costing of mileage used for taxation purposes). The NHS schedule of service costs was used to estimate the cost of bortezomib administration. Cost of delivery of Bortezomib for healthcare providers is a sum of these individual costs.
Results: We identified 127 patients who incurred a total of 2 134 visits whilst receiving Bortezomib therapy at the Churchill Hosptial in Oxford during this 2 year period. Median age was 70 years-old (yo) (39-95); Male 74 patients (58%) 53 patients (42%). We restricted the analysis to 110 patients who started and completed therapy during the study period. Median number of patient visits was 16 (range 11-52). The median travel distance (return journey) for each patient was 33 miles (53 km) (range: 1.2-224 mi; 1.9-360 km). Median travel time was 90 min (range: 8-300 min). The range travel cost per patient was £8.35-£13.20. Twenty-seven patients (21%) required use of specialist hospital transport services, which resulted in 295 transport-episodes (14%) in total. In order to assess the time spent in the day therapy unit, a subgroup of 589 patient-episodes were analysed to assess time from arrival to administration of Bortezomib: the median time from patient registration to bortezomib administration was 63min (range: 5-433min). Pharmacy cost for preparation of Bortezomib was £50 per dose. The cost of delivery of bortezomib (not including cost of drug) was £1,160 per cycle, which equated to a total median cost of £4,640 per patient (range: £290-£15,080). Drug procurement costs for Bortezomib is estimated at an additional £12,261 per course of therapy (BNF 2016). Delivery costs therefore added an additional 38% to the procurement costs.
Summary/Conclusions: We provide the first time-in-motion data on myeloma patients treated with Bortezomib. The 'real-world cost' of delivering therapy is 37% higher than the drug-costs alone. In addition the impact on patients is substantial: over a two year period 127 patients required 2 134 visits with a median time in the day unit of 63 minutes and a median travel time of 90 minutes per visit. Our data highlights the burden of both time and economic costs to patients during therapy. Novel oral proteasome inhibitors offer the potential to reduce this resource impact in the future. This data could be used by health care providers and reimbursing agents for economic modeling of the potential benefits of oral proteasome inhibitors.

E1458

HOSPITAL CARE AT HOME ADMINISTRATION OF SUBCUTANEOUS AZACITIDINE IS FEASIBLE AND PREFERRED BY PATIENTS COMPARED TO HOSPITAL ADMINISTRATION: A FRENCH REGIONAL HEMATOLOGY NETWORK EXPERIENCE
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Background: In France, azacitidine (AZA) is indicated for the treatment of adult patients affected by Myelodysplastic Syndrome with intermediate-2 or high risk according to the International Prognostic Scoring System (IPSS), Chronic Myelomonocytic Leukemia (CMML) with 10-29% medullary blasts and Acute Myeloblastic Leukemia (AML) with 20-30% blasts. It's also a drug treatment of adult AML patients over 65 years with>30% of medullary blasts. Azacitidine is a hypomethylating agent administered by subcutaneous route. Though effective, treatment cycles require frequent hospital visits which could decrease patient comfort and increase medical personnel workload. Limousin is a region with the oldest population of France and with a very low population density. There is one university hospital and two local state-run hospitals each with a hematology department. In 2009, HEMATOLIM, the Limousin hematology network, set up a protocol called ESCADHEM (externalization and securitization of injectable chemotherapy at home for malignant hematological diseases) that facilitates chemotherapy administration via local Hospital at Home (HaH) establishments, which is an alternative to conventional hospitalization in France (www.fnehad.fr). The aim was to minimize the frequent hospital visits that these treatments require. This organization includes the three previously mentioned hospitals, four HaH structures, and three central pharmacies with an integrated preparation unit for cancer treatments. From 2009 to 2015, a total of 11 367 infusions were administred at home for 464 pts. In 2016, we demonstrated the feasibility of ESCADHEM and the medico-economic interest of such care with Bortezomib, another injectable chemotherapy at home (Touati *et al.* Support Care Cancer. 2016 Dec; 24(12):5007-5014). In 2014, we have conducted a satisfaction survey with a cohort of 84 pts who received treatment in HaH structures via ESCADHEM (20% pts with AML/MDS were treated by AZA). The overall satisfaction rate was 95%.
Aims: Our work aimed to demonstrate that HaH administration of AZA is feasible and well preferred by patients compared to hospital administration.
Methods: Chemotherapy at home obeys to strict rules. The first chemotherapy cycle (C1) and the first injection (D1) of subsequent cycles were administered at the outpatient care unit. The following injections were administered at the patient's home and carried out by HaH, according to a predefined procedures (Fig 1) to comply with safety rules essential to the protection of the professional, the patient, the entourage and the environment. Subcutaneous AZA injections were administered at a dose of 75mg/m^2 for 7 days of each 28-day cycle. Between 2009 and 2015, 169 patients were treated with AZA by a combination care in outpatient unit and HaH and were included in the study. In February 2017, we set up a satisfaction survey with an anonymized questionnaire for all patients alive at the date of dispatch. The aim is to assess several criteria such as the degree of satisfaction, quality of care, well-being, personal anxiety, advantages and disadvantages. Each item will be analyzed using a Likert scale to realize a satisfaction survey.
Results: From 2009 to 2015, a total of 6369 subcutaneous injections of AZA were administered at home for 169 pts with AML/MDS received AZA therapy. Among all pts, 110 were males and 59 females with a median age of 75 years (range 41-92) there are 88 (52%) MDS patients and 81 pts (48%) with AML. Patients received a median number of 5 cycles (1-41) and 26 injections of AZA (1-244) at home. The total duration of HaH management lasted from less than 1 day to more than 3.4 years with a mean of 6.3 months. During the period of HaH administration of AZA, 101 out of 169 pts (60%) had to return to conventional hospital for a non programmed rehospitalization: 90% of the time the patient needed a transfusion, 4% because of infection and 6% for other reasons.

Figure 1.

Summary/Conclusions: This important number of subcutaneous injections (n=6369) in a large cohort (n=169) over a period of time of 6 years activity show that home administration of subcutaneous AZA is feasible without serious adverse event. The first satisfaction survey demonstrates a high rate of satisfaction by all patients (20% with AML/MDS) treated by injectable chemotherapy at home. The results of the satisfaction survey, focused on pts treated with subcutaneous AZA, is in process and results will be presented at the EHA meeting.

E1459

USE OF COMBINED ORAL FENTANYL CITRATE (ACTIQ®) AND MIDAZOLAM AS PREMEDICATION FOR BONE MARROW BIOPSY IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES: A RANDOMIZED CONTROLLED PATIENT BLINDED CLINICAL TRIAL

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Background: Bone marrow aspiration and biopsy (BMAB) is a painful procedure, and the commonly adopted local infiltration anesthesia (LIA) with lidocaine is unable to relieve the pain during the most uncomfortable phases, or the anticipatory anxiety related to pain recalling thereafter. As there are no formal guidelines for adding a sedoanalgesic premedication before beginning the BMAB, many combinations have been adopted by several authors.
Aims: Our randomized and patient blinded trial aimed to evaluate, as primary end point, the efficacy and safety of opioid and benzodiazepine agent combination plus LIA in patients who underwent BMAB for hematological malignancies. Two secondary end points were: 1) define if patients who already underwent to BMAB without LIA prefer sedoanalgesia; 2) sedoanalgesia can influence the quality of the biological specimen harvested.
Methods: Patients were randomly assigned into two arms for receiving either sedoanalgesic placebo plus LIA (standard group, 48,6%) or oral fentanyl citrate 200 mcg plus oral midazolam 5mg in addition to LIA (combo-group, 51,4%) during BMAB. Pre-procedural anxiety and procedural pain were assessed according to the Numered Rating Scale (NRS: 0-10), dividing the time of the procedure into five intervals (T0, T1, T2a, T2b, and T3) and evaluating discomfort grade during each moment of procedure in both groups. Cognitive function was measured before and 30 minutes after the procedure. Possible side effects were recorded, as well as the adequacy of tissue samples harvested. A telephone interview was performed 24 hours later. A total number of one-hundred-sixteen (n=116, Table 1) were enrolled in the study. Nine (n=9) patients did not meet inclusion criteria and were excluded. Fifty-two (n=52) patients were randomized and assigned to standard group and fifty-five (n=55) to combo group.
Results: At T2b and T3 (corresponding to the biopsy time and time after the biopsy, respectively) there was a significantly lower ($p< 0.05$) perception of pain in the patients who received sedo-analgesia (combo-group) compared to those who did not (standard group). Moreover, 100% of the patients in combo group who had previously undergone this procedure without premedication, reported that they would prefer sedoanalgesia for the subsequent procedures, thus showing the effectiveness of this combination also in relieving anticipatory anxiety. Finally, the histological specimen was found to be high in quality, as defined by standards.

Table 1.

Table 1. Baseline characteristics of the population undergone to BMAB

Characteristic	No.
Number of patients	107
Sex	
Male/Female	9/5
Age, years	
Median (range)	61 (19-84)
Type of hematologic neoplasm	
Acute myeloid leukemia	19
Non Hodgkin lymphomas	16
Myelodisplastic syndromes	16
Multiple Myeloma	13
Essential thrombocythemia	11
Polycythemia vera	10
Chronic lymphatic leukemia	7
Hodgkin lymphoma	6
Primary myelofibrosis	5
Acute lymphoblastic leukemia	5
Chronic myeloid leukemia	4

Summary/Conclusions: Administration of oral analgesia and anxiolysis is a safe and feasible option to be used in outpatient setting; sedo-analgesia is very effective in reducing pain during the biopsy and diminishes the anticipatory anxiety related to a painful procedure. Patients should have the possibility to choose between local anesthesia alone or sedo-analgesia plus local anesthesia.

E1460

ASSESSMENT OF THE ECONOMIC IMPACT OF HORSE-ATG IN SWEDEN FOR APLASTIC ANAEMIA

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Background: Aplastic anaemia (AA) is a rare, potentially fatal haematopoietic stem-cell disorder that can either be inherited or acquired. AA is graded according to disease severity, from non-severe to very severe and is linked to immune-related responses such as the destruction of the bone marrow. Cases of severe and very severe AA are considered to be a haematological emergency requiring urgent treatment. Extended hospitalisations and the cost of treatments and disease management are associated with the economic impact of AA.

Aims: To assess cost-effectiveness of ATGAM (horse antithymocyte globulin) in comparison to rabbit antithymocyte globulin (r-ATG) in the treatment of moderate to severe aplastic anaemia (sAA) patients in Sweden.
Methods: A semi-Markov state-transition cohort model was developed to estimate longer-term (up to five years) clinical and economic outcomes for patients with AA receiving either ATGAM or r-ATG as first-line IST treatment. The following key assumptions were included in the model: responders who relapse are assumed to be re-treated with no expected change in survival. Patients that do not respond to first-line treatment move onto a second-line treatment comprised of either IST, IST + eltrombopag or hematopoietic stem cell transplantation (HSCT). Although response rates are lower, those who respond to second-line treatment are assumed to have the same outcomes as those who respond to first-line. Patients who continue to not respond receive standard supportive care with a significant decrease in expected survival. Efficacy data for ATGAM and r-ATG were obtained from published literature. Adverse events were not included due to lack of evidence of any difference between the two comparators. Medication, administration, and disease management costs were obtained from published literature, publicly available sources and clinical expert opinion. As resource utilization for disease management changes over time and differs considerably between responders and non-responders, three distinct phases have been included in the model: short-term (first 6 months post-IST administration), medium-term (6-12 months) and long-term (greater 1 year), for patients in either of the response categories.
Results: Response to treatment was calculated to be seen in 67% of ATGAM patients' *vs* 35% in r-ATG (accounting for mortality). Over 5 years, the model estimated that patients gained 4.15 life-years (3.28 quality-adjusted) on ATGAM *vs* 3.52 (2.56) on r-ATG. Short-term disease management costs were estimated to be SEK 880,144 (€96,816) in responders *vs* SEK 1,264,016 (€139,041) in non-responders. Medium and long-term costs also followed the same pattern. Overall costs (drug plus disease management), were significantly lower for patients receiving ATGAM *vs* r-ATG; making ATGAM cost-saving by being both more effective and less costly than r-ATG. When considering treatment costs only (including cyclosporine and HSCT), the model estimated a cost of SEK 107,097/life-year gained (approx. €11,781) and SEK 135,655/quality-adjusted life-year (approx. €14,922), showing ATGAM is highly cost-effective. The analysis showed that when treatment and disease management costs are considered, ATGAM dominates r-ATG as the gain in QALYs and LYs are achieved at a lower cost. Therefore making ATGAM cost-saving with greater health benefits in comparison to r-ATG.
Summary/Conclusions: Due to improved treatment response, survival, and quality of life outcomes, the model shows that ATGAM is at least more cost-effective, if not cost-saving, in comparison to r-ATG for the treatment of patients with aplastic anaemia.

E1461

NEUROPSYCHOLOGICAL ANALYSIS OF LONG-TERM CONSEQUENCES OF ANTINEOPLASTIC TREATMENT

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Background: Study of long-term consequences of conducted antineoplastic treatment is becoming on the front burner because of significant increase in survival rates of Acute Lymphoblastic Leukemia (ALL) and Hodgkin's Lymphoma (HL) patients. Modern therapeutic protocols (cranial irradiation and chemotherapy) can negatively affect patients' cognitive functions and consequently decrease patients quality of life. It is therefore necessary to carry out qualitive and quantative analysis of neuropsychological impairments in patients conducted antineoplastic treatment.
Aims: The aim of the research to study neuropsychological status (memory and executive funcions) of adult patients after antineoplastic therapy in childhood.
Methods: Patients in a present clinical remission (5-14 years remission duration). N=60 (ALL), N=50 (HL). Mean age-14 (ALL), (HL) – 19 years. N=60 control group. Qualitative, quantitative neuropsychological scales developed in Luria's approach methodology (tests for dynamic praxis and memory domains) and one-way ANOVA on ranks were used.
Results: The main neuropsychological impairments were found in auditory-verbal memory and auditory-motor coordination (memory narrowing, violations in selectivity, inertia in mental process, perseverations, contaminations, difficulties in praxis automatisation). 20% of patients didn't recall all words (auditory-verbal memory tests) after four representations (3,3% in control group, p<0,005). 19% of patients failed or had severe difficulties in dymanic praxis tests (2% in control group, p<0,005). The results point at possible deficits in temporal and frontal parts of the brain (predominantly posterior frontal and mediobasal systems).
Summary/Conclusions: Neuropsychological impairments in audioverbal, auditory motor and dynamic praxis spheres, qualitive and quantative analysis showed deficits in temporal and posterior frontal areas of the left hemisphere, that can be as a result of antineoplastic therapy in childhood. Such findings can improve understanding the nature of patients impairments and difficulties they experience. Rehabilitation programs should take into account patients neuropsychological changes and cognitive decline. Indeed psychological interventions are necessary in long-term period after clinical remission.

E1462

A CLINICAL AUDIT OF NUTRITIONAL SCREENING AND SUPPORT OF HOSPITALIZED PATIENTS WITH HEMATOLOGIC DISEASES

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Background: Poor food intake is a common problem in patients with hematologic diseases. Recurrent infections and chemotherapy complications are some of the possible causes. Malnutrition is correlated to slow recovery, prolonged hospitalization, and higher mortality. Audits about the nutritional support of hospitalized patients may detect significant failures in patient care and help towards the correct application of the international guidelines.
Aims: We performed a prospective observational audit on hospitalized patients with hematologic diseases to investigate their nutritional status and whether they received the appropriate nutritional support.
Methods: The initial population consisted of 122 consecutive patients with hematologic diseases admitted from March 31, 2016 to June 8, 2016 in two Hematologic Units of a Tertiary University Hospital in Athens, Greece. We designed a special questionnaire based on the Malnutrition Universal Screening Tool (MUST) with additional questions on demographic, somatometric and medical data (Table 1). The questionnaire was applied by 6th-year medical students to all patients within 48 hours of admission. Patients were classified as high, intermediate, and low-risk per the MUST score and were reassessed at predefined intervals. During reassessment, we examined the food intake and the nutritional interventions (nutritional supplements, enteral or parenteral nutrition) applied.
Results: Ninety-three patients were included in the final analysis (5 refused to participate, 22 were excluded due to short-term hospitalization, 2 were absent during reassessment). Forty-one (38%) patients had a MUST score ≥2 (high risk) but none of them received nutritional supplements. One patient was supported with parenteral nutrition (Table 1).

Table 1.

Table 1: Patients' characteristics and results

Number of patients, N (%)	93 (100)
Age (years), median (range)	57.5 (17-87)
Gender (M/F)	1.4
BMI (kg/m^2), median (range)	25.39 (16.95 – 40.64)
% of unplanned weight loss in past 6 months, median (range)	3.6 (0-25.3)
Disease, N (%)	
Lymphoproliferative disorders/ Multiple myeloma	45 (49)
Acute leukemia/ Myeloproliferative disorders	38 (41)
Benign hematologic disorders	5 (5)
No confirmed diagnosis	5 (5)
MUST, N (%)	
0	41 (44)
1	14 (15)
≥2	38 (41)
Patients receiving nutritional support, N (%)	1 (1) *
Recent chemotherapy/radiotherapy, N (%)	45 (48)/ 3 (3)
Reported food intake (last 5 days), N (%)	
Increased	1 (1)
Normal	61 (66)
Decreased	29 (31)
No intake	2 (2)
Serum albumin levels on admission/at discharge (g/dl), median(range)	4.1 (2-4.9)/ 3.6 (2.3-4.5)
Other variables: estimated food intake in 5 days, reduced appetite, type of diet, calorie intake, duration of hospitalization, ECOG score, recent surgery, dysphagia, nausea, mucositis, infection, neurological deficits, head trauma etc.	
*a 65-year-old woman with 12% weight loss over the last month, BMI=21 kg/m^2, albumin=2 g/dl, a hospital-acquired infection and no food intake.	

Summary/Conclusions: Our audit revealed a lack of nutritional support of the hospitalized patients. A meeting with the involved health professionals was organized and an oral presentation of the results and the possible causes (lack of sensitization of the staff, high regimen cost, shortness of staff) was performed. Proposals to change the current situation were made such as detection of high risk patients by medical students and further assessment by a nutritional specialist. A brief MUST-based questionnaire was also proposed to be used for all patients upon admission. A re-audit was programmed and is already in progress.

E1463

ASSESSING REAL-WORLD TREATMENT PATTERNS, OUTCOMES AND RESOURCE USE IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) POST AUTOLOGOUS STEM CELL TRANSPLANT ACROSS EUROPE

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Background: Autologous stem cell transplant (ASCT) is the standard of care for first line (1L) treatment (tx) for patients (pts) with MM deemed of suitable fitness to safely undergo the procedure. More recently introduced tx options have significantly increased the life expectancy of pts with MM and continue to provide further promise for the future in this devastating disease. The increasing therapeutic armoury across the MM pathway allows for varied tx patterns providing both potential differences in outcomes and healthcare resource use (HCRU).
Aims: The aim of the analysis was to determine current management of pts in the post ASCT setting, assess outcomes of pts and HCRU.
Methods: A retrospective chart review was conducted in France, Germany, Italy, Spain and the UK. Data collection took place in Q1 2017. Physicians provided data on consecutive pts with MM who had undergone an ASCT as part of 1L tx on or after 1st January 2014, to specifically examine the HCRU post 1L SCT. Data collected pertained to pt characteristics, tx patterns, duration of tx and outcomes (including time to progression (TTP) and best response achieved (IMWG updated criteria), HCRU in terms of hospitalizations, additional supportive drugs prescribed and healthcare professional (HCP) visits. Pt records included in this interim analysis were completed by Feb 17th 2017, with data collection continuing in all countries.
Results: 214 record forms have been reviewed to date. Pts' mean age at diagnosis was 59 (±7.8 SD) years; 43% female and 57% male. Mean duration from diagnosis, to receiving an ASCT was 9.6 months (±13.3 SD). Of the pts included in the study, 62%, 28% and 8% had received 1st, 2nd and 3rd line tx respectively. In the 1L setting, 72% of pts did not receive any drug therapy post ASCT, 21% received consolidation and 8% maintenance therapy. Of the pts who did not receive maintenance therapy, 42% and 34% went onto receive 2L and 3L drug therapy respectively; whereas, only 24% of pts who received maintenance therapy went onto 2L, and none onto 3L. The most frequently prescribed regimens at 1L maintenance were Lenalidomide (82%), Bortezomib (12%) and Thalidomide (1%). The mean TTP from start of 1L tx was 22.2 months (±11.1 SD) for pts not receiving maintenance and 33.0 months (±8.1 SD) for pts receiving maintenance. Overall 43% of pts achieved a sCR and CR, 51% achieved a VGPR and PR. During the period from 1L post ASCT to start of 2L, 54% of pts required supportive tx (bisphosphonate (55%), blood transfusions (24%), G-CSF (21%), ESAs (11%), radiotherapy (4%) and dialysis (3%)). 64% of pts were hospitalized at least once during this period, with a mean duration of 7.2 days (±18.1 SD). The mean number of visits to Hematologists was 7.1 times in 24.8 months (between start of 1L to start of 2L tx); mean visits to a HCP during this period were 17. The mean TTP from start of 2L tx was 11.2 months (±6.8 SD). 20% of pts achieved a sCR and CR, 52% achieved a VGPR and PR.
Summary/Conclusions: The sample is reflective of the pt demographics data reported in Raab *et al.* 2016. Furthermore, the TTP for pts not receiving any active ongoing tx post ASCT in this real-world study is comparable to findings in other studies. Limited or no data exists on HCRU post ASCT. This study demonstrates that there is ongoing HCRU impact even if pts are not receiving any active ongoing tx post first ASCT. Prolonging the remission period post ASCT may therefore spread the marginal cost of HCRU whilst simultaneously enhancing a pt's quality of life by deferring future tx lines.

E1464

NUMBER-NEEDED-TO-TREAT (NNT) AND COST OF RESPONSES ACHIEVED IN TYROSINE KINASE INHIBITOR (TKI) TREATMENT OF REFRACTORY CHRONIC-PHASE CHRONIC MYELOID LEUKEMIA (CP-CML) IN THE UNITED STATES (US)

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Background: The emergence of targeted therapies with high efficacy in small patient populations such as TKI-refractory CP-CML has challenged decision-makers.
Aims: To demonstrate a simple and intuitive approach to assessing the value of available TKIs (nilotinib, dasatinib, ponatinib and bosutinib) in this setting.
Methods: Using synthesized efficacy data from a published meta-analysis (Lipton 2014), we calculated NNT to achieve one additional response, defined as complete cytogenetic response (CCyR), for CP-CML patients treated with a TKI after failing ≥2 prior TKIs. NNT represents the expected number of treated patients required to achieve one additional response—*i.e.*, the multiple of treated patients to responders. We assumed response is not evaluated prior to 3 months, per National Comprehensive Cancer Network (NCCN) guidelines. Therefore, the cost of achieving an additional response was estimated as the product of NNT and 3-month cost, based on US Wholesale Acquisition Costs (WAC) and recommended dosing for each TKI from US prescribing information (USPI).
Results: To achieve one expected response, the NNT is 1.7 (95%CrI: 1.5-1.9) patients for ponatinib, 3.8 (3.1-4.8) for nilotinib, 4.2 (2.2-11.1) for dasatinib, and 4.5 (3.4-6.7) for bosutinib (based on CCyR of 60%, 26%, 24% and 22%, respectively). With a 3-month WAC for ponatinib of $49,683, nilotinib: $33,892, dasatinib: $33,897 and bosutinib: $36,045, the estimated 3-month cost per response achieved is $82,800 ($73,100-$95,500) for ponatinib, $130,000 ($106,000-$161,000) for nilotinib, $141,000 ($75,300-$377,000) for dasatinib, and $164,000 ($124,000-$240,000) for bosutinib.
Summary/Conclusions: Using published, synthesized efficacy estimates, the NNT to achieve response with ponatinib in TKI-refractory CP-CML is less than with nilotinib, dasatinib or bosutinib. Despite a higher WAC, ponatinib has the lowest estimated 3-month cost per response achieved. Therapy choice should, however, consider both treatment cost and the benefit-risk profile of the individual patient.

E1465

THE COST-EFFECTIVENESS OF PEGASPARGASE FOR FIRST-LINE TREATMENT OF ACUTE LYMPHOBLASTIC LEUKAEMIA: A COST-UTILITY ANALYSIS

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Background: Asparaginase is a key component in the multi-agent chemotherapeutic regimen for the treatment of children, adolescents, and adults with acute lymphoblastic leukaemia (ALL). Compared to native asparaginase (native ASP), pegaspargase (PEG-ASP) has a longer half-life, can be given less frequently, and is less immunogenic, which leads to fewer hypersensitivity reactions. In the UK, patients with newly diagnosed ALL are treated with PEG-ASP followed by Erwinia-derived asparaginase (ERW-ASP) in cases of hypersensitivity, based on the UKALL protocols. Although native ASP is no longer used as the first choice of asparaginase therapy, it was the standard of care before PEG-ASP was available. A cost-utility analysis (CUA) was conducted to evaluate overall cost-effectiveness of PEG-ASP in comparison to native ASP when utilized as part of antineoplastic combination therapy for treating newly diagnosed ALL in children, young people, and adults.
Aims: To evaluate the cost-effectiveness of a treatment strategy including PEG-ASP in a multi-agent in patients with newly diagnosed ALL compared to regimens that include native ASP.

Table 1.

Technologies	Total		Incremental		ICER (£)
	Cost (£)	QALYs	Cost (£)	QALYs	
PEG-ASP > ERW-ASP	£7,871	17.3431	—	—	—
Native ASP > ERW-ASP	£12,612	17.2926	–£4,741	0.0504	Dominated*
ERW-ASP > Native ASP	£48,149	17.3396	–£40,277	0.0035	Dominated*
ERW-ASP > PEG-ASP	£48,234	17.3477	–£40,362	–0.0047	£8,627,243

* Dominated indicates more costly and less efficacious compared to the PEG-ASP > ERW-ASP sequence.

Methods: In line with accepted National Institute for Clinical Excellence (NICE) methodology, a combined decision tree and health state transition Markov model was developed to compare treatment sequences starting with PEG-ASP *versus* native ASP, followed by ERW-ASP in case of hypersensitivity. Although ERW-ASP is not used first-line in the United Kingdom, alternative switching scenarios could be clinically possible, and therefore all scenarios were modelled. Paediatric, young adult (≤25 years), and adult (26-65 years) patients were modelled separately using the UKALL 2003 and UKALL14 protocols, respectively. Further splits were made between high-, intermediate-, and standard-risk patients in the paediatric model, between patients aged ≤40 *vs* ≥41 years, and patients eligible *versus* not eligible for transplant in the adult model. Key model parameters (survival, risk of hypersensitivity) were based on published data and clinical expert input. In the base-case analysis, overall survival and event-free survival were assumed to be equivalent for PEG-ASP, native ASP, and ERW-ASP, with 1,000IU/m^2 dosing (per UKALL protocols) used for PEG-ASP. In the scenario analyses, the 2,500 IU/m^2 dosing (per SmPC) of PEG-ASP was examined, as well as variations in comparative survival and hypersensitivity rates. Incremental cost-effectiveness ratios (ICER; defined as incremental costs/quality-adjusted life years [QALYs] gained) were produced.

Results: The base-case scenario demonstrated that PEG-ASP followed by ERW-ASP dominated (*i.e.*, was both less costly and more effective than) native ASP followed by ERW-ASP in adults, children, and the whole (combined) population (Table). Scenario analyses highlighted the robustness of the cost-effectiveness results. Differences in total QALYs between PEG-ASP and native ASP were driven primarily by the difference in hypersensitivity rates.
Summary/Conclusions: This analysis demonstrates that PEG-ASP, as part of multi-drug chemotherapy, is a cost-effective treatment option compared to native ASP for treating ALL in children, young people and adults with newly diagnosed ALL.

E1466

IMPACT OF VENETOCLAX ON THE QUALITY OF LIFE OF PATIENTS WITH RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA: RESULTS OF A PHASE 2, OPEN-LABEL STUDY OF VENETOCLAX (ABT-199/GDC-0199) MONOTHERAPY

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Background: Chronic lymphocytic leukemia (CLL) is associated with reduced health-related quality of life (HRQoL), with progressive severe fatigue being a particularly relevant burden. Disease-related symptoms, toxic effects of therapy, and the awareness of living with an incurable disease can have a profound impact on HRQoL. Venetoclax (VEN) is an oral BCL-2 inhibitor that has demonstrated deep and durable responses in relapsed/refractory (R/R) CLL patients.
Aims: To assess whether Venetoclax has a sustained impact on health related quality of life among patients with relapsed/refractory CLL based on a second interim analysis (first interim results through week 24) of patients treated with VEN monotherapy.
Methods: Patients ≥18 years of age with R/R CLL received VEN monotherapy until disease progression, unacceptable side effects, or discontinuation for any other reason. Patient-reported HRQoL measures included the EORTC QLQ-C30 and EORTC QLQ-CLL16, which were assessed at Baseline (BL), at 4 weeks and every 12 weeks thereafter. Mean change in the HRQoL measures from BL to each assessment are reported. Clinical relevance was based on minimum important difference (MID) of values from BL at different assessment points. The lower bound of 5–10 point changes, considered a "little" change for EORTC-QLQ-C30 was used for MID acceptance for both measures.
Results: Clinically meaningful improvements from BL were observed early and were sustained through week 96 in VEN treated patients in the EORTC-QLQ-C30 global health status and the role, social, and emotional functioning scales. Improvements in VEN treated patients in EORTC-QLQ-CLL16 disease effects, social problems, and future health worries scores were statistically significant and exceeded the MID at all assessment points. Furthermore, early and sustained improvements in fatigue through week 96 were seen in both EORTC-QLQ-C30 and EORTC-QLQ-CLL16 (Table 1). The changes observed in patient EORTC-QLQ-CLL16 future health views were considered large (>20 points) at Weeks 12, 24, and 48.

Table 1.

Table 1. Change from Baseline Results in Key Domains on EORTC QLQ-C30 and EORTC QLQ-CLL16

EORTC QLQ-C30 parameter (n)	BL Mean	Visit Mean	Mean Change from BL (95% CI)
Global Health Status*			
Week 12 (111)	60.4	68.5	8.1 (3.6, 12.6)
Week 48 (92)	62.5	73.2	10.7 (6.3, 15.0)
Week 96 (50)	58.2	68.8	10.7 (4.7, 16.7)
Fatigue†			
Week 12 (118)	35.8	27.4	-8.3 (-12.5, -4.2)
Week 48 (99)	33.3	25.3	-8.0 (-12.7, -3.3)
Week 96 (52)	35.3	26.5	-8.8 (-16.0, -1.5)
EORTC QLQ-CLL16 parameter (n)	**BL Mean**	**Visit Mean**	**Mean Change from BL (95% CI)**
Fatigue†			
Week 12 (117)	29.6	19.2	-10.4 (-15.1, -5.7)
Week 48 (97)	29.0	20.1	-8.9 (-14.2, -3.6)
Week 96 (53)	30.5	21.4	-9.1 (-16.8, -1.4)

* A positive change in score represents improvement.
† A negative change in score represents improvement.

Summary/Conclusions: These updated interim results suggest that patients receiving VEN monotherapy experienced early and sustained clinically relevant improvement in several key aspects of functioning and HRQoL for up to 96 weeks in a very symptomatic and difficult to treat patient population. These results are important to consider when making treatment decisions in the R/R settings.

E1467

WHICH HAEMATOLOGICAL CONDITIONS CAN THIRD YEAR MEDICAL STUDENTS RECOGNISE INTERPRETING FULL BLOOD COUNT RESULTS?

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Background: Haematology is often seen by medical students as a niche specialty, however interpreting full blood count results is a daily job for most hospital doctors. Furthermore a recent review showed that survival for haematological malignancies is worse in UK than in other European countries, late diagnosis being one the possible causes. Educate future doctors in interpreting symptoms and blood results correctly to suspect haematological condition should be considered essential. In the UK the Education Subcommittee of British Society for Haematology wrote the "A Haematology Curriculum for Medical Students" as a guide to the knowledge of haematology expected from medical students.
Aims: The aim of the study was to evaluate the ability of a group of third year medical students in recognising ten haematological conditions as indicated in the curriculum proposed by the BSH. The students had all attended a haematology course during their second year of medical school.
Methods: A multiple choice test "best of four" containing ten clinical cases including full blood count results was given to the students. According to the Team Based Learning "TBL" model the students completed the test first individually, "i-RAT" and then after discussing the results in small groups "t-RAT". The topics and the percentage of correct answers are shown in table 1.
Results: Twenty four students participated. In the i-RAT none of the scenarios were correctly interpreted by 100% of the students, the scenarios interpreted correctly by at least 70% of the students were only two: B12/folate deficiency and iron deficiency; less than 30% of the students could identify CML, NHL and Multiple myeloma; the remaining topics: thalassemic trait, MDS AML, CLL and lymphocytosis due to viral infection were correctly diagnosed by a variable number of students, between 66% and 42%. When the test was repeated after discussion in small groups "t-RAT" there was an improvement of the percentage of correct answer with the exception of the topic AML. In the t-RAT 100% of the groups diagnosed correctly thalassemic trait and MDS, at least 70% of the groups identified correctly CML, CLL and multiple myeloma in addition to B12/folate and iron deficiency, there was an improvement in diagnosis of Multiple myeloma, but still less than 30% of the groups could identify NHL.

Table 1.

Table 1: topics and percentage of correct answers

TOPICS	% OF CORRECT ANSWERS	
	i-RAT	t-RAT
B12/folate deficiency	83	86
Iron deficiency	71	86
Thalassemic trait	66	100
MDS	66	100
AML	58	43
CLL	46	86
Lymphocytosis due to viral infection	42	43
CML	21	71
NHL	21	29
Multiple myeloma	17	57

Summary/Conclusions: This group of medical students found it difficult to correctly diagnose some of the haematological conditions presented, even though they had studied all the conditions before, however the use of a "Team Based Learning" approach where students could discuss the cases in small groups did improve their results. Interestingly for two conditions, for CML and Multiple myeloma the number of correct answers was the same for i-RAT and t-RAT, possibly the students who responded correctly during the i-RAT were each in a different group during the t-RAT and worked as peer-to-peer teachers for the other students. The t-RAT results for AML were actually worse than for the i-RAT, probably the students who replied correctly in the i-RAT were concentrated in fewer groups. To the author's knowledge this is the first study on the effect of applying the Team Based Learning method to haematology teaching. This study showed that TBL could be a useful teaching tool to improve teaching of haematological conditions in medical schools, however the size of the sample was small and the results should be validated with a bigger study.

E1468

LONGITUDINAL ASSOCIATIONS BETWEEN HEALTH-RELATED QUALITY OF LIFE AND HEALTHCARE UTILIZATION IN AL AMYLOIDOSIS

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Background: Light chain (AL) amyloidosis is a rare, complex disease associated with significant organ dysfunction, disability, and death. AL amyloidosis patients interact with the healthcare system in a myriad of ways; however, few studies have quantified healthcare utilization (HCU) in this condition.
Aims: To prospectively examine the association between health-related quality of life and healthcare utilization among patients with AL amyloidosis.

Methods: A non-interventional, longitudinal online study was conducted among patients with AL amyloidosis who were recruited with assistance from patient advocacy groups. Initial (n=341) and six-month follow-up (n=226) surveys assessed demographics, disease and treatment characteristics, and health-related quality of life (HRQoL), measured by the SF-36v2® Health Survey physical and mental component summary scores (PCS and MCS). HCU (e.g., outpatient doctors' visits, emergency room [ER] visits, hospitalizations, and insurance coverage) was measured during the six-month follow-up. Prevalence of HCU and its bivariate associations with patient characteristics were evaluated. Multivariable logistic regression models were used to test for associations between HRQoL and having an ER visit or hospitalization in the past six months.
Results: Overall, visits with specialists and other healthcare providers during the previous six months were nearly ubiquitous (92.0% and 94.6%, respectively). Collectively, 56.0% of patients reported having ≥1 ER visit or hospitalization. ER visits and hospitalizations were not associated with the numbers or types of organs affected by the disease or the duration of disease. There were significant associations between PCS and ER visits ($p<0.05$) and between both PCS and MCS and hospitalizations ($p<0.05$ for all) based on multivariable analyses.
Summary/Conclusions: There is a lack of real-world evidence regarding HCU among patients with AL amyloidosis. This research identified longitudinal associations between HRQoL and HCU, indicating there is potential for using HRQoL surveys as screening tools to predict future HCU for AL amyloidosis patients. The development of prediction models for HCU in AL amyloidosis should consider incorporating HRQoL, as well as disease staging and treatment type.

E1469

SAFETY, FEASIBILITY AND EFFECTIVENESS OF ELECTRICAL MUSCLE STIMULATION IN HOSPITALIZED PATIENTS UNDERGOING AUTOLOGOUS OR ALLOGENEIC STEM CELL TRANSPLANTATION AND INTENSIVE CHEMOTHERAPY

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Background: Autologous and allogeneic stem cell transplantation (HSCT) or intensive chemotherapy are the only treatment option for many patients with haematological malignancies. Even after complete remission many patients are physically and psychologically impaired because of intensive treatment and weeks of immobilisation. Electrical muscle stimulation (EMS) is a verified training tool to prevent muscle decline in seniors and helps improving physical performance in patients with chronic disease.
Aims: This prospective, randomized and controlled study tested the safety, feasibility and efficacy of EMS in 72 patients (EMS=42, control=30) undergoing autologous HSCT (n=21), allogeneic HSCT (n=17) and intensive chemotherapy (n=34).
Methods: A Myopuls 2000 device (Curatec Services GmbH) was used. Targeted training time was 15 minutes 5 days a week on both thighs and arms from start of therapy (T1) to time of discharge (T2). Adverse events and treatment adherence were documented. Impact on psychological and physical functioning was evaluated using the Multidimensional Fatigue Inventory (MFI), the EORTC QLQ-C30, the Short Physical Performance Battery and the 6 Minute Walking Distance test at T1 and T2.
Results: Seven patients died in the EMS- (n=4) and control-group (n=3). 6 of 42 EMS patients withdrew because of sepsis (n=4) or loss of motivation (n=2). 32 patients from the EMS group completed our study with 22 accomplishing>66% of the pre-set training time. EMS related adverse events were hematoma (n=1) and muscle pain (n=2). No bleeding events (WHO bleeding scale>1) or ventricular arrhythmias occurred. Difference in 6-minute walking distance between both groups was 23 meter (p=0,2). SPPB test results differed by one point (p=0,08). MFI and EORTC QLQ-C30 both favoured the EMS group, but showed no statistical significance.
Summary/Conclusions: EMS is feasible and safe in patients undergoing intensive chemotherapy regimens. It also may improve physical fitness, fatigue and quality of life, indicated by favourable test results in the EMS group. To verify positive effects of EMS in patients with haematological malignancies, further research is needed, with more patients and sham EMS stimulation.

E1470

MYELOMA PATIENT VALUE MAPPING: A DISCRETE CHOICE EXPERIMENT

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Background: Myeloma is a life threatening haematological cancer. Although myeloma is responsive to treatments, there remains no cure. In recent years, there have been improvements in survival due to the use of high dose therapies, stem cell transplant, and other novel therapies. However, while myeloma patients are living longer, they are also living with symptoms and treatment side-effects. Therefore, myeloma patients face difficult decisions about the benefits and risks of treatment. The purpose of this study was to assess myeloma patient preferences for treatment.
Aims: The study aimed to answer the following questions: What treatment attributes do myeloma patients value? What is the relative importance of different treatment attributes to myeloma patients and the maximum acceptable risk they are willing to accept? What risk-benefit trade-offs characterise patients' decision-making around treatment options, including not to treat? What, if any, influences and predictive factors are found in the way patients assess benefits and risk?
Methods: Participants were 475 Myeloma patients in the UK. Data were collected using discrete choice experiments (DCEs) through an online survey. The DCEs presented patients with a traditional treatment choice experiment (*e.g.,* treatment A *vs* treatment B), focusing on the clinical benefits of treatments and the associated risks. The attributes and levels of the attributes were selected based on previous research, literature review and expert opinion. The DCE data were modelled using a Latent Class Model (LCM), and the effect of demographic characteristics were also examined.
Results: Findings revealed two classes (groups) of patients with different preferences for treatment attributes. Patients in class one placed greater importance on overall survival and mild-to-moderate side effects, whereas patients in class two placed greater importance on how the treatment was administered and the average out-of-pocket costs. Patients living with others and those diagnosed in the last five years were more likely to be to be in class one (those more concerned with overall survival) than class two (those more concerned with quality of life).
Summary/Conclusions: Findings from this study suggest that not all myeloma patients value the same treatment features equally. This finding has important implications for healthcare policy decisions and could be used to guide decisions around the value of new myeloma medicines. For example, to establish more patient-aligned endpoints in clinical trials or as evidence which is incorporated into the Heath Technology Assessment process.

E1471

COST-MINIMIZATION ANALYSIS OF RITUXIMAB SUBCUTANEOUS FORMULATION *VERSUS* INTRAVENOUS ADMINISTRATION OF RITUXIMAB FOR THE TREATMENT OF NON-HODGKIN'S LYMPHOMA IN THE REPUBLIC OF MACEDONIA

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Background: Rituximab, an anti-CD20 monoclonal antibody, in combination with chemotherapy is a standard of care for non-Hodgkin's lymphoma (NHL), in a standard dose of 375mg/m^2 body surface area (BSA), administered by intravenous (IV) infusion, or fixed dose of 1400mg administered as subcutaneous formulation (rituximab SC). Intravenous infusion of rituximab typically last for three to four hours, while subcutaneous application last approximately five to seven minutes. The evidence to support the use of rituximab SC as an alternative to rituximab IV is primarily based on the phase III, randomised, non-inferiority, open-label SABRINA study. Recent studies demonstrated therapeutic and pharmacokinetic non-inferiority of rituximab SC to rituximab IV.
Aims: The aim of the study was to identify and compare the total costs of subcutaneous (SC) *vs* intravenous (IV) administration of rituximab for the treatment of NHL patients in the Republic of Macedonia.
Methods: Cost-minimization analysis was used to evaluate pharmacoeconomic impact of the use of subcutaneous *vs* intravenous administration of rituximab in the treatment of NHL patients. The total of 220 NHL patients (mean body surface area 1.9 m^2, middle aged 59.6 years) were enrolled in the study. Evaluated healthcare resources included drug treatment costs, infusion chair occupying cost, active Healthcare Professional time cost and consumable disposals.
Results: Direct costs of administering one course of rituximab, including cost of drug, cost of administration and cost of consumables in all treatment phases (premedication, medication and post medication), for intravenous administration of rituximab were 1621€ compared to 1546€ for subcutaneous administration of rituximab. Average time for intravenous administration is 6 hours, 12 minutes and 13 seconds, compared to 10 minutes and 13 seconds for subcutaneous administration. Subcutaneous rituximab incurred less non-drug related costs than intravenous rituximab under the observed clinical practice: 14.62€ *vs* 1.76€ regarding active healthcare professional time and 10.10€ *vs* 1.2€ as infusion chair occupying cost.
Summary/Conclusions: Subcutaneous administration of rituximab is a cost-saving therapy in comparison with intravenous administration of rituximab for the treatment of NHL patients in the Republic of Macedonia.

E1472

QUALITY OF LIFE AND ABILITY TO WORK OF PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA TREATED WITH THYROSINE KINASE INHIBITORS

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Background: Thyrosine kinase inhibitors (TKIs) are now standard treatment for chronic myelogenous leukemia (CML), but little is known about quality of life (QoL) of the patients.
Aims: The purpose of this study is to evaluate QoL of CML patients receiving TKIs, a disease requiring strict daily compliance with taking these drugs orally, as well as regular clinical and biological controls.
Methods: The study included patients with CML followed in three hospitals in west Algeria between 2004 and 2016. The measure of QoL was performed by the tool of functional assessment of chronic illness therapy (Functional Assessment of Chronic Illness Therapy, FACIT) for leukemia. We have established QoL scores given by the questionnaire, FACIT, consisting of three levels: TOI for leukemia trial outcome index, FACT-G for general score, and FACT-LEU for the total score of leukemia. Specific areas of the questionnaire were associated with QoL of patients such as fatigue and ability to work. The correlation between these data and QoL scores was assessed using Spearman's test. The test is significant if $p<0.05$.
Results: 67 patients with CML have agreed to answer to the questionnaire of QoL, medications in use, and their side effects. The mean QoL of the patients was 93.7 (out of 124 total points) for the TOI, 77,2 (out of 108) for the FACT-G, and 128.9 (out of 176) for the FACT-LEU. Patients who presented with TKIs side effects had a low score of QoL ($p=0.0006$), especially when these effects are severe ($p=0.003$). Stopping TKIs medication was noted in 41.3% of patients with severe side effects. Severe fatigue was observed in 14 (22.9%) patients, having low QoL scores in all scales ($p<0.0001$). 44 (65.8%) patients were able to work with higher QoL scores in the three FACIT scales ($p<0.0001$, Spearman correlation).
Summary/Conclusions: QoL is an important aspect in the management of CML, its assessment is necessary and must be regular. The ability to work and fatigue are important components of QoL of patients receiving TKIs and should be specifically taken into account during the treatment. Adverse effects of TKIs can interfere with QoL of patients and can lead to discontinuation of CML therapy.

E1473

QUALITY OF LIFE AND EMPLOYMENT AFTER AN HEMATOPOIETIC STEM CELL TRANSPLANTATION IN A MEXICAN POPULATION

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Background: Hematopoietic stem cell transplantation (HSCT) is a consolidation therapy for multiple hematological malignances and its goal include patients achieve levels of quality of life (QOL) similar that general population. However, studies developed in Europe and United States have shown that patients on long-term follow-up after HSCT reported lower levels of QOL, more unemployment and lower household income than before the procedure. These relationships have not been examined in Mexican HSCT patients.

Table 1.

Table 1. Levels of quality of life reported.

	Mexican patients The EORTC range is from 0 to 100 for each category		Europe and USA patients		
	Median (range)	Percentage of participants with level ≥ 80 points	Boland (2013) EORTC (0 to 100) Mean (s.d.)	Janicsák (2013) FACT-BMT Mean (s.d.)	Niederbacher (2012) FACT-BMT Mean (s.d.)
Global QL	91.67 (50 - 100)	79.4		Scale range from 0 to 148	
				142 (27)	109.79 (18.16)
Physical QL	93.33 (60 -100)	85.3	60.6 (25.4)	Scale range from 0 to 28	
				21 (4.6)	23.18 (4.16)
Role QL	100 (33.33 - 100)	85.3	55.2 (31.2)		
Cognitive QL	100 (50 - 100)	64.7	71.8 (25.9)		
Social QL	100 (16.67 - 100)	64.7	46.9 (28.3)	Scale range from 0 – 28	
				17 (6.5)	19.61(4.89)
Emotional QL	83.33 (25 - 100)	58.8	68.8 (23.9)	Scale range from 0 – 24	
				17.9 (4.3)	19.32 (3.32)

Aims: To describe the QOL (EORTC-QLQ), level of employment and household income in Mexican patients on follow-up after HSCT
Methods: This was a cross-sectional study with patients ≥18 years old with at least one year of follow up after HSCT at the National Cancer Institute, Mexico.
Results: 30 participants were included, with a median age of 34 years (range 21-65), 56% male, and 41% married. Regarding educational level 68.7% had basic education, 25% had a college education and 6.3% postgraduate education. Mean time after HSCT was 36 months, 10% had active chronic graft *versus* host disease (GVHD). Patients reported moderate to high levels of QOL (Table 1). With respect to employment, 52% had a job (56% had a full time job, 13% work part-time and 31% had an informal job) and 48% were unemployed (50% could not find a job and 50% did not want to have a job). Finally, 56% had lower household income than before HSCT.
Summary/Conclusions: Mexican patients showed similar or higher levels of QOL in comparison with samples from other countries, with the exception of higher impact in emotional QOL and better social QL in our sample. Additionally, a substantial minority of patients were unemployed and over half had lower household income after HCST. More work is needed to identify risks associated with changes in QOL, employment status and income among long-term survivors of HCST.

E1474

ANTHRACYCLINE INCREASES THE RISK OF DEVELOPING DIABETES IN B CELL LYMPHOMA

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Background: Treatments of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) or R-CHOP like regimens have made B cell lymphoma to be one of the most curative hematological malignancies. Among the effective chemotherapeutic agents in B cell lymphoma treatment, anthracycline plays an important role. However, anthracycline associated bone marrow suppression and cardiotoxicity limit its clinical application. Whether anthracycline would further increase the risk of developing diabetes in B cell lymphoma remains unclear.
Aims: The aim of this study was to compare the cumulative incidences of diabetes in B cell lymphoma patients treated with and without anthracycline. We also investigated the dose effect of anthracycline on diabetes development. Additionally, whether anthracycline would increase the severity and complication of diabetes in B cell lymphoma patients were also studied.
Methods: We conducted this population-based study by using Taiwanese National Health Insurance Research Database. From 2004 to 2011, medical records from a total of 3984 B cell patients were analyzed. To understand whether anthracycline therapy was associated with more diabetes in B cell lymphoma, we compared the cumulative incidence of newly diagnosed diabetes between patients with (n=3147) and without (n=837) anthracycline treatments. Impact of anthracycline on diabetes was further studied by multivariate Cox proportional hazard regressions in a dose-dependent manner.
Results: Log-rank test did not show the difference of cumulative incidences of newly diagnosed diabetes between B cell lymphoma patients with and without anthracycline treatments ($p=0.1446$). However, anthracycline remained associated with more diabetes [hazard ratio (HR): 1.59; 95% confidence interval (CI): 1.05–2.39; $p=0.0278$] after adjustment for age, gender, and comorbidities. Moreover, cumulative anthracycline doses of 253-400mg (HR: 1.94; 95% CI: 1.23–3.05; $p=0.0043$) and 401-504mg (HR: 1.83; 95% CI: 1.11–3.01; $p=0.0180$) increased the incidence density of diabetes in a dose-dependent manner ($p=0.0438$). Notably, patients with and without anthracycline treatment had similar yearly adapted diabetes complications severity index alteration (0.58±1.89 *vs* 0.75±1.85; mean±standard deviation), suggesting anthracycline did not deteriorate outcome of diabetes in B cell lymphoma patients ($p=0.4924$).

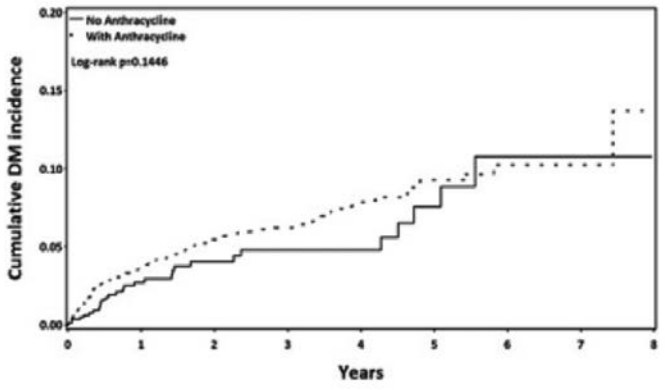

Figure 1.

Summary/Conclusions: Anthracycline therapy was responsible for more diabetes in B cell lymphoma in a dose-dependent manner. More intensive blood sugar monitoring and control should be recommended to B cell lymphoma patients, especially those who received anthracycline treatment.

E1475

THE COST-EFFECTIVENESS OF LENALIDOMIDE PLUS DEXAMETHASONE FOR THE TREATMENT OF RELAPSED OR REFRACTORY MULTIPLE MYELOMA IN CHINA

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Background: The introduction of lenalidomide plus dexamethasone (RD), and bortezomib-contained regimens, has improved the management of relapsed or refractory multiple myeloma (rrMM) in China. However due to the absence of both head-to-head (direct) comparative efficacy and local economic data, stakeholders still face hard choices to make when choosing one therapy over another. Indirect treatment comparisons and health economic modeling can help support local decision-making by enabling the incorporation of country-specific unit cost data, in the comparison of cost-effectiveness of one treatment *vs* another where treatments have not been directly compared in clinical trials.
Aims: To assess the cost-effectiveness of RD relative to bortezomib/dexamethasone (VD) and bortezomib/cyclophosphamide/dexamethasone (VCD) for rrMM in Chinese patients.
Methods: The Markov-based decision analytic model was constructed to simulate lifetime health benefits and direct medical costs associated with RD, VD, and VCD for rrMM in Chinese patients. A systematic literature review was conducted (in both Chinese and English databases, from 2005 to 2016) to obtain efficacy data of the three treatment regimens. The risk of progressive disease associated with RD and VD were estimated from available Chinese trials. The efficacy of VCD and the mortality associated with progressive disease after treatments with RD and VD were lacking in China, therefore were estimated from the published international randomized clinical trials. Published quality of life data was adapted to Chinese rrMM patients with health utility adjustment. The model took into account (i) drug acquisition costs, (ii) treatment administration costs derived from Chinese urban claims data, (iii) serious adverse events management costs based on a survey of seven MM centers across China, and (iv) rrMM management costs estimated from a Chinese real-world hospital setting. Quality-adjusted life years (QALY) and direct medical costs in the model were discounted at 3% per annum. Base case analysis calculated incremental cost-effectiveness ratio (ICER) per QALY for RD relative to VD and VCD, respectively from the Chinese healthcare payer's perspective. One-way sensitivity analysis and probabilistic sensitivity (PSA) with 5,000 Monte Carlo simulations assessed the impact of the model uncertainty on the cost-effectiveness of RD. A scenario analysis was conducted by meta-analyzing the published international randomized trials for the efficacy associated with RD, VD, and VCD, to verify the base case analysis.
Results: Based on the model simulation without discounting survival outcomes over a lifetime horizon, RD could obtain longer average PFS years than VD (2.37 *vs* 0.78) and VCD (2.37 *vs* 1.36). RD was associated with longer discounted QALY (3.06 *vs* 1.58 *vs* 1.41) and more discounted lifetime medical costs (¥494,060 *vs* ¥272,135 *vs* ¥ 244,220) than both VD and VCD. The ICERs per QALY for RD relative to VD (¥149,706) and VCD (¥150,774) were less than the cost-effectiveness threshold of China (three times of estimated 2016 China GDP per capital ¥166,920/QALY, ¥1= €0.138). The cost-effectiveness of RD relative to VD and VCD was mainly driven by the mortality risk associated with the progressive disease after treatment. The scenario analysis generated comparable ICER per QALY associated with RD relative to VD (¥120,974) and VCD (¥117,191), therefore supports the robustness of base case analysis.
Summary/Conclusions: The local data-based health economic model estimates that RD could gain longer PFS and OS with acceptable cost-effectiveness, when compared to VD and VCD in Chinese rrMM patients.

E1476

DEVELOPMENT OF A NEW HAEMATOLOGICAL MALIGNANT PATIENT-REPORTED OUTCOME MEASURE FOR USE IN CLINICAL PRACTICE: HM-PRO

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Background: Health-related quality of life (HRQoL) of patients with haematological malignancy (HM) is greatly affected by the disease and the treatment and this has not been captured in a systematic manner in routine clinical practice.
Aims: The aims of this study were to identify issues important to patients with HM and development of a new patient reported outcome measure for use in daily clinical practice.
Methods: A conceptual framework was developed using preliminary literature search and discussions with physicians and patients. Patients with HM were then interviewed to produce a comprehensive item pool reported important by them. The generated items were then discussed in the data definition panel meeting to be included in the prototype version of the HM-PRO. Subsequently, a panel of experts and a panel of patients were asked to rate the items of the prototype HM-PRO for its language clarity, completeness, relevance and scaling followed by cognitive interviews with the patients to pilot test the HM-PRO.
Results: The preliminary literature search revealed that there is no PRO specifically developed for patients with HM for use in daily clinical practice. The conceptual framework comprised of two main themes: QoL (impact); and symptoms. 129 patients (male=76; mean age=61.1 years; SD=15.3; median age =64.9 years; age range =18-88 years; diagnosis –AML, ALL, CML, CLL, MM, ANHL, INHL, HL, MPN, and MDS) with mean duration of the HM of 3.6 years (SD=4.3; and range= 19 days-23 years) from 5 haematology centres were interviewed to identify the issues important to HM patients. A prototype version of HM-PRO was developed after data definition panel meeting with 34 items in impact category (Part A) and 23 items representing disease symptoms (Part B). Nine- member panel of experts and 7-member panel of patients, rated the items and discussed them for its language clarity, completeness, relevance and scaling to reach consensus. 60 patients (male=36; mean age=63.8 years; SD=16.61; median age =69.2 years; and age range =18-91 years) with mean duration of the HM of 4.9 years (SD=6.4; and range= 14 days-26 years) were recruited for the pilot testing where 34 of which were involved in cognitive interviews. 92% of the patients reported that the statements were easy to understand and all issues important to them were covered; 95% stated that they were able to respond spontaneously and expressed their willingness to complete the instrument during their visit to the clinic; 97% reported that the statements were easy to read; 98% did not wish to delete any item; and 88% did not see the need to add any item.
Summary/Conclusions: The findings of the content validation, pretesting and cognitive interviews indicate that HM-PRO possesses a strong content validity in different HMs, includes all the issues important to these patients and the statements are easy to read, understand and respond to spontaneously. HM-PRO will undergo further psychometric testing to support its psychometric properties across different types of HMs.

E1477

OVARIAN TISSUE CRYOPRESERVATION IN PEDIATRIC AND ADOLESCENT PATIENTS UNDERGOING CANCER CHEMOTHERAPY AND/OR HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Ovarian tissue cryopreservation (OTC) and subsequent reimplantation is the only option available for fertility preservation in prepubertal females, but this approach remains unestablished in pediatric and adolescent patients with cancer. After the experience of OTC for more than 200 patients with primary ovarian failure and more than 50 patients with breast cancer in our center over 5 years, we have started OTC for pediatric and adolescent cancer patients since 2015.
Aims: To define safety and benefits of OTC in pediatric and adolescent patients with undergoing cancer chemotherapy and/or hematopoietic stem cell transplantation.
Methods: From December of 2015 to February of 2017, OTC was performed in 6 girls (median age 14 years, range 11-15): 2 patients with myelodysplastic syndrome, 2 with lymphoma, 1 with acute lymphoblastic leukemia, and 1 with primary immunodeficiency. Indications for OTC were 5 hematopoietic stem cell transplantation and 1 sterilizing chemotherapy. Two patients with myelodysplastic syndrome and 1 with immunodeficiency received no previous chemotherapy and the other 3 had received prior chemotherapy. Laparoscopy was used to collect one of a pair of ovarium that was frozen by vitrification method.
Results: Ovarian tissue was successfully collected from the 6 patients studied without major postoperative complications and this procedure did not delay chemotherapy or hematopoietic stem cell transplantation. Histological analysis of ovarian tissue revealed primordial follicles, even in the patients with previous cancer chemotherapy. No malignant cells were identified. Median post-harvest

follow-up was 9 months (0-14) and all patients were alive. Hormonal results were evaluable for 3 patients: 2 patients were in premature ovarian insufficiency. Re-implantation of ovarian tissue has not yet been performed.
Summary/Conclusions: Although OTC and subsequent re-implantation is experimental, this approach may be the best method for restoration of ovarium function and fertility preservation in pediatric and adolescent cancer patients. A risk of reseeding malignant cells is a problem still to be conquered.

E1478

A MULTI-DISCIPLINARY APPROACH TO CHEMOTHERAPY PRESCRIBING AT NEWCASTLE UPON TYNE HOSPITALS NHS FOUNDATION TRUST
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Background: Newcastle Upon Tyne Haematology service has made numerous changes in recent years to provide streamlined care for patients, focusing on reduced wait times & improve quality of care. The original pathway was costly in time, involving several waits for the patient: for urgent venepuncture, physician consultation, prescribing of chemotherapy, specialist pharmacist screening of prescriptions & then a separate trip to pharmacy for dispensing. Patients then returned home & waited for a call from the Clinical Nurse Specialist (CNS) to confirm if blood results were appropriate for chemotherapy administration. If a dose adjustment was required the drug was wasted & patients needed to return to hospital for another prescription. Pharmacy waiting times for oral outpatient chemotherapy or supplementary medications are approximately 30 minutes.
Aims: We introduced a weekly multi-disciplinary chemotherapy prescribing meeting in 2013 with the aims of improving prescribing safety; minimising time spent prescribing in clinic & reducing patient waiting times. Present at each meeting is a Haematology Specialist Pharmacist, Haematology CNS, Consultant & Specialist Registrar. Chemotherapy is planned a week in advance on ChemoCare (an electronic chemotherapy prescribing package). Chemotherapy is prescribed & immediately screened by the pharmacist; oral chemotherapy is collected from pharmacy by a CNS prior to clinic. All prescription queries are resolved during this meeting. Deferred oral chemotherapy can be returned to pharmacy stock, minimising waste. Intravenous chemotherapy is pre-planned with authorisation on the day of treatment if the patient is fit to proceed.
Methods: In the latest update to the care pathway, we focused on delivery of care to myeloma patients receiving oral chemotherapy, including setting up a nurse-led clinic. Data have been collected to assess service impact, particularly on patient satisfaction. The latter was assessed using a patient survey. Between July-Dec 2014, 66 patients received oral chemotherapy in the Myeloma Consultant-led clinic, Lenalidomide based regimens accounted for 86% of the oral regimens prescribed. On average, 7 patients per week were on maintenance therapy. During this period 8% of chemotherapy courses were deferred due to low blood counts or side-effects. Drugs were not wasted due to the pharmacy agreement.
Results: A patient satisfaction survey was undertaken from Jan-June 2015, post-introduction of the nurse-led clinic paired with the MDT chemotherapy prescribing meeting. Patients were asked about a wide-range of quality parameters. Results showed 89% of patients noted a reduction in wait times & 89% felt they spent more time in consultations as a result. All patients noted they spent more time with the nurse specialist & benefited from not attending pharmacy. All patients rated the service as more efficient.
Summary/Conclusions: The MDT approach to prescribing & dispensing oral chemotherapy & supportive medication has streamlined our way of working & led to greater efficiency for both staff & patients. The new model has changed how patients are seen & assessed and minimised drug wastage, an issue incurred in the old system. However, it must be noted that during Jan-June 2015 the cost of wasted drug, due to patients being unfit for treatment on the day, potentially incurred by pre-prescribing was £57,775. It is, therefore, critical to ensure that medication that has not yet been given to patients can be returned to pharmacy if this type of pre-prescribing model is to operate efficiently.

E1479

FINANCIAL TOXICITY OF THE MANAGEMENT OF MULTIPLE MYELOMA
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Background: Advances in supportive care and the development of novel treatments have helped to double the life expectancy of patients with newly diagnosed multiple myeloma (MM). Financial toxicity is increasingly recognized as adversely affecting the quality of life and medication adherence, and patients with MM might be particularly vulnerable because of extended treatment duration.
Aims: Our aim was to measure financial toxicity and its effects on patients undergoing treatment for MM.
Methods: Between October 2016, and January 2017, we did a cross-sectional survey of individuals receiving at least 3 months of ongoing treatment for MM at our department. The survey included the 11-item COST measure (financial toxicity score range 0-44). A paper survey was offered to eligible patients on arrival for routine follow-up visits or treatment, and participants were asked to complete the survey before or after their visit or stay in day hospital. The data were postponed by two psychologists. The COST questionnaire was validated with internal consistency (Cronbach's coefficient) and item correlation (Pearson's r coefficient) tests, especially those of Quality of life (EORTC QLQ 30).
Results: Of 47 patients approached for the study, 44 individuals completed the survey and 40 (91%) were insured. Analysis of the internal consistency of the COST questionnaire showed an overall Cronbach's alpha coefficient of 0, 84. According to COST data, 26 (59, 1%) patients have a score<22. Patients with financial difficulties have a negative impact on their quality of life (P=0, 02, r=0, 32), and low scores of physical and role functioning (P<0,001, r >0, 5). 29 (66%) patients feel financially stressed, and 23 (52, 3%) did not control their financial situation. After a logistic regression, lower household income (P=0,009) and Poor response to treatment (P=0, 0037) were associated with higher financial burden as measured with the COST score.
Summary/Conclusions: Despite insurance and free care, financial toxicity is common in many myeloma patients, especially those with lower income and refractory disease. Strengthened collaboration among patients and health-care stakeholders is needed to promote healthcare reforms that promote high value and affordable myeloma care.

E1480

THE IMPLICATIONS OF NON-PROPORTIONAL HAZARDS FOR THE MEASUREMENT OF SURVIVAL BENEFIT IN HEALTH TECHNOLOGY ASSESSMENT: CURRENT APPROACHES AND THE ROLE OF RESTRICTED MEAN SURVIVAL TIME
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Background: Median survival and hazard ratios (HR) calculated from Cox proportional hazard (PH) models for progression-free survival (PFS) and overall survival (OS) are principal survival endpoints in oncology clinical trials. The advent of novel agents, including immuno-oncologics, has seen increasing reports of non-proportional hazards (non-PH). When non-PH are present, it is challenging to evaluate the true clinical significance of survival differences expressed as median and HR since these measures do not represent the comparative benefit over the full period of observed data. In such situations, additional metrics such as restricted mean survival time (RMST) may be valuable.
Aims: To determine current methods used by health technology assessment (HTA) agencies when non-PH are observed in assessments of hematology/oncology drugs, and the extent to which RMST is accepted as an alternative measure of treatment benefit in these circumstances.
Methods: Methodological guidelines published by 10 HTA agencies in 8 major developed countries (Australia, Canada, France, Germany, Italy [Emilia Romanga, Veneto], Spain, Sweden, and the United Kingdom [NICE, SMC]) and by influencing organizations in the US (including the Institute for Clinical and Economic Review, ICER) were reviewed to establish recommended approaches for presenting survival benefit from clinical trials, particularly the use of RMST where non-PH were reported. To determine how these guidelines are implemented in practice, published HTA reports were examined across the 8 countries for 23 oncology agents (including 4 in hematology) approved by the FDA and EMA since 2014, to identify instances where testing for non-PH was conducted and RMST data reported.
Results: Guidelines from only 2 agencies (PBAC in Australia and NICE in the UK) described formal testing for non-PH. Testing was reported in 15 (of 23) NICE assessments and 4 (of 10) PBAC assessments. For the hematology drugs, non-PH testing was conducted in 3 (of 4) NICE assessments; it did not hold in 2 instances. Of the agencies (from France, Germany, Italy and Spain), which focus on comparative clinical benefit, only 1 (GENESIS in Spain) discussed the concept of RMST in their guidelines; 5 (of 45) assessments from these countries included RMST. Of the agencies (from Australia, Canada, Sweden, and the UK) which focus on cost-effectiveness, all except the TLV in Sweden include RMST in their guidelines; RMST was reported in 13 (of 81) HTA assessments from those countries. Of the 3 hematology drugs where non-PH was tested within the NICE process, only one (ofatumumab, where there were PH) reported RMST (utilized during economic model sensitivity analyses). Non-PH is not a widely reported issue in US guidelines; however, the ICER has acknowledged it and PH testing was conducted in both ICER reports in oncology.
Summary/Conclusions: Testing for non-PH is not widely reported in clinical trials or incorporated into assessments by HTA agencies except by UK NICE. RMST as a metric to assess OS has played a role in assessing clinical benefit within the context of HTA assessments, although not consistently within countries (across drugs) or across countries (for the same drug), as was seen with the hematology agents. As treatments for cancer expand to new classes and indications, instances of non-PH will likely increase; alternative survival metrics such as RMST may have an increasingly important role to play in describing survival benefit in such cases.

Sickle cell disease

E1481

DISEASE SEVERITY AND SLOWER PSYCHOMOTOR SPEED IN ADULTS WITH SICKLE CELL DISEASE

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Background: Psychomotor slowing is common in children with sickle cell disease (SCD), but little is known about its severity in adult patients. While the primary risk factor for psychomotor slowing is stroke, there has been mounting evidence that cognitive impairment also occurs in patients without a history of overt or silent stroke. Risk factors for cognitive impairment in patients with SCD without stroke are, however, not completely known, particularly in relationship to the SCD genotype.
Aims: We conducted a cross-sectional study to quantify psychomotor slowing, measured with the Digit Symbol Substitution Test (DSST), a pencil and paper test of executive function, in relationship with disease severity in adult patients with SCD attending an outpatient clinic. We also examined whether demographic, behavioral, physiologic, and pathologic factors that are known to be related to SCD severity and cognitive function in other settings are also related to psychomotor speed in these patients.
Methods: Genotype was used to group patients with SCD (n=88, age: 36.3 years, 33 males) in "severe" (homozygous for the mutated sickle hemoglobin HbS [HbSS], or compound heterozygous with β^0 thalassemia [HbS/β^0]) or "moderate" groups (compound heterozygous for HbS, with either HbC [HbSC], or β^+thalassemia [HbS/β^+]). Standardized DSST scores based on published norms were used to define mild cognitive impairment, defined as ≤1.5 standard deviations (SD) below the DSST T-score (T-scores had a mean of 50 and SD of 10). Data on demographics, hematological parameters, hydroxyurea and opiate intake, stroke (including silent cerebral infarcts (SCI)) and transfusion history were collected concurrently with DSST. Analyses were repeated after exclusion of patients with a history of stroke (n=12). Age-adjusted p-value was calculated with logistic regression for all variables except age (unadjusted) and DSST T-score (already adjusted for age, sex and education in calculation of T-score).
Results: Among our patients, 56 (63%) had a "severe" genotype and 32 (27%) had a "moderate" genotype. Mild cognitive impairment was detectable in both the "severe" and the "moderate" group (30% and 9%, respectively, age-adjusted p=0.15). Compared to the "moderate" group, those in the "severe" group, had significantly lower DSST scores (age, sex and education adjusted p value=0.006), independent of adjustment for factors that differed between groups: hemoglobin, ferritin, hydroxyurea use, blood pressure parameters and stroke history. Results were similar after excluding patients with stroke.

Table 1.

Table 1. Predictor variables of interest

	"Severe"	"Moderate"	P†
Age (years)	33.7 (10.8)	40.9 (12.8)	**0.009**
Male sex*	21 (37.5%)	12 (37.5%)	1.00
Education (years)	13.1 (1.8)	13.2 (1.7)	0.80
Mild Cognitive Impairment*	17 (30.4%)	3 (9.4%)	0.14
DSST T-score	47.6 (14.5)	51.0 (13.4)	**0.006**
O^2 Saturation (%)	97.5 (1.8)	98.1 (1.7)	0.14
WBC count (X 10^9/L)	9.7 (3.8)	9.2 (3.7)	0.87
Hemoglobin (g/dL)	9.2 (1.5)	11.5 (1.5)	**<0.0001**
Platelet count (X 10^9/L)	344.1 (179.8)	263.3 (115.1)	0.16
Reticulocytes (%)	1.7 (4.0)	1.1 (1.9)	0.51
LDH (IU/L)	321.2 (142.3)	269.2 (149.1)	0.18
Ferritin (ng/mL)	1141.6 (1864.4)	403.4 (1042.2)	**0.05**
Creatinine (mg/dL)	0.7 (0.3)	0.8 (0.2)	0.91
SBP (mm/Hg)	111.3 (13.4)	118.9 (13.6)	0.08
DBP (mm/Hg)	68.8 (7.7)	73.5 (9.8)	**0.02**
MAP (mm/Hg)	83.1 (8.4)	88.6 (10.0)	**0.03**
Hydroxyurea use*	32 (57.1%)	10 (31.2%)	**0.04**
Opiate use*	15 (26.8%)	10 (31.2%)	0.57
Transfusion history*	17 (31.5%)	5 (16.1%)	0.15
Stroke history*‡	10 (18.2%)	2 (6.2%)	0.08

* Mean (SD) unless otherwise noted. † Age-adjusted p. ‡ Includes SCI

Summary/Conclusions: Psychomotor slowing in SCD differs in relationship to genotype; this difference appears unrelated to history of stroke or severity of anemia and other risk factors examined cross-sectionally. Although relatively infrequent, mild cognitive impairment was also detectable in patients with a less severe genotype. Longitudinal studies of SCD should include all diseases genotypes, and examine factors that would reduce the risk of cognitive impairment in each subgroup.

E1482

MONITORING OF CHRONIC HEPATIC DAMAGE IN SICKLE CELL DISEASE: LONGITUDINAL OBSERVATION OF A COHORT OF ADULT PATIENTS

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Background: Acute vaso-occlusive events (VOCs) in Sickle Cell Disease (SCD) is an important cause of hepatic damage which can result in catastrophic consequences as acute hepatic failure and contribute to early mortality. In addition, sickle hepatopathy may be the consequence of SCD's treatment as liver iron overload or viral hepatitis due multiple blood transfusions that these patients require in their lifetime. Therefore both SCD itself and related therapies may lead liver to fibrosis/cirrhosis.
Aims: We evaluated liver fibrosis using Transient Elastography (TE) in patients with SCD, exploring possible correlation with clinical, laboratory and imaging findings in longitudinal way.
Methods: SCD patients with at least one stiffness evaluation were retrospectively evaluated in the decade 2006-2016 using biochemical markers (liver damage, cholestatis, liver syntetic capacity, iron overload, viral hepatitis and hemolitytic index), TE and liver imaging (ultrasound, MRI-R2*).
Results: 37 adult patients were evaluated: 32% HbSS, 68% HbSβ°, median 39yrs, 46% male, median stiffness 6.6 KPa IQR: 5.1-9.1 KPa (Table). There were not differences of stiffness value for gender, genotype. A positive moderate correlation was observed between TE and serum ferritin values (Rp=0.43, p=0.008), ALT (Rp=0.42, p=0.01), AST (Rp=0.49, p=0.0022), conjugated bilirubin (Rp= 0.59, p<0.001), ALP (Rp=0.51, P=0.002); a positive strong correlation was observed between TE and GGT (Rp=0.79, p< 0,001), negative moderate correlation with the albumin (Rp=-0.47, p=0.0048). We found that the group of patients on eritroexchange programmes had a value of stiffness lower than the group transfused (p=0.007). No significant correlation was found between stiffness and LIC (Rp=0.11, p=0,67). For 24 patients all record were available at time of first observation until last follow up (f.u.): 75% HbSβ°, median age 39.5yrs, male 42%, median f.u. 6 yrs, median stiffness 7.3 KPa IQR: 5.3-11.9 KPa. At the first evaluation we documented a significative positive-moderate correlation of TE with serum ferritin (Rp=0.43, p=0,037), AST (Rp=0.54, p= 0,006), conjugated bilirubin (Rp=0.52 values 0,009) and positive-strong correlation with GGT (Rp=0.68, p<0,001); these parameters except of ferritin (Rp=0.3, p=0.15) and AST (Rp=0.39, p=0.058) have maintained the correlation with last f.u.; albumin and ALP showed a significant strong correlation only at f.u. (albumin Rp=-0.64, p=0.004; ALP Rp=0.7, p=0.0017). To remove factors associated with liver fibrosis we also conducted this analysis in the subset of patients HCV negative without liver iron overload: 26 patients, HbSβ° 73%, median age 40.5yrs, male 50%, median f.u. 6 yrs, median values of stiffness 6.1 KPa IQR: 4.6-7.4 KPa. All significant correlations previously described were confirmed also in this group. Three patients in this cohort presented stiffness value according to F4 METAVIR since their first evaluation: all these patients showed pauci-symptomatic disease in terms of VOCs, however they had a severe hepatic damage due to sickle cell disease.

Table 1.

Table Mean characteristics of the 37 patients

	median	IQR
Age (yrs)	39.0	30-50
BMI (kg/m2)	23.1	20.3-25.7
Hb (g/dL)	10,0	9.3-10.7
AST (IU/L)	35.2	26.8-50.4
ALT (IU/L)	25,0	19-36.3
GGT (IU/L)	27.4	17.8-51.7
Total bilirubin (mg/dL)	1.9	1.4-3.0
Conjugated bilirubin (mg/dL)	0.63	0.5-0.75
Ferritin (ng/mL)	670	320-1146
ALP (IU/L)	87.3	68.3-109.7
Albumin (g/L)	4.2	4.0-4.3
Prothrombin Time Activity %	84.4	79.1-89.9
LDH (IU/L)	617	505-869
Reticulocytes (%)	6.45	3.9-10.9
Hb Fetal (%)	5.6	1.9-13.5
T2* (ms)	8.6	6.7-13.7

Summary/Conclusions: Early identification of chronic hepatic disease sometimes pauci-symptomatic in terms of VOCs but able to lead to advanced stage and progressive fibrosis is crucial for suitable clinical management to avoid cirrhosis in SCD patients. The combination of TE with specific serum markers (GGT, ALP, albumin) is a valid tool to early detection of sickle hepatopathy.

E1483

MICROSTRUCTURAL ANALYSIS OF RETINO-CHOROID LAYERS USING OPTICAL COHERENCE TOMOGRAPHY IN ADULT PATIENTS WITH SICKLE CELL DISEASE

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Background: Retinopathy is one of the ophtalmological complications of patients with Sickle cell disease (SCD), due to microvascular occlusions; occasionally, proliferative sickle cell retinopathy (PSR) can lead to severe vision loss.
Aims: a. to analyze macular alterations in patients with Sickle Cell Disease (SCD) by spectral-domain optical coherence tomography (SD-OCT), using the automated software for retinal segmentation; b. to investigate relationship between OCT abnormalities and the severity of proliferative sickle cell retinopathy (PSR); c. to elucidate the role of potentially contributory systemic factors on the development of macular thinning.
Methods: This is a prospective, observational case-control study. Ophthalmological evaluation, fluorescein angiography and SD-OCT were performed. Central and temporal retinal layers were measured by the SD-OCT Automatic Segmentation software. SCD eyes were divided into two groups based on the presence of visible macular thinning areas. Clinical data and blood samples were collected.
Results: Thirty consecutive adult SCD outpatients were studied (median age 38.7±9.89 (M:F 12:18), including 9 patients with Sickle Cell Anemia (SCA), 17 with Sickle Cell β°-Thalassemia and 4 HbS/HbC. One HbS/HbC eye was not considered due to retinal detachment and severe refractive defect. Nineteen out of 59 eyes (32.2%) and 13 out of 30 SCD patients (43%) were noted to have patchy areas of macular thinning on SD-OCT, mostly seen temporally to the fovea. Among these patients, 6 had SCA, 4 had β°-Thalassemia and 3 HbS/HbC. More severe PSR was present in 16/59 eyes (29%); the prevalence of temporal macular thinning was higher (10/16) in eyes with more severe PSR (62.5%). Both inner and outer retinal layers thinning of the foveal region and of the central and temporal macula was found in the overall SCD patients compared with normal controls ($p<0.0001$). SCD eyes with patchy retinal thinning showed a significant reduction of inner nuclear layer (INL) and outer plexiform layer (OPL) in the temporal region. Univariate analysis revealed a significant correlation between patchy areas of severe retinal thinning on OCT and SCD need for transfusions, need for chelation, HbF, ferritin, and transferrin saturation (p-value<0.10). However, the most predictive variables for retinal thinning as assessed after multivariate regression analysis were the need of chelation ($p=0.0187$) and the HbF ($p=0.0775$). More specifically, the odds of retinal thinning is 94.2% lower when chelation is present, and the odds of retinal thinning decreases by 12.9% when HbF increases by 1 unit.
Summary/Conclusions: In this study SCD eyes of all patients showed both inner and outer retinal thinning in the central and temporal macula. Ischemia caused by chronic occlusion of the deep and superficial capillary plexus could explain the different retinal layers' damage and the pattern of thinning. No major statistical differences were found between the three sickle genotypes because of small sample. Because severe retinal thinning affects visual function and can lead to irreversible visual loss, regular ocular checkups are essential for SCD patients.

E1484

NON ABLATIVE TRANSPLANT CONDITIONING WITH TREOSULFAN IS CURATIVE IN A MURINE MODEL OF SICKLE CELL DISEASE

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Background: Hematopoietic stem cell transplantation (HSCT) for patients with sickle cell disease (SCD) is curative, though significant toxicity from myeloablative conditioning is limiting. We have previously developed knock-in mice producing normal (AA) or sickle (SS) human hemoglobin recapitulating severe anemia, hyposthanuria and limited lifespan found in SCD. Reduced-intensity conditioning regimens decrease transplant toxicity and are preferable in non-malignant disorders. Novel approaches have been proposed including targeted depletion of stem cells (ACK2), co-stimulation blockade (anti-CD40L), and combination therapy with less toxic alkylating agents (Treosulfan).
Aims: Optimize non-myeloablative conditioning in a murine model of SCD that allows for sufficient donor RBC chimerism.
Methods: Control (AA) and SCD (SS) animals were treated with varying conditioning regimens (+/- rescue with AA or SS marrow), including Treosulfan (2-5g/kg), ACK2 (100-500ug), anti-CD40L, and low-dose radiation, alone or in combination. Short and long-term toxicities, including survival, were monitored over a 12 month period. Hematologic effects were determined by assaying CBCs, reticulocytes, bone marrow (BM) cellularity and RBC chimerism (isoelectric focusing). Myeloid/lymphoid chimerism was monitored by FACS combined with droplet-digital PCR. Renal tubular function was assessed by measuring urine osmolality, and moribund animals underwent necropsy to assess organ damage.
Results: Erythroid hyperplasia was noted in the BM of SS, relative to AA mice. Treosulfan, in a dose-dependent manner, decreased BM cellularity and induced cytopenia in AA and SS mice. AA mice were able to tolerate Treosulfan at non-myeloablative doses of 6g/kg. In contrast, SS mice were unable to tolerate doses of 3g/kg unless RBC transfused by d+3. At 3g/kg dose, erythroid engraftment was transient in SS transplanted mice and most often absent by 2 months post-transplant, with only 25% of animals having sustained RBC chimerism at one year. In SS mice achieving 50% AA in peripheral blood, fertility was preserved, urine osmolality normalized and organ pathology was comparable to age-matched controls. ACK2, anti-CD40L, or low-dose radiation, in combination with Treosulfan (3g/kg), failed to improve engraftment. In contrast, increasing Treosulfan to 3.6g/kg resulted in donor-erythroid chimerism at 3 months post-transplant in all mice, with improvement in hematologic parameters and normalization of hyposthanuria. These animals are currently being observed for fertility, organ toxicity and survival.
Summary/Conclusions: SCD mice closely mimic human disease in phenotype and ablative conditioning intolerance. Treosulfan, at sub-myeloablative dosing, sustained erythroid chimerism and reversed the SCD phenotype. Our data suggests that pre-transplant conditioning with Treosulfan alone may be permissive for engraftment, in an allogeneic and gene-corrected autologous clinical transplant setting.

E1485

SILENT CEREBRAL ISCHEMIA AND THROMBOEMBOLIC EVENTS IN SICKLE CELL DISEASE: ANALYSIS OF COAGULATION PARAMETERS AND THROMBOELASTOGRAPHY

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Background: The complications of Sickle Cell Disease (SCD) include stroke and silent cerebral infarcts (SCI). The increased incidence of thromboembolic events in SCD has only recently been recognized. Apart from red cell sickling other pathogenetic mechanisms have been proposed but they have not been clarified completely. Coagulation factors have been analysed in several studies in SCD but very limited data exist about global coagulation assays such as thromboelastography, which evaluates the contribution of platelets, coagulation factors and cellular elements in clot formation.
Aims: The aim of the present study was to assess the incidence of cerebral ischemia and TEEs in SCD patients and to investigate their pathophysiology with analysis of coagulation parameters, including thromboelastography.
Methods: 61 adult SCD patients were included in the study and underwent brain MRI. Measurements of fibrinogen, D-Dimers, antithrombin III, proteins S and C were performed (SIEMENS BCS) and thromboelastography ROTEM^R was performed in order to analyse NATEM CT(Closure Time), MCF (Maximum Clot Firmness), EXTEM CT, MCF and FIBTEM CT, MCF. Brain imaging as well as all clotting assays were performed in steady state and not during the course of an acute thrombotic or ischemic event
Results: The median age of the patients was 51 yrs (range 27-70), 40 of them were female and 21 male. Abnormal findings were revealed in the brain MRI of 35/61 patients (57.4%). The findings consisted mainly of microischemic lesions. Only 5/35 patients had a previous history of overt stroke, 1/5 TIA. In the remaining 30/35 patients ischemic lesions were considered SCIs, in the abscence of neurologic manifestations. In 3/5 patients with a previous stroke the size of the infarcts in brain MRI was larger (with maximum diameter up to 4,5 cm). 14/61 patients had a previous history of venous TEE (23%), in 7/14 the event was pulmonary embolism and 2/14 had recurrent TEE. 14/61 (23%) of patients had a previous history of acute chest syndrome (ACS). In total 48/61 patients (78.7%) were already on treatment with hydroxyurea when they underwent the analysis. Elevated platelets were found in 22/61 patients (36%), elevated fibrinogen in

4/61 (1.6%), positive D-Dimers in 57/59 (96.6%), decreased protein S in 10/61(16,3%) and decreased protein C σε 13/61 (21,3%). NATEM MCF was increased in 27/61(44,3%) patients while EXTEM MCF was increased in 31/61(50.8%) patients. Patients with a history of TEE had higher mean values of NATEM-MCF and EXTEM–MCF and those differences were statistically signifinact (p=0.023, and p=0.011 respectively). There was a statistically significant association between the presence of ischemic lesions in brain MRI and the history of previous TEE (p=0.01). On the contrary the history of ACS was not correlated with the presence of ischemic lesions in MRI. Chronic Hydroxyurea treatment did not correlate with the abscence of ischemic findings in brain MRI. Among patients with ischemic lesions those who were already on chronic hydroxyurea treatment had a shorter NATEM-CT compared to patients without treatment. In patients with ischemic lesions in MRI and a history of TEE NATEM-MCF and EXTEM MCF were higher (p=0.03, και 0.03, respectively).
Summary/Conclusions: The presence of microischemic encephalopathy is very common in SCD patients and is associated with a history of TEE, which is also frequent in SCD. There seems to be a permanent activation of the coagulation mechanism in SCD. In SCD patients with SCIs and a history of TEE, apart from clotting factors and natural inhibitors there seems to be a contribution of platelets and cellular elements, possibly sickle cells. The impact of chronic hydroxyurea treatment on the pathogenesis of silent infarcts and TEEs needs further evaluation.

E1486

Abstract withdrawn.

E1487

INVASIVE BACTERIAL INFECTIONS IN GAMBIAN PATIENTS WITH SICKLE CELL ANEMIA IN AN ERA OF WIDESPREAD PNEUMOCOCCAL AND HAEMOPHILUS INFLUENZA TYPE B VACCINATION

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Background: Bacterial infections cause significant morbidity and mortality in patients with sickle cell anemia, especially in populations without reliable access to antimicrobial prophylaxis and treatment. The longstanding use of penicillin prophylaxis and vaccination for *Streptococcus pneumoniae* and *Haemophilus influenzae* type b in resource-rich settings has minimised the additional risk of invasive bacterial infections associated with sickle cell anemia. However, these interventions are not routinely implemented in much of Africa, despite this region having the greatest burden of disease, with over 80% of people with sickle cell anemia born on the continent. The Gambia has well established vaccination programmes for pneumococcal and *Haemophilus influenzae* type b, which is rare in the region. There is little data on the identity of bacterial infections in African sickle cell anemia populations, and we believe (until this study) there were no data from countries with comprehensive vaccination programmes against *Streptococcus pneumoniae* and *Haemophilus influenzae* type b.
Aims: Primary: to determine the predominant pathogens causing invasive bacterial infections in a population of sickle cell anemia patients admitted to the Medical Research Council Unit Gambia. Secondary: to review the characteristics of this sickle cell anemia population.
Methods: A retrospective analysis of the clinical and laboratory records relating to 161 admissions of 126 patients with sickle cell anemia admitted to the Medical Research Council Unit Gambia over a five-year period (between April 2010 and April 2015) when there was high coverage of pneumococcal and *Haemophilus influenzae* type b vaccination.
Results: Pathogenic bacteria were cultured from blood in 11 of the 131 admissions which had blood cultures taken (8.4%, 95% CI 4.5-14.1%). The most frequent organism isolated was *Salmonella typhimurium* (6/11; 54.5%), followed by *Staphylococcus aureus* (2/11; 18.2%) and other enteric Gram-negative pathogens (2/11; 18.2%) and there was one case of *Haemophilus influenzae* non-type b bacteremia (1/11; 9.1%). No cases of bacteremia caused by *Streptococcus pneumoniae* or *Haemophilus influenzae* type b were identified. The most common diagnosis causing the admission was vaso-occlusive crisis (53/161; 32.9%), followed by infective complications including pneumonia (16/161; 9.9%) and osteomyelitis (12/161; 7.5%). The median length of admission was five days and the median age of patients was five years (IQR: 2-13 years). A new diagnosis of sickle cell anemia was made during the admission in just under half of patients.

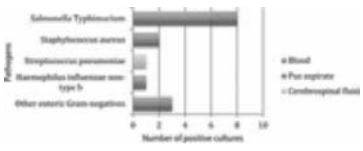

Figure 1.

Summary/Conclusions: The predominance of non-typhoidal *Salmonella* and other enteric Gram-negatives as the causative agents of invasive bacterial infections in our study is striking. Despite its success in resource-rich settings, penicillin may not be the optimal prophylaxis for sickle cell anemia patients already vaccinated for pneumococcal and *Haemophilus influenzae* type b in The Gambia. For sickle cell anemia patients with suspected bacterial sepsis, empirical treatment must be effective against both non-typhoidal *Salmonella* and *Staphylococcus aureus*, and account for local resistance patterns. As other countries in the region adopt pneumococcal and *Haemophilus influenzae* type b vaccination programmes, they may see a change in the spectrum of pathogens found in sickle cell anemia patient populations. Local research may be needed to determine appropriate antimicrobial treatment and prophylaxis regimens for patients with sickle cell anemia.

E1488

THE ASSOCIATION OF IGF-1 AND IGFBP-3 SERUM LEVELS AND GENE EXPRESSION WITH THE PATHOGENESIS OF INFLAMMATION IN SICKLE CELL DISEASE

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Background: Sickle cell disease (SCD) is one of the chronic inflammatory diseasesSerum markers of inflammation have provided evidence for a state of chronic inflammation in sickle cell disease (SCD). Inflammation promotes endothelial adherence to sickle erythrocytes.
Aims:. We aimed to investigate the serum insulin-like growth factor 1 (IGF-1) and insulin-like growth factor binding protein 3 (IGFBP-3) levels and gene expression in the pathogenesis of inflammation in sickle cell disease and to determine its role in painful crises.
Methods: A total of 71 patients aged 2 to 18 years, who were followed with the diagnosis of SCD in our department, were included in the study between April 2012 and April 2013. Patients were divided into two groups; Group 1: Patients who had a painful crisis during the study (41 patients, mean age: 11.5 years) and Group 2: Patients who were in steady state during the study (30 patients, mean age: 11 years). Blood samples were taken from the patients for complete blood count, serum levels of C-reactive protein (C-RP), interleukin-1 β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), IGF-1, IGFBP-3 and IGF-1, IGFBP-3 gene expression.
Results: When the patients in both groups were compared in terms of serum IGF-1 level; serum IGF-1 levels were normal in all patients (100%) in group 2 and 33 patients (80.5%) in group 1, and the difference was considered to be statistically significant (p <0.001). When the groups were compared in terms of serum IGFBP-3 level; serum IGFBP-3 level in Group 2 was found to be significantly lower in Group 1 (p <0.001). Also, when the patients were examined for IGF-1 and IGFBP-3 gene expression, no significant difference was found between the groups (Table 1). A negative correlation was found between leukocyte level and IGF-1 in group 1, and IGF-1 gene expression and CRP in group 2. Serum IGFBP-3 and IL-6 levels were found to be significantly lower in patients without any painful crisis than those with painful crisis in the last year (p <0.05).

Table 1.

	Group 1 (n:41) Median [min.-max.]	Group 2 (n:40) Median [min.-max.]	P Value
Leukocytes (µ/dL)	14,150 [4,6-33,8]	12,9 [6,9-18,2]	0,376
CRP (mg/L)	4,90 [0,20-159,16]	3,03 [0,36-10,36]	0,067
IL-6 (pg/ml)	11,31 [4,35-160,17]	8,12 [0,10-48,32]	**0,001**
IL-1β (pg/ml)	7,89 [5,71-95,65]	6,84 [5,20-30,76]	**0,018**
TNF-α (pg/ml)	10,75 [4,52-45,28]	7,20 [5,44-11,03]	**0,019**
IGF-1 (ng/ml)	215,07 [37,27-751,82]	231,09 [62,66-550,31]	0,334
IGFBP-3 (ng/ml)	2538,02 [1028,82-5625,40]	1762,11 [124,94-3050,49]	**0,010**
IGF-1 Gene Expression	0,18 [0,00-1,19]	0,27 [0,01-3,71]	0,224

Summary/Conclusions: The clinical manifestations of SCD were thought to be associated only with hemoglobin polymerization for a long time. However, recent studies have shown that SCD is a chronic inflammatory disease. The pro-inflammatory cytokines and IGF are in a state of equilibrium in the human body. It has been reported that IGF-1 plays a major role in the production of NO, which is produced in the endothelium and causes a vasodilatory response, and that it increases antioxidant systems and reduces oxidative stress, thereby decreasing inflammation by reducing pro-inflammatory cytokines. In our study, we found that the serum levels of IGF-1, an important growth factor that has not been studied previously in SCD and has recently been evaluated on the effects of inflammation, decreased in SCD patients with painful crisis compared to patients in steady state. It was also found that the levels of inflammatory cytokines, evaluated during the same period, such as IL-6 and TNF-α increased. In conclusion, IGF-1 was thought to play a role especially in the pathogenesis of acute inflammation in SCD.

E1489

UNIVERSAL NEWBORN SCREENING FOR SICKLE CELL DISEASE: PRELIMINARY RESULTS OF THE FIRST YEAR OF A MULTICENTRIC ITALIAN PILOT PROJECT

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Background: Sickle cell disease (SCD) is the most common monogenetic disease worldwide. Although it is most prevalent in Africa, in parts of the eastern Mediterranean and Asia, as a result of migration, the prevalence is also continuously increasing in central and northern Europe. It is established that early detection and appropriate prophylactic measures prevent potentially fatal complications and many European countries have already introduced newborn screening programs for SCD. In Italy it is estimated that 6.5% of the total population is represented by carriers of hemoglobinopathies[1], nevertheless, there isn't a national newborn screening program for SCD nor a plan to establish it. Selective newborn screening programs for SCD are currently active in three regions of Italy, and a pilot universal newborn was terminated due to lack of funding[2]; the benefit of universal newborn screening is still not clear n the Italian context. Since May 2016 a pilot program of universal newborn screening for SCD is active at the birth center of the Department of Women and Child- Health in Padova, which has about 3,500 births a year. In September 2016 the screening program was extended to San Gerardo Hospital in Monza, also located in Northern Italy, with centralization in Padova for samples analyses
Aims: To study the feasibility and efficacy of a universal newborn screening for SCD and to evaluate SCD epidemiology in the areas of Padova and Monza. To identify S gene carriers and affected newborns for a timely taking in charge and to provide genetic counseling for the family. To reduce morbidity and management costs of children with SCD
Methods: Guthrie cards collected after parents' informed consent, from an unselected cohort of newborns, are analyzed by HPLC (Variant Newborn Screening-NBS-BioRad). The abnormal hemoglobin fractions identified by HPLC are confirmed by molecular analysis of the β-globin gene (HBB) by PCR and sequencing of the DNA extracted from the dried blood spot. Genetic counselling is offered to the families of infants carriers of the S gene, after notification of the result; families of all infants with SCD are contacted within two months for enrollment in comprehensive care.
Results: Over a period of nine months and five months 1364 and dried blood spots were collected in Padova and Monza respectively. Two families in Padova and 19 in Monza refused the test. The ethnic origin of newborns was similar in the two sites: 64.08% were children of Italian couples, 8.21% of mixed couples and 27.71% of foreign couples in Padova. And 69.45% were of Italian couples, 9.29% of mixed couples and 21.26% of foreign couples in Monza. None of the collected samples has been excluded from the analysis. The main results are summarized in Table1.

Table 1. Summary of screening results.

PADOVA

Number of newborns	Sex		Hemoglobin pattern						
1364	Male	Female	FA	FAS	FAC	FAE	FAD	FS	FSC
	716	648	1350	**8**	1	0	1	**1**	**1**
	52.50%	47.50%	98.97%	**0.58%**	0.07%	0	0.07%	**0,07%**	**0,07%**

MONZA

Number of newborns	Sex		Hemoglobin pattern					
1077	Male	Female	FA	FAS	FAC	FAE	FAD	FS
	555	522	1060	**1**	4	2	2	**0**
	48.47%	51.53%	98.42%	**0.092%**	0,37%	0.19%	0,19%	**0**

Summary/Conclusions: These preliminary data indicate the feasibility and effectiveness of a multicentric universal newborn screening program in Italy. The incidence of S abnormality is in agreement with the only previous Italian universal newborn screening experience conducted in a single city[2]. Moreover, the results confirm the high incidence of other hemoglobin variants such as HbC, HbD and HbE. The affected children received dedicated medical care from birth. Our preliminary data confirm and support the need for a universal newborn screening that should be extended to the whole country.

References

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E1490

EXTENDING ACCESS TO CARE FOR CHILDREN WITH SICKLE CELL DISEASE THROUGH TELEHEALTH

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Background: Sickle Cell Disease (SCD) is the most common inherited blood disorder in the United States and is highly prevalent in South Carolina. Previous work using administrative databases have shown that 25% of affected individuals live in the more rural PeeDee region and seek acute care from community hospitals. As a result, many of these patients have to travel >90 minutes for routine SCD care. Due to the difficulty in travel, many patients from this region were seen less frequently which made more time-intensive interventions difficult. Hydroxyurea, the only drug FDA-approved to modify the course of SCD, requires monthly laboratory assessments in the firstyear and every 3 months in subsequent years. Thus, in addition to concerns with medication side effects, the frequency of visits limits this option for individuals in rural areas with SCD.
Aims: The primary aim of this project was to evaluate the feasibility and acceptability of using a telehealth clinic to provide SCD care for children living in a designated rural area. The secondary aims were to improve the clinic adherence for patients living in the rural PeeDee region, decrease the burden of care and expense of travel for affected families and improve Hydroxyurea acceptance and uptake.
Methods: The Medical University of South Carolina (MUSC) Center for Telehealth agreed to sponsor the necessary equipment including the video communication system, moveable camera and tele-stethoscope. A regional partner was identified in the target area willing to host the telehealth clinic. Nurses and Advanced Practice Providers were trained in using the equipment and also trained in spleen palpation techniques which cannot be performed using the telehealth system. A local hospital was also identified where routine laboratories can be performed for children seen in the local clinic. Pediatric patients currently seen at the central academic center (MUSC) living in this region were contacted to assess their interest in coming to the telehealth clinic.
Results: The pediatric SCD telehealth clinic was initiated in November, 2014 and data reflects the first 16 months of practice. There were originally 21 patients identified from MUSC of whom 4 families declined interest in participating. Two additional children with known SCD were referred from the local pediatric group for the telehealth clinic who had been designated as "lost to follow up (LTFU)." The clinics were originally scheduled monthly however three clinics were cancelled during the first 16 months and a total of 13 clinics were conducted. There were 64 total visits scheduled of which 50 visits were completed. The overall no-show rate was 14% (range 0-34%) and six clinics had a no-show rate of 0%. The scheduling rate was 78% (range 60-100%). The primary aim was to assess the feasibility and acceptability of a telehealth clinic measured by patients' and families' adherence to scheduled appointments. Of the original cohort of 19 patients, 100% (19) have continued participating in clinic (defined as attending more than 3 clinics in the 16-month period). Prior to starting the telehealth clinic, 10 of the 19 patients had only been seen once in the previous calendar year and 5 of those patients had not been seen in >18 months. Three new patients were started on Hydroxyurea. Two additional referrals to the telehealth clinic were made during the first 13 months (young adults with SCD who had been LTFU for over 3 years). These young adults were seen once by telehealth and then referred to MUSC for the young adult clinic.
Summary/Conclusions: The pediatric SCD telehealth clinic met its primary aim and has continued monthly operations. Hydroxyurea initiation has improved and decreased travel has been welcomed by participating families. Challenges have included equipment issues, difficulties in post-clinic care coordination and assuring caregivers received discharge information. Future directions include a tele-transcranial Doppler program from children with SCD at risk for stroke and additional telehealth clinics for adults with SCD that will be utilized for both routine care as well as acute care through the state sickle cell network, (SC)[2]. This approach will both harness the resources of the state to approach SCD and will also use a technology-based approach to increase education of providers.

E1491

EMERGING NEED FOR SICKLE CELL DISEASE NEWBORN SCREENING PROGRAM IN ITALY, A EUROPEAN COUNTRY WITH INTENSE MIGRATION FLUXES

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Background: The incidence of the Sickle Cell Disease (SCD) has increased in Europe because of the high rate of migration from areas in which carriers of the sickle cell allele account for 19-27% of the entire population. Although SCD is endemic in Southern Italy, the recent migration fluxes spread SCD all over Italy with the number of carriers at about 6.5% of the whole population. The distribution of SCD patients has dramatically changed. The large part of resident immigrants are young with an high fertility rate. Neonatal screening combined with timely diagnostic testing, parental education and comprehensive care markedly reduces morbidity and mortality of SCD. Up to now, a national newborn screening program for SCD is not active in Italy and only few pilot studies have been carried out (Ballardini E *et al.* Blood Transfus. 2013 Apr: 11(2): 245-9.;Venturelli D et al.. Blood Transfusion 2014; 12: 346-51.; Rolla R *et al.* Clin Lab 2014; 60 (12): 2089-93).
Aims: To provide recommendation for newborn screening program for SCD in Italy.
Methods: A panel of experts was identified by Italian Society of Thalassemia and Hemoglobinopaties (SITE) and Italian OncoHematology Pediatric Association (AIEOP).The panel has rigorously revised the literature (from 1990 to 2016), the exhisting recommandations/guidelines of other countries where newborn screen for SCD already exhists. The GRADE system (Grading of Recommendations Assessment, Development and Evaluation) was used to score levels and grades of evidence. The working group produced the draft guideline, and the final version has been revised by external (international) reviewers and the national patients association (UNITED).
Results: The recommendations were divided into in five sections according to the newborn screening program as well as: 1) testing of newborns and specific screening methods, 2) evaluation of screening results for a definitive diagnosis, 3) enrollment of affected newborns in comprehensive care programs, 4) evaluations of the efficacy of follow-up and interventions, and assessment of the benefit to the patient, family, and society. The on line access for recommendations will be available for clinicians and healthcare providers.
Summary/Conclusions: The recommendations for SCD newborn screening program will be an important tool (i) in discussion of strategical new born screening panel at national level; (ii) to early identify patients to be treated in comprehensive SCD centers and (iii) to produce epidemiological data required for future design of SCD map in Europe.

E1492

GENETIC HEMOLYTIC MARKER IN SICKLE CELL ANAEMIA

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Background: The heterogeneity and complexity of the phenotypic profile among individuals with sickle cell anemia (SCA) its one of the principals focus of current research. The SCA, a homozygous condition for Hb S, is a hereditary haemolytic anemia wich severe clinical consequences. The intravascular hemolysis is a chronic clinical subphenotype and has been associated as an independent risk factor related to complications such as pulmonary hypertension, leg ulcer and more recently with progress of vasculopathies. Researches has already shown that the heterogeneity of the hemolytic profile can be due to the presence of different beta S-globin gene cluster haplotypes among the individuals, which suggests the participation of genetic factors in the characterization of this subphenotype. Thus, search for genetic variants has been a promising strategy to assist in the individualization of treatments, and favoring clinical evolution. Recent studies showned that the presence of at least one rs7203560 SNP allele (G) of the NPRL3 gene plays a protective role at hemolysis in individuals with SCA, suggesting this variant as a genetic marker of hemolysis.
Aims: Our objective were to evaluate the association between different genotypes of the SNP rs7203560 and the intravascular hemolysis in patients with SCA.
Methods: We evalueted 76 Brazilian people with SCA, all with a Bantu / Bantu haplotype profile, and in a steady state. The patients were divided into two groups: wich with use or without use of hydroxycarbamide (HC): 22 individuals using (Bantu / Bantu + HC) and 54 without (Bantu / Bantu - HC), respectively. The plasmatic hemoglobin (cell-free hemoglobin - Hb) was measured by enzyme-linked immunosorbent assay (ELISA) to evaluated intravascular hemolysis. The association between categorical variables (with or without use of HC and diferents SNP genotypes) and cell-free Hb levels was performed by univariate covariance analysis (GLM), followed by Fisher's Post Hoc, considering the gender and age covariables. Statistic software was used and assumed p <0.05 as significant
Results: Evaluating the recessive model (GG / GT *versus* TT), we found a significant difference between the different genotypic patterns (p=0.026), and not for HC treatment. Therefore, we performed an analysis to evaluate the participation of SNP in the variation of cell- free Hb levels and hemolysis markers commonly used as hemolysis parameters (relative reticulocytes, the enzymes lactate dehydrogenase and aspartate aminotransferase and unconjugated billirubin), and we found that the individuals genotypic profile was responsible for 50.7% of this variation (Wilk's λ: 0.507; F: 4.08; p-value <0.001), suggesting that the SNP may play a role in characterizing the hemolytic profile of our patients with SCA.
Summary/Conclusions: The SNP here studied is located in the intronic region of the NPRL3 gene, where the main regulatory elements of the alpha globin gene cluster (HS-48, HS-30 and HS-33) are also found. Studies have already suggested that the protective effect of the G allele of the SNP on the hemolytic profile may be probably related to the role of this genetic variant in the expression of the alpha globin genes. Its promissing that aditional analyzes in other ethnic groups and models of hemolytic anemias, such as those of an acquired character are realized. This is one of our next step in the attempt to suggest this variant as a genetic marker capable of assisting in the characterization of the hemolytic and prognostic profile of people with SCA.

E1493

ASSESSMENT OF INTERNATIONAL DAY HOSPITALS/INFUSION UNITS FOR THE EVALUATION AND TREATMENT OF SICKLE CELL DISEASE

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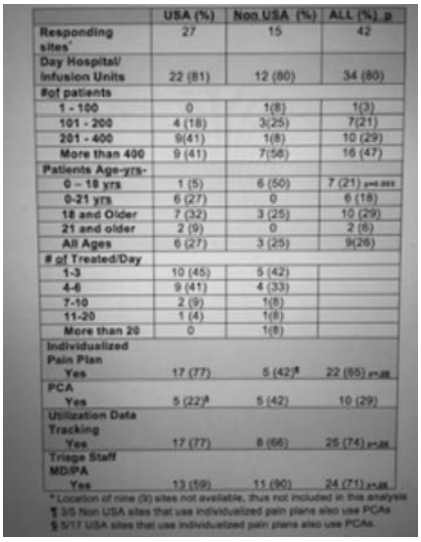
Background: A Sickle Cell Disease (SCD) Day Hospital is defined as a "dedicated facility for the treatment of SCD uncomplicated painful crises, operating on principle-based pain management". SCD Day Hospital/Infusion Units (SCD-DH/IU) play a positive role in improving pain management, preventing emergency room visits, hospitalizations, and readmissions. No study to date has systematically surveyed the availability, organization, diagnostic tools/therapy provided, and number of SCD patients treated at these facilities as well as compared these facilities' practices based on location
Aims: To evaluated and compare availability and characteristics of key SCD-DH/IU components with the overarching goal of enhancing and standardizing across facilities, guidelines and standard of care and help supporting the development of alike outpatient-care units at other health care institutions and countries.
Methods: A Web-based survey was developed and link to the survey sent via email in September 2016 and January 2017, to 120 health care providers (80 in the USA and 40 in other countries) identified via the Global Sickle Cell Disease Network as caring for individuals with SCD. Responses were collected between September 4 and February 10, 2017. Data was analyzed by descriptive statistics and T tests using Graphpad.
Results: Fifty seven surveys were completed (41% response rate) from 51 unique institutions. Responders from the USA sites, 27 (53%) were, mostly, from long-standing sickle cell institutions in the East, West, and South. Non USA sites, 15 (29%) included Canada, Oman, France, Kuwait, and England. Location of nine sites (18%) was not available. Data from only 42 sites showed: 34 (80%) sites reported having a SCD-DH/IU facility. Thirty-one (73%) sites care for 200 or more individuals with SCD, including 17(40%) caring for more than 400 SCD patients. Self-standing units accounted for 30% of SCD-DH/IU, while most (63%) were part of a multi-specialty unit. Only three site operated 24 hours/day, 7 days/week, while 50% of the sites functioned Monday-Friday, 8am-5pm. Half of the SCD-DH/IU sites treat 1-3 SCD patients, 34% treat 4-6 and only 8% treat more than 10 daily. Treatments a vailable at SCD-DH/IU varied among sites. All performed blood tests, but not all were able to provide IV hydration, IV pain management, and blood transfusions. SCD-DH/IU data such as utilization, therapy outcomes, and admissions/readmissions were tracked by 74% of the sites. Only 44% have standard post-discharge/follow-up procedures, ¾ of those were Non-USA sites. Most (69%) sites provide individualized care plans for pain management. Only 29% use Patient Controlled Analgesia (PCA). Most 85% allowed direct hospital admissiol for patients initially evaluated in the SCD-DH/IU. Seven (19%) sites do not have a dedicated provider (MD/PA/NP) available to triage SCD patients presenting to the SCD-DH/IU. Twelve SCD-DH/IU sites have both a SW and a psychologist on site to address patients' psychosocial issues, 21 sites have only a SW, one has only a psychologist, and six neither available. When presented with three different clinical scenarios, sites significantly differed in services availability. Data analyzed based on geographical location, *i.e.* USA *vs* Non-USA showed similar results independently of location; trends for higher protocols use and data tracking in the USA sites; but higher availability of triage medical staff in Non-USA sites. Notably, 50% of Non USA sites, only treated patients 18 years and younger, p=.003.

Table 1.



	USA (%)	Non USA (%)	ALL (%) p
Responding sites*	27	15	42
Day Hospital/ Infusion Units	22 (81)	12 (80)	34 (80)
#of patients			
1 - 100	0	1(8)	1(3)
101 - 200	4 (18)	3(25)	7(21)
201 - 400	9(41)	1(8)	10 (29)
More than 400	9 (41)	7(58)	16 (47)
Patients Age-yrs-			
0 – 18 yrs	1 (5)	6 (50)	7 (21)
0-21 yrs	6 (27)	0	6 (18)
18 and Older	7 (32)	3 (25)	10 (29)
21 and older	2 (9)	0	2 (6)
All Ages	6 (27)	3 (25)	9(26)
# of Treated/Day			
1-3	10 (45)	5 (42)	
4-6	9 (41)	4 (33)	
7-10	2 (9)	1(8)	
11-20	1 (4)	1(8)	
More than 20	0	1(8)	
Individualized Pain Plan Yes	17 (77)	5 (42)§	22 (65)
PCA Yes	5 (22)‡	5 (42)	10 (29)
Utilization Data Tracking Yes	17 (77)	8 (66)	25 (74)
Triage Staff MD/PA Yes	13 (59)	11 (90)	24 (71)

* Location of nine (9) sites not available, thus not included in this analysis
‡ 3/5 Non USA sites that use individualized pain plans also use PCAs
§ 5/17 USA sites that use individualized pain plans also use PCAs

Summary/Conclusions: This is the first study highlighting key healthcare practice data for the small but significant number of SCD Day Hospital/Infusion Units around the globe. Our data suggest that among institutions with SCD-DH/IU there is no consensus regarding clinical practice or data collection. We conclude that there is a significant need to further evaluate SCD DH/IU patient-based value, and to develop operational standards / benchmarks to ensure dissemination, adaptability, and sustainability of these alternative care models

E1494

REDUCED SERUM HAEMOPEXIN LEVELS IN HAEMOGLOBIN SC DISEASE OCCUR INDEPENDENTLY FROM THE DEGREE OF HAEMOLYSIS

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Background: In intravascular haemolysis, saturation of haptoglobin leads to haemoglobin oxidation and the release of free haem, whose main scavenger is haemopexin. In sickle cell mice, excess free haem has been shown to cause vaso-occlusion that can be reversed by haemopexin, implicating that knowledge on how haemolysis changes haemopexin production may influence the applicability of clinical use of haemopexin for sickle cell disease and other haemolytic states. Recent studies have reported reduced haemopexin levels in children with sickle cell disease (Santiago et al., 2016) and adults with beta thalassemia (Vinchi et al., 2016) in association with elevated haem levels, thus suggesting haemopexin decreases due to chronic haemolysis. No data are available in adults with milder sickling disorder haemoglobin SC (HbSC) disease.
Aims: In this study, we examined haemolytic markers, haem, and haemopexin levels in samples from HbSC patients with varying degrees of haemolysis in comparison with healthy subjects with no abnormal haemoglobins (HbAA group).
Methods: Forty HbSC patients (age range 25-68 years, 15 men) and forty HbAA controls (age range 18-66 years, 28 men) participated in this study. Exclusion criteria were pregnancy, other cause of haemolysis, history of blood transfusion or sickle cell pain crisis in the past 3 months. Venous blood samples were collected for complete blood counts (Advia 2120, Siemens) and measurement of lactate dehydrogenase (LDH), bilirubin (Roche Hitachi), haem (Bioassay Systems), and haemopexin (Abcam) levels. Statistical analysis was performed with GraphPad Prism v.5 and data are expressed as mean±standard deviation.
Results: As expected, serum LDH, total and indirect bilirubin, and reticulocyte counts were increased in HbSC patients (*P*=0.0001). Despite this, no significant difference in total circulating haem was found between HbSC and HbAA (39±2.6 *vs* 35±1.8 μM, respectively, *P*=0.30), but haemopexin was surprisingly elevated in HbSC patients when compared to HbAA (15,080±488 *vs* 8,407±684 ng/mL, *P*= 0.0001), contrary to what has been reported in other haemolytic diseases. Haemoglobin (Hb) was higher in the HbAA group when compared to the HbSC group (15±0.2 *vs* 12±0.3 g/dL), and considering World Health Organization definitions of anaemia for men (Hb below 13g/dL) and women (Hb below 12g/dL), 20 (50%) patients in our HbSC cohort were not anaemic, thus fulfilling criteria for compensated haemolysis. HbSC patients with compensated haemolysis were not significantly different from their anaemic counterparts, with similar reticulocyte counts, LDH, bilirubin, haemopexin (9,836±948 *vs* 7,739±813 ng/dL, *P*= 0.10), and total haem levels (33.92±2.4 *vs* 37.55±2.9 μM, *P*=0.30). We also found an unexpected negative correlation between haemoglobin and haemopexin, r=-0.42 (Pearson), *P*=0.007).
Summary/Conclusions: Despite the putative importance of reduced haemopexin in the pathophysiology of sickle cell disease, HbSC patients do not always present with haemopexin deficiency, regardless of the intensity of the haemolytic state, and possibly to due to a lesser importance of intravascular haemolysis. Our data support that non-anaemic HbSC patients may be equally affected by haemolysis, but intravascular haemolysis does not predominantly regulate haemopexin production. We suggest that excessive free haem and low haemopexin probably represent a lesser contribution to the pathophysiology of complications found in this subgroup of sickling disorders.

E1495

ASSOCIATION OF TOLL-LIKE RECEPTOR 2 GENE POLYMORPHISM WITH THE INCIDENCE OF BACTERIAL INFECTIONS IN SICKLE CELL DISEASE

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Background: Despite antimicrobial prophylaxis and immunization, bacterial infection remains a leading cause of morbidity and mortality in sickle cell disease (SCD) patients. Functional hyposplenia/asplenia partially explains their susceptibility, since even young SCD children with functional spleen are at raised infectious risk. Toll-like receptors (TLR), that recognize pathogen molecular patterns, are at the forefront of immune protection. The interaction between TLR and infectious diseases in SCD patients has never been explored.
Aims: To evaluate if functional polymorphisms in TLR confer susceptibility/resistance to infections in SCD.
Methods: 160 SCD patients followed either in France (n=104) or Senegal (n=56) with recorded history of infections were tested for SNPs in TLR-1, TLR-2, TLR-4, TLR-6 and TLR-10 by TaqMan 5'-nuclease assay for their association with infectious history. Comparisons between groups were evaluated by x^2 or Fisher exact T-test with Bonferroni corrections of P-value (Pc); associations were measured by odds ratio (OR).
Results: 76 patients were positive for at least one bacterial infectious episode (IP) and 84 had no infection (NIP). Eleven IP had more than one episode of infection. Median age was 25 years (range 4-49) for IP and 23 years (range 3-52) for NIP with no distribution bias in gender (p=0.24). All patients had vaccinations against *Streptococcus pneumoniae* and *Haemophilus influenza B,* and patients under 10 years had received penicillin prophylaxis. Etiological agent was identified in 58 cases with encapsulated bacteria (EB) occurring in 35; the most common agents consisted of *Mycobacterium tuberculosis*, *Streptococcus pneumoniae*, *Salmonella spp*, *Escherichia coli* and *Klebsiella pneumoniae*. Sites of infection included respiratory tract (n=24), bone and joints (n=21), blood stream (n=17), urinary tract (n=11), central nervous system (n=8) and abdominal (n=5). TLR-2 *rs*4696480 TA genotype was less represented in IP than in NIP [45% *vs* 98%, OR=0.02, 95%CI=0.01-0.09, Pc<0.003] and in particular TLR-2 *rs*4696480 TA genotype was significantly less frequent in the group of patients infected by EB as compared to NIP+IP with other known etiological agents [51% *vs* 85%, OR=0.19, 95%CI=0.08-0.44, Pc<0.003]. Other TLR SNPs, genotype and haplotype showed no significant difference between groups.
Summary/Conclusions: *rs*4696480 TA genotype apparently confers protection against infections especially for EB. Given the previously demonstrated association of AA genotype with exacerbated expression of inflammatory cytokines as well as association of T allele with lower expression of cytokines it is tempting to postulate that TA genotype can be considered as a compromise between deleterious effects of over inflammatory response (TLR-2 AA genotype) and under response (TLR-2 TT genotype) to infectious agents. Such balanced selection effect is probably reflected by the observed deviation from HWE.

Stem cell transplantation - Clinical

E1496

HIGH PROGNOSTIC VALUE OF PRE-SCT MOLECULAR MINIMAL RESIDUAL DISEASE ASSESSMENT BY WT1 GENE EXPRESSION IN AML TRANSPLANTED IN CYTOLOGIC COMPLETE REMISSION

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Aims: We analyzed the outcome of allogeneic Stem Cell Transplantation (allo-SCT) in AML patients according to molecular Minimal Residual Disease (MRD) at the pre transplantation (pre-SCT) workup, assessed by the quantitative expression evaluation of the panleukemic marker Wilms' tumor gene (WT1), according to LeukemiaNET validated method.
Methods: 122 consecutive AML patients received allo-SCT while in cytologic Complete Remission (cCR), between 2005 and 2016, at our Center. The median age at SCT was 53 years (18-70). The quantitative analysis of the WT1 gene expression (bone marrow samples) was available in 100% cases, both at diagnosis (100% overexpressing WT1 with a mean of 8607±8187 copies/10^4 Abelson) and before allo-SCT (81/122-66% MRD-WT1-negative and 41/122-44% MRD-WT1 positive cases at the pre-SCT workup). We evaluated post-SCT Overall Survival (OS), Disease Free Survival (DFS) and Relapse Rate, according to MRD-WT1 pre-SCT status.
Results: Both post-allo-SCT OS and DFS were significantly better in patients who were MRD-WT1 negative (WT1<250 copies) at the time of SCT compared with those who were MRD-WT1 positive (WT1>250 copies), with a median OS and DFS not reached in the MRD-WT1 negative group and 9 and 8 months, respectively, in the MRD-WT1 positive group (OS log-rank p<0.0001; hazard ratio [HR]=3.9, 95% confidence interval [95% CI]=2.0-7.38; DFS log-rank p<0.0001; HR=3.73, 95% CI=2.0-6.72). The relapse rate after allo-SCT was 15% (12/81) in pre-SCT MRD-WT1 negative cases and 44% (18/41) in MRD-WT1 positive cases (p=0.00073). At univariate analysis, MRD-WT1 negativity before allo-SCT and grade <2 acute GVHD were significant prognostic factors for improved OS and DFS. However, at multivariate analysis, MRD-WT1 negativity before allo-SCT was the only independent prognostic factors for improved OS and DFS.
Summary/Conclusions: These data show that pre-allo-SCT molecular MRD evaluation through WT1 expression is a powerful predictor of post-SCT outcome (OS, DFS, relapse rate). Patients with both cCR and a MRD-WT1 negativity before allo-SCT have a very good outcome with a very low relapse rate and better survival. The pre-SCT MRD-WT1 stratification in AML is a valuable tool to identify patients, transplanted in cCR, who are at high risk of relapse and who could be considered for conditioning regimen intensification and/or for post-SCT preemptive strategies (donor lymphocyte infusion, tapering of immunosuppression, azacitidine or new target drugs).

E1497

GOOD IMMUNOLOGICAL RECONSTITUTION IN ADULTS WITH ACUTE LEUKEMIA AFTER ALFA-BETA TCR/CD19+ DEPLETED HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)

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Background: Haplo-HSCT based on the infusion of high numbers of T cell depleted (TCD) hematopoietic progenitor cells and no post-transplant immunosuppression controls both graft rejection and GvHD in patients with acute leukemia. One major remaining issue is the delay in the post-transplant immunological reconstitution because of the minimal residual T lymphocytes in the graft and *in vivo* ATG-linked T cell depletion. Current studies are focussing on rebuilding posttransplant immunity to improve clinical outcomes separating GvHD from favourable donor immune responses. Selective elimination of αβ+ T cells retains in the graft NK, dendritic cells, monocytes and γδT lymphocytes. Under this approach, a rapid immunological reconstitution and very promising outcome have been reported in pediatric patients.
Aims: With the aims of confirming these results in adults, we tested this approach in adults with acute leukemia.
Methods: Thirty-two patients, median age 51 years (range 19-74), with AML (n=27) or ALL (n=5) entered to study. Twenty were in CR (12 CR1; 8 CR≥2), 12 in advanced-stage disease at transplant. Conditioning consisted of ATG 1,5mg/kg from day -13 to -10, Treosulfan 12 gr/sqm from -9 to –7, Fludarabine 30mg/sqm from -6 to -2 and Thiotepa 5mg/Kg on days -5 and -4. PBPCs from haplo-donor (3 mothers, 9 siblings, 13 sons/daughters and 7 cousins) underwent αβTCR/CD19+ depletion by CliniMACS. No post-transplantation immunosuppression was given. Ganciclovir was given over the conditioning regimen in the 22 patients who were CMV seropositive; L-AmB was used as anti-mold active prophylaxis over the neutropenic phase
Results: Grafts contained a median of $11x10^6$/kg (range 5-19) CD34+ cells, $4.3x10^6$ $CD3^+$Tcells/kg (range 1-36), $4.9x10^4$/kg (range 0.4-62) $\alpha\beta^+$T cells, $4x10^6\gamma\delta^+$Tcells/kg(range 1-34), $5x10^4$B cells/kg (range 1.5-32) and $22x10^6CD56^+$NKcells/kg (range 5-91). All patient achieved a full donor sustained engraftment. Median time to reach 500 neutrophils and 20,000 platelets was 13 (range 10-18) and 11 days (range 6-30), respectively. Two patients developed and died from severe acute GVHD. One of them had received the highest dose of αβ+T cells ($3.7x10^5$/kg) and the second one affected by 6GPDH deficiency experienced a late onset hepatic GVHD. Eight patients had skin limited grade II aGVHD that required short course steroids. Only two patients have so far developed mild cGvHD that recovered completely after steroid and cyclosporin treatment. Tending to confirm our working hypothesis, there was a rapid, sustained increase in peripheral blood T-cell subpopulations (Fig. 1). Naïve and memory T-cell subsets increased significantly over the first year after transplantation. B-cell reconstitution was rapid and sustained and immunoglobulin serum levels normalized within 3 months. CMV reactivation occurred in 9 of the 30 patients who were at risk (only 3 had 2 or more CMV reactivations). One with unfavorable serology (donor negative into recipient positive) developed and died of CMV disease 8 months after transplant. Relapse was the main cause of failure (8/12 in relapse, 3/20 in CR). NRM was 15% (4/12 in relapse, 4/20 in CR), 13 patients survive at a median follow-up of 29 months (range 5-53).

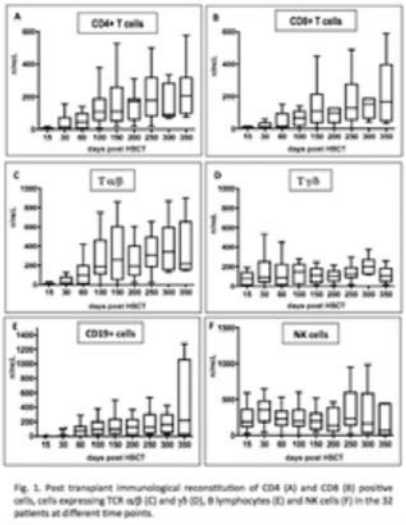

Fig. 1. Post transplant immunological reconstitution of CD4 (A) and CD8 (B) positive cells, cells expressing TCR αβ (C) and γδ (D), B lymphocytes (E) and NK cells (F) in the 32 patients at different time points.

Figure 1.

Summary/Conclusions: The infusion of αβ/CD19-depleted grafts confirmed a fast immunological reconstitution also in adults. Relapse is still a major concern in patients already in relapse at transplantation.

E1498

UNMANIPULATED HAPLOIDENTICAL TRANSPLANTATION CONDITIONING WITH BUSULFAN, CYCLOPHOSPHAMIDE AND ANTI-THYMOGLOBULIN FOR ADULT SEVERE APLASTIC ANEMIA: GOOD OUTCOME AND PROGNOSIS ANALYSIS

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Background: Severe aplastic anemia (SAA) is a life-threatening disorder for which allogeneic hematopoietic stem cell transplantation (HSCT) is the available curative approach. Recently, more and more studies have focused on the feasibility of haplo-identical transplantation in SAA patients because of donor availability.
Aims: To evaluate the outcomes and prognosis of haploidentical hematopoietic stem cell transplantation (HSCT) in adult patients with acquired severe aplastic anemia (SAA), we conducted a retrospective analysis.
Methods: A total of 49 SAA adults received haplo-identical transplantation without *in vitro* T-cell depletion between May 2011 and December 2016. Patients were administered busulfan (BU), cyclophosphamide (CY) and anti-thymoglobulin (ATG) as conditioning regimens and then bone marrow plus peripheral blood transplantation.
Results: The patients' median age was 25 years (ranging from 18 to 45). All of 47 cases surviving for more than 28 days achieved donor myeloid engraftment. The median time for myeloid engraftment was 13 (range, 10-21) days and for platelet was 17.5 (range, 7-101) days with the cumulative incidence of 93.88±0.17%. The cumulative incidence of grade II-IV and III-IV acute graft-versus-host disease (aGVHD) were 20.89±0.35% and 4.17±0.08%, respec-

tively. For patients who survived more than 100 days, the incidence of chronic graft-versus-host disease (cGVHD) were 14.94±0.40% and 27.40±0.77%, and that of extensive cGVHD were 2.57±0.07% and 7.18±0.27% at 1 year and 3 year. With a median follow up of 20.1 (2.1-70.1) months for alive patients, 3-year estimated overall survival (OS) and failure-free survival (FFS) were both 82.5±5.7%. Multivariate analysis showed hematopoietic cell transplantation-specific comorbidity index (HCT-CI) score of ≥3 was significantly associated a worse 3-year survival outcome (86.0% *vs* 50.0%, *P*=0.035, Hazard ratio [95% Confidence interval]: 6.266 [1.139-34.463]).
Summary/Conclusions: Haplo-identical transplantation without *in vitro* T-cell depletion conditioning including BU/CY+ATG is a feasible strategy for adult SAA patients, with successful engraftment, acceptable GVHD, and inspiring survival outcomes. HCT-CI might be an outcome predictor in these patients.

E1499

PLERIXAFOR EFFICIENTLY AND SAFELY MOBILIZES PERIPHERAL BLOOD STEM CELLS: HOVON-107 RESULTS IN HLA-IDENTICAL SIBLING DONORS AND TRANSPLANTED RECIPIENTS

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Background: Plerixafor (PFX) is a reversible inhibitor of stem cell-stroma cell interactions, by interrupting SDF-1 binding to CXCR4. A single subcutaneous (sc) injection results in direct release of hematopoietic stem and progenitor cells (HSPC) with limited side effects and therefore could be of advantage for allogeneic stem cell donors.
Aims: We set out to address the feasibility of sc PFX in family donors and their recipients. Feasibility was defined by the percentage of HLA-matched sibling donors in whom at least 2x 10^6/kg CD34+ cells/kg recipient weight could be harvested after 1 or 2 gifts of PFX (320µg/kg).
Methods: Currently, data of 23 donors and 23 transplanted patients are available, All donors (16 male; 7 female, median age: 47, range: 24-60) received PFX sc 9-11 hours before stem cell collection. The median age in patients was 50 (21-64), diagnoses included: AML/MDS RAEB (n-9), ALL(n-3), MM (n=4), Hodgkin/NHL (n=3), CLL (n=2), other (n=2).Transplant conditioning regimen was non-myeloablative in 17 patients and myeloablative in 6. Grafts obtained after the first gift PFX were analyzed for the total number of CD34+ cells, CD34 subsets: CD34+/CD45RA-/CD90+ and 90- cells, T-cells and distinct CD4+ T cell subsets including regulatory T cells (Treg), Th1, Th2, and Th17 cells. Median cell numbers assessed in 10 G-CSF-mobilized grafts were used as controls.
Results: Criteria for feasibility were met as in 22 out of 23 donors ≥2x 10^6kg CD34+ cells were collected. PFX was administered twice in 10 donors. Side effects CTC grade 2 occurred in 39% of donors and included gastrointestinal (17%), headache or tingling (17%), fatigue/myalgia (17%). CTC grade 3 fatigue was observed in 1 donor; in 2 donors grade 3 clotting occurred occurred during the leukapheresis procedure. All side effects resolved. The median number of CD34+ cells in the graft was 189 x 106 (range:106-548) after PFX *versus* 438 x 106 (range:360-840) with G-CSF. Within the CD34+ cells, 31% CD34+/45-/90+ cells were present after PFX *vs* 14% after G-CSF. With PFX the median number of CD3+ cells in the graft was 22.4 (11-57) x10^9 *versus* 12.8 (7.6-21) x10^9 after G-CSF. CD4/CD8 ratios were similar. The percentages of T-reg within the CD4+ cells were 6.3% (2.7-10.4) after PFX and 5.8% (2.6-10.9) after G-CSF. The Th1 cells within the PFX grafts were 24.2% (3-40.7) *vs* 16.6% (2.5-27.5) with G-CSF. The Th2 and Th17 cells after PFX and G-CSF were, respectively, 2.5% (0.4-7.5) *versus* 2.3% (0.5-4.4) and 1.3% (0.2-25.9) *versus* 1.4% (0.2-5). The unmanipulated grafts were infused according to local protocol and contained a median of 3.5x 106/kg CD34+ cells (range:1.9-6.5). All patients engrafted with prompt recovery of granulocytes >0.5x 10^9L at a median of 19 days (0-27) and platelet recovery >50 x 10^9L at day 13 (0-23). Acute graft *versus* host disease (GVHD) grade 1-4 was observed in 6 out of 23 patients, including 2 with grade 3 and no grade 4. Chronic extensive GVHD was observed in 9 patients.
Summary/Conclusions: Stem cell mobilization by sc PFX is feasible in HLA identical sibling donors with limited side effects and results in sufficient numbers of CD34+ cells for transplantation. While absolute numbers of CD34+ cells were higher after G-CSF mobilization, the subset of phenotypic stem cells was similar after both types of mobilization. No graft failures were observed and all 23 patients showed prompt recovery of neutrophils and platelets. So far, despite the infusion of higher numbers of T-cells, no increase of GVHD was apparent.

E1500

A FEASIBILITY STUDY OF THE FULL OUTPATIENT CONDUCT OF HEMATOPOIETIC TRANSPLANTS IN PERSONS WITH MULTIPLE SCLEROSIS EMPLOYING AUTOLOGOUS NON-CRYOPRESERVED PERIPHERAL BLOOD STEM CELLS

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Background: With the goal of immune system reset, autologous hematopoietic stem cell transplantations have been done in patients with multiple sclerosis (MS).
Aims: With the goal of immune system reset, autologous hematopoietic stem cell transplantations have been done in patients with multiple sclerosis (MS).
Methods: 131 consecutive patients with MS were autografted in a single center using non-frozen peripheral blood stem cells, on an outpatient basis and conditioning with cyclophosphamide (Cy) and rituximab. The protocol was registered in ClinicalTrials.gov identifier NCT02674217. The PBSC mobilization schedule was done with Cy and Filgrastim (G-CSF). Intravenous Cy (50mg/Kg) was delivered on days -11 and -10. Sucutaneous G-CSF (10ug/Kg/BID) was delivered on days -9 to -1. The apheresis procedure was performed on day -2. The apheresis objective was to reach at least 1 x 106 viable CD34+ cells/Kg. As outpatients and after collecting the PBSC, intravenous Cy (50mg/kg) was delivered over a 120 minute period, on days -2 and -1 followed by MESNA (1000mg/m^2 over a 180 minute period). After the intravenous Cy, oral ondasetron, oral cotrimoxazole, oral fluconazole and oral acyclovir were used in all patients until granulocytes were greater than 0.5x109/L. After the recovery of the granulocytes, patients were given rituximab (375mg/m^2 over a 3 h period) and subsequently rituximab (100mg) every two months over a 12-month period. The cumulative dose of Cy is 200mg/Kg.
Results: 80 females and 51 males were included; median age was 47 years. 28 have PPMS, 42 RRMS, and 61 SPMS. All procedures were started on an outpatient basis and two persons were admitted to the hospital during the procedure. In order to obtain at least 1x106/Kg viable CD34 cells, one to four apheresis were performed (median 1). Total number of viable CD34+ cells infused ranged between 1 and 9.6x106/Kg (median 2.2). Patients recovered above 0.5 x109/L absolute granulocytes on median day 9 (range 6 to 12). Two individuals needed red blood cells but none needed platelet transfusions. There were no transplant related deaths and the 125 month overall survival of the patients is 100%. In a subset of 78 persons followed for 3 months or more the EDSS (Expended Disability Status Scale) was assessed three months after the graft and means diminished from 5.2 to 4.9. The EDSS score improved in 33 patients (42.3%), remained stable in 29 (37.17%) and worsened in 16 (20.51%). Best results of EDSS were found in Relapsing Remitting (82%) and Primary Progressive (80%) type of MS compared to Secondary Progressive (71.4%).

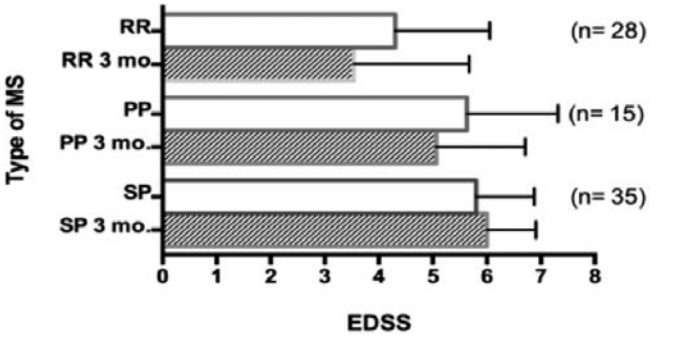

Figure 1.

Summary/Conclusions: It is possible to conduct autotrasplants for patients with MS employing non-frozen peripheral blood stem cells and outpatient conduction. Additional information is needed to assess the efficacy of these procedures in the treatment of patients with MS.

E1501

VEDOLIZUMAB IN STEROID REFRACTORY INTESTINAL GRAFT-VERSUS-HOST DISEASE

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Background: Steroid refractory intestinal graft-versus-host-disease (GvHD) is a serious complication after allogeneic stem cell transplantation (allo-SCT), and treatment options are limited. We have previously described successful treatment of this condition with the antibody vedolizumab, targeting the homing of allogeneic T-cells to the intestinal mucosa by inhibiting the binding of T-cell integrin α4β7 to mucosal addressin MadCAM-1.
Aims: Explore outcome of all patients treated with vedolizumab in our department.
Methods: Prospective case series of 13 patients with steroid refractory gastrointestinal GvHD. Patients received 300mg of intravenous vedolizumab at weeks 0, 2 and 6, followed by infusions every 8 weeks if deemed necessary.

Patients were endoscopically evaluated at time of GvHD diagnosis and follow-up. Treatment characteristics are provided in table 1.

Results: All 13 patients experienced clinical responses, which were confirmed by endoscopies and in mucosal biopsies. 10 patients (77%) achieved a clinical response within 28 days, and half of these were complete responses. At last follow-up 10 patients (77%) had achieved sustained complete responses, 2 patients (15%) had responded partially and 1 patient (8%) suffered disease progression. 7 patients (54%) were alive after a median follow up of 35 weeks. The causes of death were transplantation related toxicity, GvHD in other target organs and infectious complications. Increased relative counts of CD25++ CD127low regulatory T-cells prior to treatment were observed in peripheral blood of 7 of 9 evaluable patients, and the relative counts decreased in all 7 patients during follow-up.

Table 1.

Table 1.

Age, median (range)	50 (18-67)
Time from allo-SCT to intestinal GvHD, median (range)	36 days (9-97)
Time from diagnosis to vedo, median (range)	14 days (6-374)
Intestinal GvHD clinical grade prior to vedo, mean (range)	2.7 (1-4)
Histological GvHD grade prior to vedo, mean (range)	2.9 (1-4)
Doses of vedo, median (range)	3 (1-21)
Observation time, median (range)	35 weeks (9-120)

Summary/Conclusions: Our results indicate that vedolizumab may effectively treat steroid refractory cases of intestinal GvHD and is well tolerated. The mechanism of action is believed to be inhibition of allo-reactive T-cells interacting with intestinal endothelial cells. It is unclear why regulatory T-cells were initially increased in our steroid refractory GvHD patients and subsequently normalized. This might initialle reflect a response to the alloreactive inflammation and subsequent redistribution to affected tissues and/or its resolution after successful treatment.

E1502

RISK FACTORS, OUTCOMES AND CHARACTERIZATION OF 'AUTOLOGOUS GRAFT *VERSUS* HOST DISEASE': THE MAYO CLINIC EXPERIENCE

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Background: Graft *versus* Host Disease (GVHD) is a common complication of allogeneic stem cell transplantation (SCT) which is caused by the recognition of recipient antigens by the donor T lymphocytes. Acute GVHD remains a major cause of morbidity and mortality and half of the cases are refractory to steroids. The development of GVHD after autologous SCT (ASCT) is a poorly understood phenomenon. While some experts suggest that such an entity does not exist, some ASCT recipients develop clinical and histo-pathological changes similar to GVHD after allogeneic SCT.

Aims: In this analysis, we aimed to elucidate the factors that affect the outcomes of patients with autologous GVHD.

Methods: This is a retrospective analysis of patients that received ASCT at Mayo Clinic between January 2006 and December 2015. Autologous GVHD was defined as the development of clinical and histo-pathological findings indicative of GVHD in ASCT recipients, as determined by pathology review. Survival was estimated and compared using the Kaplan Meier and Log Rank tests. The study was approved by the institutional review board.

Results: Between 2006 and 2015, 3,891 consecutive patients underwent ASCT. Of these, 35 patients (0.9%) developed symptoms suggestive of GVHD warranting biopsies. In 19 of these 35 patients (54%), the histopathological changes were consistent with GVHD. The most common underlying disease in patients who developed GVHD was multiple myeloma (14 patients, 73.7%) and the most common conditioning regimen used was melphalan (16 patients, 84.2%). The median age at ASCT was 61.9 (range 49.2-72.6) years and the median time from disease diagnosis to ASCT was 3.1 (0.3-9.6) years. The median number of prior therapeutic regimens was 2 (range 1-7). GVHD manifested with gut involvement in all 19 patients, skin involvement in 8 patients (42.1%) and liver involvement in 2 patients (10.5%). The median time to symptom onset was 11 (range 3-80) days and the median time to GVHD diagnosis was 12 (range 2-162) days. Most patients (14, 73.7%) had grade 3 or 4 GVHD and the clinical grading correlated with the histopathologic grading in all patients. All but one patient received steroids with an average dose of 0.6-2.2mg/kg prednisone equivalents. The median time to symptom resolution was 15 (range 3-162) days and 14 patients (73.7%) achieved a complete resolution of symptoms. The median overall survival (OS) from the time of ASCT was not reached and 53% of patients were alive 3 years after ASCT. Of the 19 patients diagnosed with autologous GVHD, 5 (26.3%) died due to complications of GVHD or its treatment. Delay in initiation of steroids beyond 1 week was associated with lower response rates to treatment (30.8% *vs* 69.2%, p=0.03), longer duration of symptoms of GVHD (median 28 *vs* 4 days, p=0.02), and a trend towards worse 1-year OS (64.5% *vs* 83.3%, p=0.1). Higher steroid doses (>1mg/kg) were associated with a trend towards better complete response rates (76.9% *vs* 23.1%, p=0.5), although this difference did not reach statistical significance.

Table 1.

Table 1 – Baseline Characteristics and Outcomes

	Total (n=19)
Age at ASCT	
Median, y (range)	61.9 (49.2-72.6)
Gender, n (%)	
Male	11 (57.9)
Underlying disease, n (%)	
Multiple myeloma	2 (10.5)
Diffuse Large B-cell Lymphoma	14 (73.7)
AL Amyloidosis	1 (5.3)
POEMS	1 (5.3)
Lymphomatoid granulomatosis	1 (5.3)
Stem cell collection time, n (%)	
>1 year before ASCT	5 (26.3)
Conditioning regimen, n (%)	
Melphalan	16 (84.2)
BEAM	3 (15.8)
Graft source, n (%)	
Peripheral blood	19 (100)
Organ involved by GVHD, n (%)	
Gut	19 (100)
Skin	8 (42.1)
Liver	2 (10.5)
GVHD grading, n (%)	
1	2 (10.5)
2	3 (15.8)
3	8 (42.1)
4	6 (31.6)
GVHD treatment, n (%)	
Steroids	18 (94.7)
Tacrolimus	1 (5.3)
Response to steroids, n (%)	
Yes	14 (73.8)
Time to event, Median, d (range)	
Time to GVHD symptoms onset from ASCT	11 (3-80)
Time to GVHD diagnosis from ASCT	12 (2-164)
Time to steroid initiation from symptoms onset	7 (0-47)
Time to GVHD symptoms resolution from steroid initiation	15 (3-162)
Outcomes	
OS, Median, y (95% CI)	NR (4.6-NR)
12-month OS, %	70%

Summary/Conclusions: Our findings suggest that autologous GVHD is associated with significant mortality and early initiation of treatment with steroids results in improved outcomes. Further studies into the mechanisms of the disease are warranted.

E1503

CNS DEMYELINATION AFTER HAPLO-HSCT AND ITS ASSOCIATION WITH THE IGG INTRATHECAL SYNTHESIS INDEX AND ANTI-MYELIN OLIGODENDROCYTE GLYCOPROTEIN ANTIBODY IN CEREBROSPINAL FLUID

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Background: Haploidentical haemopoietic stem cell transplant (haplo-HSCT) is an upfront and effective therapy for haematological patients, but it usually has many complications such as neurological complications. As one of the neurological complications following haplo-HSCT, immune-mediated demyelinating diseases of the central nervous system (CNS) seriously affect the patient quality of life. However, the incidence, risk factors and pathogenesis of CNS demyelination are not very well understood.

Aims: To analyse the incidence, risk factors, and prognosis of CNS demyelination after haplo-HSCT.

Methods: A study was conducted in 1,526 patients who underwent haplo-HSCT between January 2013 and June 2016. The definition of CNS demyelination during haplo-HSCT was confirmed by neurologic signs, MRI abnormality corresponding to the neurologic signs, abnormal CSF studies and the presence of systemic GVHD or the response to immunosuppressive therapy (Grauer O *et al. Brain*. 2010; 133(10): 2852-2865, *Chronic graft versus host disease*.

Page 243-51, 2009, *Thomas' Hematopoietic Cell Transplantation*. Page 766-75, Fifth Editon, 2016, Polman C H *et al. Ann Neurol*. 2011; 69(2): 292-302). Patients who did not meet these criteria and were determined to have CNS infection (bacterial, fungus, and viruses), neurotoxicity or malignancy relapse, based on clinical and laboratory findings, were excluded. The CSF immunoglobulin index includes BBB permeability, the IgG index, the CSF IgG intrathecal synthesis index, CSF and blood myelin basic protein, CSF and blood anti-myelin basic protein antibody, CSF and blood anti-myelin oligodendrocyte glycoprotein antibody.

Results: Thirty patients developed CNS demyelination after haplo-HSCT. The cumulative incidences of the diseases at 100 days, 1 year and 2 years post transplantation were 0.6%, 1.6% and 2.3%, respectively. The mean age at the time of presentation was 26.5 years (range, 10-52 years), and the mean time from transplant to the onset of neurologic symptoms was 216 days (range, 17-844 days). Nineteen patients received a corticosteroid pulse, five patients received immunoglobulin, and six patients received supportive treatment and an increase in immunosuppressive therapy. The symptoms improved in all patients. The mean duration from the time of improvement to deterioration was 5 days (±4). In univariate analysis, we found that BBB permeability and the CSF IgG intrathecal synthesis index were related to the occurrence of CNS demyelination ($p<0.1$). In multivariate analysis, the CSF IgG intrathecal synthesis index (OR=1.017, 95% CI: 1.003-1.031, p=0.019) and CSF myelin oligodendrocyte antibody (OR=12.059, 95% CI: 1.141-127.458, p=0.038) were independently associated with the onset of CNS demyelination. We also studied the possible pathogenesis of CNS demyelination. Immune reconstitution (the cell proportion of CD19+B cells, CD3+T cells, CD4+T cells), the count of leucocytes, lymphocytes, monocytes and platelets, as well as the level of immunoglobulins A, G and M +30 days, +60 days, and +90 days after HSCT showed no significant differences between CNS demyelination and no demyelination ($P>0.05$). The probabilities of overall survival showed no significant differences between patients with and without demyelination.

Summary/Conclusions: The CSF IgG intrathecal synthesis index and CSF anti-myelin oligodendrocyte glycoprotein antibody are independently risk factors for the onset of CNS demyelination after haplo-HSCT and have no influence on long-term survival. Immune reconstitution may not be pathogenesis of CNS demyelination.

E1504

BASELINE CREATININE CLEARANCE AND ALBUMIN ARE POWERFUL RISK FACTORS FOR ALLOGENEIC TRANSPLANTATION RELATED MORTALITY

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Background: The course following allogeneic hematopoietic stem cell transplantation (HSCT) varies between individuals. Baseline comorbidities, commonly scored by the Hematopoietic Cell Transplantation-Comorbidity Index (HCT-CI), are important determinants of transplant risk. However, their prognostic utility varies and only partially accounts for transplantation-related mortality (TRM). Standard pre-HSCT laboratory carries objective physiologic information which can be used for TRM risk estimation.

Aims: Determine the value of pre-HSCT estimated creatinine clearance (CrCl), albumin, and alkaline phosphatase (Alk-p) for TRM prediction.

Methods: The study population included 1,217 patients from two European centers. Indications for transplantation and conditioning regimens were diverse. Donors were either HLA-matched sibling donors (54%), matched unrelated donors (30%), or 9/10 HLA-mismatched unrelated donors (15%).The impact of CrCl, albumin, and Alk-p on TRM was evaluated in a univariate and multivariate analysis, adjusted for age, HCT-CI, disease status, donor-recipient sex mismatch, donor type, cytomegalovirus serostatus, and conditioning intensity. Relapse was considered a competing event for TRM. The predictive benefit of adding the laboratory markers to the HCT-CI score was estimated by calculating the area under the receiver operating curves (AUC) of TRM prediction models, with and without the markers.

Results: Patients had a median age of 55 years and HCT-CI scores of 0 (24%), 1-2 (39%), and >=3 (37%). A cut-off of CrCl<60 ml/min, albumin<3.5 g/dl, and Alk-p>180 IU/l corresponded with 8.8%, 8.3%, and 6.5% of the population, respectively. CrCl and albumin were associated with increased risk and higher cumulative incidence of day-100, 1-year, and 2-year TRM, regardless of whether they were continuous or categorized (Figure-panel a). A similar pattern was observed with Alk-p, except for day-100 TRM. In a multivariate analysis, a CrCl<60 ml/min and albumin <3.5 g/dl were consistently among the top risk factors for early and late term TRM. Hazard ratios for 2-year TRM of CrCl<60 ml/min and Albumin <3.5 g/dl were 2.00 (1.37-2.95) and 2.329 (1.58-3.43). Interestingly, age did not meet statistical significance in models incorporating these biomarkers, suggesting they strongly reflect patients' physiological status. Alk-p was dropped out in the multivariate analysis. Prediction models for day-100 and 2-years TRM, based only on HCT-CI, had AUCs of 56.4 and 58.6, respectively. The introduction of both albumin and CrCl, separately or combined, resulted in incremental improvement in AUC, topping at 66.1 (+17% increase) and 63.2 (+8% increase), for day-100 and 2-years TRM, respectively (Figure-panel b). The improvement was maintained in all conditioning and donor subgroups.

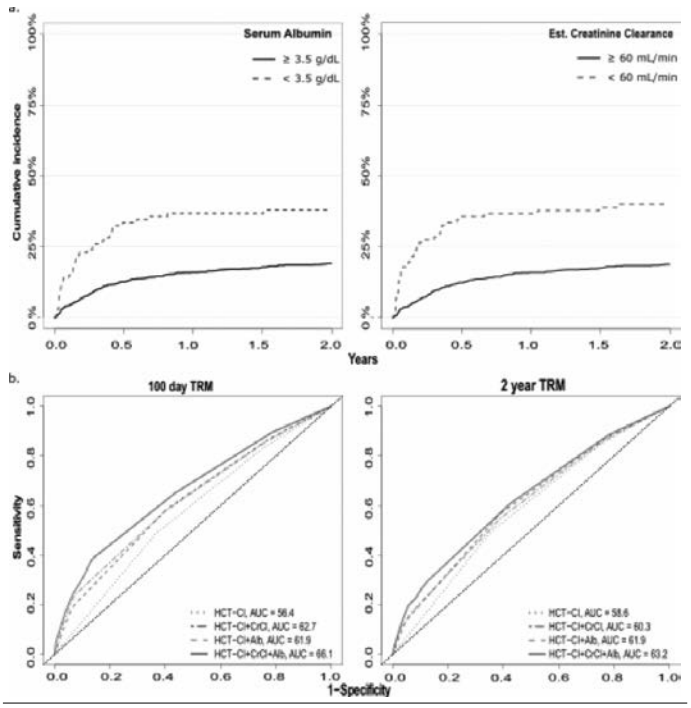

Figure 1.

Summary/Conclusions: Pre-transplantation CrCl and albumin are powerful risk factors for TRM. Deviations from normal ranges were frequent in our cohort, making them useful prognostic markers. We report for the first time the role of CrCl in HSCT prognostication, rather than the traditional HCT-CI cut-off of Creatinine >2mg/dL, which is rare in HSCT population (<1% in our cohort). We also corroborate albumin's important prognostic role. Incorporation of these simple biomarkers can improve pre-transplant risk stratification and potentially be used as a tool for treatment personalization.

E1505

CYTOGENETIC AND MOLECULAR RISK FACTORS AT DIAGNOSIS ARE OVERCOME BY WT1 AND FLOW CYTOMETRY-BASED PRE TRANSPLANT MINIMAL RESIDUAL DISEASE ASSESSMENT IN ADVANCED ACUTE MYELOID LEUKEMIA PATIENTS

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Background: Allogeneic bone marrow transplantation (BMT) offers the only chance of cure for patients with advanced acute myeloid leukemia (AML). High levels of pre BMT minimal residual disease (MRD) have been reported to predict relapse risk in patient transplanted in first complete remission (CR). WT1 expression levels and multicolor flow cytometry (MFC) are the most common tools to evaluate MRD.

Aims: Here, we analyzed the role of pre-BMT MRD assessment as predictor for the post-transplant relapse risk in patient transplanted beyond first CR.

Methods: We retrospectively analyzed the outcome of 92 consecutive AML patients receiving allo-BMT in 2nd (CR2) or 3rdCR (CR3). Pre-BMT MRD was evaluated by WT1 expression and MFC. Median age at transplant was 45 years. Disease phase was CR2 in 63 patients (68%) and CR3 in 29 (32%). Risk group according to European Leukemia Net (ELN) at diagnosis was low in 28 patients (30%), intermediate in 44 (48%) and high in 20 (22%). Sixty-six patients (71%) received myeloablative conditioning, whereas 26 (29%) were conditioned with reduced intensity regimen. Stem cell source was HLA-identical sibling in 18 (20%), haploidentical (HAPLO) in 24 (26%) and alternative donor in 50 (54%). Median follow-up was 64 months (95% CI 39.8-88.2 months). A positive MFC MRD was defined by the presence of at least $1x10^{-3}$ residual leukemic cells at four or eight (since 2011) color flow-cytometry. WT1 copy

number/Abl copy number 250x10^4 was used as cut-off value for abnormal WT1 expression.
Results: Relapse occurred in 30 patients (33%) and two years non-relapse mortality was 29%. Three-year estimate of OS was 47.9% (median19 months). The survival probability was significantly affected by donor source (better for HAPLO, p<0.05), ELN risk at diagnosis (better for ELN low risk, p<0.01), MRD status before BMT measured with any method (p <0.01 for WT1-based MRD, p<0.03 for MFC based MRD) and CR status at BMT (better for CR2, p<0.05). Specifically patients transplanted in a MRD negative status had comparable OS irrespectively of ELN risk at diagnosis (2-years OS of 62.2% and 52.7% among MFC MRD negative patient with ELN risk low or intermediate/high, respectively, Fig.1). The predictive value of MRD resulted independent from all other analyzed variables, although patients with positive MRD undergoing HAPLO BMT had a slightly better outcome. Multivariate OS analysis revealed that MRD status (evaluated by any method) was the only independent predictor of OS (p <0.05 for both). Pre BMT MRD was also a strong predictor of cumulative incidence (CI) of relapse in competitive risk analysis (p<0.01 and p<0.03, respectively, for WT1 and MFC MRD). Multivariate CI of relapse analysis showed that donor source and MRD significantly influenced relapse risk (p <0.05 and <0.01, respectively).

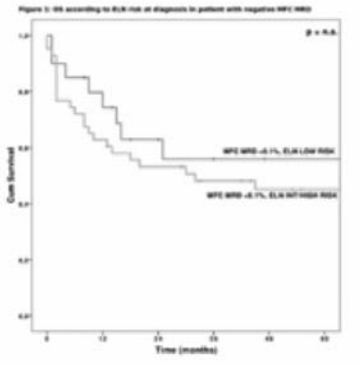

Figure 1.

Summary/Conclusions: Pre transplant MRD evaluated by both WT1 and MFC on bone marrow samples is a reliable predictor of relapse risk and OS which can overcome the ELN risk stratification at diagnosis. Pre BMT MRD negative patients had a significantly better OS, compared with MRD positive ones. MRD positive patients showed an increased risk of relapse, irrespectively of having a low ELN risk at diagnosis. In patients undergoing BMT beyond CR1 pre-BMT MRD status confirms its prognostic relevance and may help in selecting stem cell source. Pre-BMT MRD evaluation may also help in choosing pre-emptive therapeutic strategies.

E1506

IMPACT OF ALLELE SPECIFIC PATIENT:DONOR HLA DISPARITY ON OUTCOME OF REDUCED INTENSITY TRANSPLANTS PERFORMED USING HLA MISMATCHED UNRELATED DONORS: ON BEHALF OF THE ALWP OF THE EBMT

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Background: Allogeneic stem cell transplantation (allo-SCT) represents an increasingly important curative treatment strategy in adults with acute myeloid leukaemia (AML), consequent upon both the increased availability of unrelated donors and the advent of reduced intensity conditioning (RIC) regimens. Although optimal outcomes are achieved in patients transplanted using an unrelated donor matched at 10/10 HLA A, B, C, DRB1, DQ alleles it remains the case that many undergo transplantation using a donor matched at only 9/10 HLA alleles.

Aims: There are limited data concerning the impact of specific HLA mismatches on patient outcome and we therefore interrogated the EBMT database in order to characterize the impact of mismatch on transplant outcome
Methods: 937 patients with AML in CR1 or CR 2 underwent transplantation utilizing a RIC regimen using a 9/10 mismatched unrelated donor between 2001-2015. Of these 264 were transplanted using a donor mismatched at HLA-A, 127 were mismatched at HLA-B, 292 mismatched at HLA-C, 180 mismatched at HLA-DQ and 74 mismatched at HLA- DRb1. 85% of patients received *in vivo* T cell depletion.
Results: The 2 year leukaemia free survival (LFS) for the whole cohort was 45% and the 2 year overall survival (OS) was 50%. The corresponding non-relapse mortality was 26% and relapse incidence 29%. 30% of patients developed Grade 2-4 acute GVHD and 14% chronic extensive GVHD. In Cox analysis age, adverse karyotype and patient CMV seropositivity were correlated with decreased LFS and OS. There was no significant difference in LFS or OS between patients transplanted from donors mismatched at HLA-A, B, C, DRB1 or DRQ respectively. Of note mismatch at HLA-C was correlated with a lower incidence of acute GVHD but this did not translate into a survival difference. Patient:donor CMV mismatch was associated with a marked increase in transplant related mortality and concomitant reduction in LFS and OS in all groups studied.
Summary/Conclusions: To our knowledge this is the largest to date studying the impact of specific HLA mismatch on the outcome of adults undergoing a RIC allograft from an adult unrelated donor. Recipients of HLA-A, B, C, DRB1 and DQ mismatched allografts demonstrated equivalent outcomes. Patient:donor CMV disparity is an important adverse prognostic factor in HLA mismatched transplants. These data have the potential to inform donor selection in allo-mandatory adults with AML undergoing a RIC allograft who lack a 10/10 matched donor.

E1507

PRE-EMPTIVE THERAPY WITH IFN-A-2B FOR ACUTE LEUKEMIA PATIENTS WITH HIGH RISK OF RELAPSING TENDENCY POST ALLO-HSCT

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Background: Relapse still remains the most frequent cause of treatment failure and mortality. According to the data of CIBMTR, relapse has become the leading cause of death following allo-HSCT. The high risk patients with more advanced disease, have a relapse rate of 40–80%.Therefore, prevention and treatment of relapse post allo-HSCT is the most likely approach to improve survival of these patients. It is well known that IFN-α had been widely used in the field of antitumor. Recently it is shown that IFN-α also play an important role in immune modulation to enhance the effect of GVL.
Aims: To determine the efficacy and safety of IFN-α-2b pre-emptive therapy for acute leukemia(AL) patients with relapsing tendencies after allogeneic hematopoietic stem cell transplantation (allo-HSCT).
Methods: Retrospectively analyzed 986 acute leukemia patients undergoing allo-HSCT from Jan ,2006 to Mar ,2014 in our hospital. After allo-HSCT, 986 AL patients were periodically monitored the minimal residual disease(MRD) including: bone marrow smear, leukemia-associated immunophenotype (LAIP), leukemia specific or related fusion genes, and donor chimerism through multi-parameter detection to evaluate disease status. Patients were given IFN- a -2b 3 million units / day by subcutaneous injection for preemptive treatment once a relapse tendency was detected, such as: increasing proportion of blast in bone marrow between 3–5%, or MRD>1.0×10^{-3}, or leukemia specific fusion gene transfrom negative to positive, or dynamic incressing copy number of WT1 more than 200 copies/10^4 abl, or decreasing of donor chimerism(≤ 90%). There were 98 patients who presented increasing tendency of MRD and were enrolled in this study. Among them, 31 patients received IFN-α-2b pre-emptive therapy, and 67 patients received non-IFN-α-2b therapy such as: withdraw immunosupressant, traditional DLI or DC-CIK immunotherapy.
Results: There were no significant differences in disease characteristics between two groups. For the 31 patients who received IFN-α-2b pre-emptive therapy(IFN group), the median time of IFN-atreatment was 60 days (range: 5–720 days), Twenty five patients had responsed to the treatment without progressing to hematological relapse (response rate 80.6%). 2 patients developed to hematological relapse again after temporary response; 3 patients had no response and eventually progressed to hematological relapse. Regarding 67patients who received non-IFN-α-2b therapy(non IFN group), 22 patients responsed to the treatment (RR 32.8%), 45 patients failed to the treatment and progressed to hematological relapse at a median time of 35 (range: 6–940) days, There was significant difference of RR between two group(P=0.000). 31 patients of IFN group tolerate well and no patient terminated therapy due to side effects. During the treatment of IFN, 18 patients(58.1%) developed GVHD: 6 patients 19.4% with aGVHD and 14-45.2% with limited cGVHD. The median follow-up time was 21-4.5-78.5 months. 22 of 31 cases of IFN group maintained disease-free survival. The 5-year overall survival rate (OS) and the leukemia-

free survival rate (LFS) of IFN group were 47.0%±13.9% and 38.7%±13.1% respectively. However, the 5-yr OS and LFS of non IFN group were 14.5%±10.7% and12.5%±9.4% respectively.The difference were significantly (P=0.000,P=0.002 respectively). Patients with GVHD had significantly better response than patients without GVHD (88.9% *vs* 53.8%, P=0.043, P <0.05).
Summary/Conclusions: IFN-α-2b pre-emptive therapy can effectively prevent high-risk patients with relapsing tendencies for disease progression post allo-HSCT. Further large-scale investigation is warranted.

E1508

PREDICTING SURVIVAL AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION. THE GATMO SCORE

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Background: Several attempts to predict mortality after autologous stem cell transplantation (ASCT) have been made, like Hematopoietic Stem Cell Transplant Comorbidity Index (HCT-CI) score, originally described by Sorror for allogeneic HSCT. There is no score applicable to the clinical practice that integrates comorbidities with other patient characteristics.
Aims: To describe a comprehensive score that combines comorbidities with other factors and analyse the impact of this score in OS and NRM after ASCT in a cohort of patients transplanted in Argentina.
Methods: We retrospectively reviewed a cohort of 1453 medical records of adult patients who received an ASCT in our centres between October 2002 and August 2016, for Multiple Myeloma or Lymphoma. We compared NRM and Relapse with CI, OS with KM and long term MVA with fine-Gray or Cox regression. We included in the score all the factors that remained significant after MVA for NRM, and assigned a score of 1 if the Hazard ratio (HR) was around 2 (1.5-2.5) and 2 if it was around 3 (2.6-3.5).
Results: Mean age was 50.7 years (range 15-74); 57% were male, 52% had Multiple Myeloma, 29% Non Hodgkin Lymphoma and 19% Hodgkin Lymphoma. Forty-seven percent were in CR, 50% in PR and 3% SD/PD; 14% received three or more chemotherapy lines before transplant (heavily pre-treated). Regarding comorbidities, 62% had low HCT-CI score (score 0), 26% intermediate risk (1-2) and 12% high risk (≥3). Median follow up was 1.1 years (range 100 days-12 years). Early NRM (day 100) was 2.8%, long term NRM (1-3 years) was 4.3-5.8% and OS (1-5 years) was 89-67%. On multivariate analysis risk factors that showed an independent significant impact with NRM and were included in the score were: male patients (1 point), age ≥55 years (1 point), heavily pre-treated (1 point), HCT-CI ≥3 (1 point) and Non Hodgkin Lymphoma (2 points). One hundred and seventy eight patients (12%) had a score of 0, 469 (32%) 1, 381 (26%) 2, 241 (17%) 3, 137 (9.5%) 4 and 47 (3.5%) ≥5. The hazard ratio for NRM increased proportionally with the score (1 2.2, 2 3.5, 3 4.6, 4 10.1 and ≥5 32.3). Patients were grouped as low risk (LR) with a score 0 (12%), intermediate risk (IR) score 1-3 (75%) and high risk (HR) >3 (13%). The score was significantly associated with early NRM (day 100: 1.1% *vs* 1.9% *vs* 9.2 for LR, IR and HR respectively), long term NRM (1-3 years 1.1-1.1% *vs* 2.9-4.1% *vs* 15-20%, respectively, p<0.001) (figure 1) and OS (1-5 years 93-78% *vs* 91-67% *vs* 73-50% respectively, p<0.001) (figure 2). No significant association was observed with relapse rate.

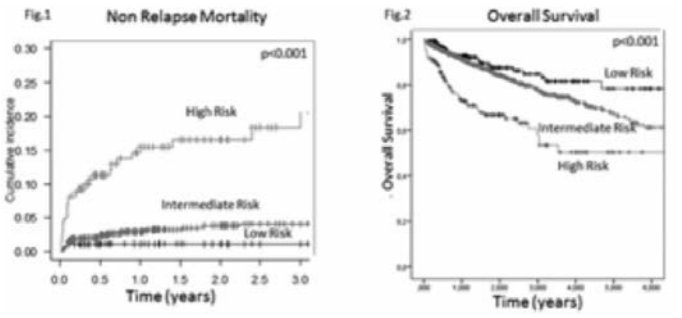

Figure 1.

Summary/Conclusions: We found that GATMO score had a significant association with long term OS due to an increase in NRM. All end-point risks increased proportionally with the score. This observation should be confirmed in larger series.

E1509

A RETROSPECTIVE ANALYSIS OF PATIENT CHARACTERISTICS AND RISK FACTORS FOR ADMISSION TO THE INTENSIVE CARE UNIT (ICU) FOLLOWING HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANT (HDC-ASCT)

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Background: HDC-ASCT is a standard treatment modality for patients with myeloma and lymphoma. It carries a low, but significant risk of morbidity and mortality. Given that the upper age limit for patient selection continues to increase, it is important to have an objective way of assessing patient suitability for HDC-ASCT. Admission to the ICU is an ominous clinical event post HDC-ASCT and carries a high risk of mortality. There are currently no standard assessment tools to predict the risk of morbidity and mortality.
Aims: To review the incidence and cause of ICU admission in patients receiving HDC-ASCT and identify pre-transplant factors that may be predictive of transplant morbidity and mortality.

Table 1.

Patient characteristics	N=169 (%)
Sex, Male/Female	96/73
Median age, Years (Range)	58 (23-74)
Diagnosis	
Plasma cell neoplasm	122 (72)
Non Hodgkin lymphoma	38 (22)
Hodgkin lymphoma	9 (5)
Conditioning regimen	
High dose melphalan 200mg/m2	103 (61)
High dose melphalan 140mg/m2	18 (11)
BEAM (BCNU, cytosine arabinoside, etoposide, Melphalan)	37 (21)
BCNU/Thiotepa	11 (6.5)
First HDC-ASCT transplant	151 (89)
Median cell dose × 10^6 CD34 cells/kg (Range)	4 (2.05-9.5)
Karnofsky performance status	
100	2(1.1)
90	94 (55)
80	57 (33)
70	14 (8.2)
60	1 (0.5)
Not available	1 (0.5)
Charlson comorbidity index	
0	107 (63)
1	36 (21)
2	17 (10)
3	8 (4)
>3	1 (0.5)
Haematopoietic cell transplantation comorbidity index	
0	91 (53)
1	38 (22)
2	14 (8.2)
3	14 (8.2)
4	9 (5.3)
>4	3 (1.7)
Left ventricular ejection fraction	
≥50%	151 (89)
<50%	18 (10)
Body mass index	
18.5-25	42 (24)
25-30	86 (50)
>30	38 (22)
Not available	3 (1.7)
Glomerular filtration rate (ml/minute)	
≥ 50	155 (91)
30-50	6 (3.5)
≤ 30	8 (4.7)

Methods: All patients receiving HDC-ASCT for myeloma and lymphoma at King's College Hospital, London between July 2015 and December 2016 were included. Data cut off was 1st February 2017. Electronic patient records were used to collect data on baseline patient characteristics, comorbidities and performance status. The Charlson comorbidity index (CCI) and haematopoietic cell transplantation comorbidity index (HCTCI) were calculated. Univariate analysis of variables was performed using Graph Pad Prism version 5.03. A p value <0.05 was considered significant.
Results: 169 patients received HDC-ASCT. The median age was 58 years (23-74). Patient characteristics are shown in the table (See Image). Thirteen patients (7.6%) required ICU admission at a median of 14 days post cell infusion (range 5-85), with all patients having a neutrophil count <1x10^9/l. The reasons for ICU admission included sepsis (n=12), severe mucositis/colitis (n=11), renal failure (n=7), hypotension and arrhythmias (n=7), respiratory distress (n=4), liver failure (n=1). The median number of days spent in ICU was 9 (range 2-16). Five patients required single organ support (non-invasive ventilation, 2; inotrope support, 2; haemofiltration; 1) and 2 required only management of

fluid balance. Six patients required multi-organ support (non invasive ventilation/intubation, haemofiltraion and inotropic support) and all died. Four patients died within 30 days of HDC-ASCT and had not engrafted neutrophils at the time of death. Two patients died late at day +120 and day +93 post HDC-ASCT. The latter had both successfully engrafted neutrophils but subsequently became neutropenic. Causes of death were neutropenic sepsis (3), cerebrovascular event (1), hepatitis E (1) and autologous graft *versus* host disease (1). By univariate analysis none of the baseline parameters, comorbidities or conditioning regimens were predictive of ICU admission. The only parameter for which there was a trend for significance was baseline cardiac ejection fraction (EF) <50% (p=0.05). Three patients that required ICU has an EF <50% and 2 were on heart failure medications prior to HDC-ASCT. Two of these 3 patients died.

Summary/Conclusions: In this retrospective series, the risk for ICU admission and death following HDC-ASCT was 7.6% and 3.5% respectively. All patients requiring more than one organ support died. The only predictor of ICU admission may be baseline ejection fraction but this would need confirmation in a larger series. Patient selection remains challenging with no definite tool to predict ICU admission or death.

E1510

AUTOLOGOUS STEM CELL TRANSPLANTATION WITH BENDA-EAM (BENDAMUSTINE, ETOPOSIDE, CYTARABINE, MELPHALAN) IN AGGRESSIVE NON HODGKIN AND HODGKIN´S LYMPHOMA

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Background: Autologous Stem Cell Transplantation (ASCT) is standard of care in relapsed diffuse large B-cell lymphoma (DLBCL) and other lymphoproliferative disorders (relapsed Hodgkin´s disease, 1st line mantle cell lymphoma (MCL) or T-cell lymphoma). BCNU, Etoposide, Ara-C, Melphalan (BEAM) is a standard conditioning regimen, but BCNU is known to be associated with interstitial pneumonia (range 2 to 20%) and an increased risk of death compared with other regimens.
Aims: Therefore a less toxic conditioning protocol might improve the results in lymphoma patients. Bendamustine showed promising results in B- and T-cell lymphoma and dose escalation is safe and feasible. Here we report promising results with bendamustine replacing BCNU in the BEAM regimen described as Benda-EAM, previously published in a phase two dose finding study (Visani, Blood 2011).
Methods: Fourty-one patients with Hodgkin's (HL)(n=9) or Non-Hodgkin (n=32) lymphoma were consecutively treated with Benda-EAM (bendamustine on two consecutive days at a dose of 200mg/m^2 per day). Eleven patients were diagnosed with DLBCL, ten patients with MCL, six patients with follicular lymphoma (FL), three patients with T-cell lymphoma (TCL) and two patients with greyzone lymphoma (GZL). Twenty-seven patients were male and fourteen female with a median age of 52 years (range 22-71) and 25% were above the age of sixty. The median lines of previous therapies were 2 (range:1-4).

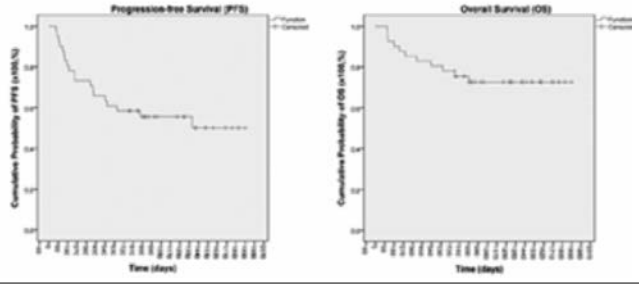

Figure 1.

Results: All patients had chemosensitive disease and before transplantation, 34 patients (83%) were in complete (CR) and 7 (17%) in partial remission (PR). A median number of 4,20*10^{6}CD34+ cells/kg (range: 1,60-13,30) were infused. All patients showed engraftment with a median time to achieve an absolute neutrophil count >1*10^9/L of 10 days (range 8-13) and to platelets >20*10^9/L of 12 days (range 7-110). The median time of fever was 5 days (range: 0-15). The median number of days on G-CSF was 7 (range 4-15) and in median 2 units of red blood cells and 5 units of platelets had to be transfused. The median duration of hospitalization was 25 days.The most common grade 3 and 4 toxicity during the whole treatment period were diarrhea (n=10), mucositis (n=7), infections (n=9) and febrile neutropenia (n=6), followedby nausea (n=4) and cardiologic toxicities (n=3). No severe pulmonary or renal toxicities were observed and no transplant related mortality occurred. After a median follow-up of 43 months 22 patients (56%) are still in CR, while 19 patients (44%) showed progression after a median time of 7 months after transplantation (range 2-29 months). Until today nine patients received an additional allogeneic transplantation. Eleven patients (27%) have died (3 DLBCL, 3 HL, 2 MCL, 1 GZL, 1 TCL and 1 FL), all due to lymphoma progression. Thus the 1- and 2-year PFS are 73.2% and 57.9% and the 1- and 2-year OVS 85.4% and 79.4%, respectively.
Summary/Conclusions: In conclusion Benda-EAM is feasible with a quite promising outcome. Currently an international randomized phase II trial comparing Benda-EAM with BEAM is recruiting. So far fifty-five of 110 planned patients are randomized and first results are expected for 2018.

E1511

THROMBOTIC MICROANGIOPATHY WITH CONCOMITANT AGVHD AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: RISK FACTORS, SEVERE OUTCOME AND TREATMENT EXPERIENCE

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Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT)-associated thrombotic microangiopathy (TA-TMA) is a significant complication after allo-HSCT. acute graft-versus-host disease (aGVHD) is one of the risk factors for the occurrence of TA-TMA, and some patients may develop both. Although there has been sufficient information available on aGVHD and TA-TMA, TMA with concomitant aGVHD after allo-HSCT remains not well understood.
Aims: To explore the possible risk factors for the occurrence and mortality of TMA with concomitant aGVHD and to investigate outcomes and treatments of this disorder after allo-HSCT.
Methods: This study was based on patients who underwent allo-HSCT at Peking University People's Hospital from January 2008 to December 2016. We included patients who showed refractory diarrhea and underwent enteroscopy and biopsy. The diagnosis of TA-TMA and aGVHD were mainly made based on the probable-TMA criteria (Byung-Sik Cho *et al.* Transplantation 2010;90:918-926) and endoscopic appearance and histologic findings (Thomas' Hematopoietic Cell Transplantation, Fifth Edition, 2016), respectively. The potential factors affecting TMA with concomitant aGVHD occurrence and markers associated with the death of these patients were identified using univariate and multivariate Cox analysis. The cumulative incidence of relapse, non-relapse mortality (NRM), overall survival (OS) and disease-free survival (DFS) were estimated using the Kaplan-Meier method and were compared by the log-rank test.
Results: Among all 3,992 allo-HSCT recipients, 276 patients showed refractory diarrhea and underwent enteroscopy; of these patients, 50 (1.93%) were diagnosed with TMA with concomitant aGVHD and were enrolled in the case group, and 150 (5.80%) were enrolled in the control group. The two groups matched well with regard to baseline characteristics. Based on the nested case-based control study, grade III-IV aGVHD (P=0.000), AKI (P=0.033) and hypertension (P=0.028) were significant independent risk factors associated with the occurrence of TMA with concomitant aGVHD. Considering the case group only, our data suggested that a haptoglobin level below normal (P=0.013), a maximum volume of diarrhea □2500 ml/d (P=0.015) and bloody diarrhea (P=0.049) were significant markers for death in both univariate and multivariate analysis. Among the case group and control group, the 9-year OS rates were 52% and 81% (P=0.001), respectively; the 9-year DFS rates were 50% and 65% (P=0.345), respectively; the 9-year cumulative incidence rates of NRM were 44% and 16% (P=0.001), and those of relapse were 6% and 19% (P=0.010), respectively. To further study the treatments of patients with TMA and aGVHD, we calculated the OS and found that plasma exchange (PE) use (PE=0, 62.5%; PE 0, 38.9%; P=0.156) had no significant influence on the patient outcome.
Summary/Conclusions: This study demonstrated that patients diagnosed with TMA with concomitant aGVHD after allo-HSCT had a significantly lower OS, higher NRM, and a lower incidence of relapse. The risk factors associated with the occurrence and mortality of TMA with concomitant aGVHD may help us assess the prognosis of patients. The findings also suggested that PE use may be ineffective to these patients.

E1512

SIGNIFICANCE OF MINIMAL RESIDUAL DISEASE MONITORING BY QUANTITATIVE RT-PCR IN CORE BINDING FACTOR AML ON TRANSPLANTATION OUTCOMES

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Background: Despite the well-defined role of minimal residual disease (MRD) monitoring in core binding factor (CBF)-AML after intensive chemotherapy, there has been, to date, a paucity of data assessing the clinical utility of MRD monitoring before allogeneic stem cell transplantation (HSCT).
Aims: We investigated the prognostic impact of MRD monitoring by real-time quantitative polymerase chain reaction (RT-PCR) for *RUNX1/RUNX1T1* and

CBFB-MYH11 transcript levels at HSCT on transplant outcomes in AML patients with CBF abnormalities.
Methods: We included 61 AML patients with CBF at diagnosis that underwent their first HSCT in complete remission (CR) from January 2007 through May 2016. Of 61, 19 (31%) had t(8;21) chromosomal translocation and 42 (69%) inv(16)/t(16;16). Diseae status at HSCT was CR1 in 19 (31%) and CR2 in 42 (69%) patients. Hematopoietic stem cell sources were peripheral blood (n=32), bone marrow (n=22) and cord blood (n=7). Conditioning regimen was myeloablative in 38 (62%) and reduced intensity in 23 (38%) patients. Donors were matched related (MR) in 24 (38%), matched unrelated (MUD) in 26 (43%), and haploidentical in 4 (7%). Quantitative real-time PCR analysis was performed on reverse-transcribed RNA for the *CBFB-MYH11(Type A)* and *RUNX1/RUNX1T1* fusion transcripts. Fusion (*RUNX1/RUNX1T1* and *CBFB-MYH11)* and internal control (*ABL1)* transcript levels were detected simultaneously and quantitative results were expressed as the percent ratio of fusion to *ABL1* transcript levels ([fusion/*ABL1*]×100).

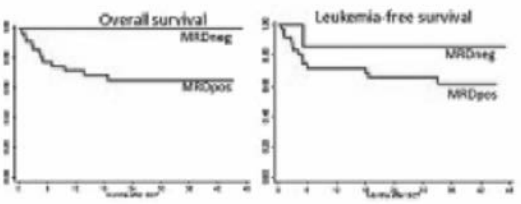

Figure 1.

Results: MRD by RT-PCR at HSCT was evaluable in 43 patients (70%) and 36 of 44 (84%) had evidence of MRD (MRDpos). RT-PCR was <0.1% in 22 patients, ≥0.1% and <1% in 7 and ≥1% in 8 patients. Overall survival (OS) and leukemia free survival (LFS) at 4-years was 100% and 85.7% in 7 MRDneg and 65.4% and 61.6% in 37 MRDpos patients respectively (p=0.09 and p=0.3). The incidence of disease progression was comparable between MRDneg and MRDpos patients, 15% *vs* 16% at 4 years. There was no increase in the risk of progression with higher levels of MRD by RT-PCR (p=0.6). None of the other variables were prognostic for OS, LFS and disease progression.There was no transplant-related mortality observed in MRDneg group while the incidence was 22.6% at 2 years in MRDpos group.
Summary/Conclusions: Durable complete remissions can be achieved in CBF AML patients with HSCT even if they are MRDpos at HSCT.

E1513

LONG-TERM OUTCOME OF ALLOGENEIC STEM CELL TRANSPLANTATION IN ADULT SEVERE APLASTIC ANEMIA WITH ABNORMAL CYTOGENETICS AT DIAGNOSIS

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Background: Cytogenetic abnormalities (CAs) have been reported at the time of diagnosis of acquired aplastic anemia (AA), up to approximately 4-15%. Considering evolution into clonal hematologic disorders and difficulty between AA and hypoplastic MDS, clinical implications of CAs in AA is important.
Aims: In this study, we investigated long-term outcome of allogeneic stem cell transplantation (SCT) in adult severe AA (SAA) patients with abnormal CAs at diagnosis.
Methods: Total of 19 patients with abnormal CAs at diagnosis who underwent allogeneic SCT at our institution between 2003 and 2015. Morphologically hypoplastic bone marrow with dysplastic cells was considered as hypoplastic MDS and excluded. Clonal CAs were defined as 2 or more cells showing the same chromosomal gain or structural abnormality, or 3 or more cells with the same chromosomal loss.
Results: The most frequent abnormality was trisomy 8 (n=11), followed by inversion 9 (n=2). Other CAs included t(3;3), t(5;18), t(1;11), t(1;8), t(1;19), -Y, +Y, -7, +9. Two patients had two or more CAs. Seven male and 12 female patients with a median age of 41 years (range, 20-59 years) were included. Patients had received SCT from HLA-matched sibling (n=12), unrelated (n=5), or haplo-identical donor (n=2). After a median follow-up of 66.3 months (range 12.3-156.3), the 5-year estimated OS rates were 94.7±5.1%. One patient died of acute GVHD. All patients engrafted and three patients developed delayed graft-failure. The incidence of acute GVHD (≥grade II) and chronic GVHD occurred in 4 (21%) and 2 (11%) patients, respectively. Among 16 patients with available follow-up data of cytogenetics after transplantation, 14 patients disappeared CAs with donor-type normal chromosome. However, in two patients, same CAs was observed after transplantation; in one patient with three CAs [+8, +9, and t(1;19)], same CAs was sustained at the most recent follow-up of 23.1 months without morphologically dysplastic cells. In another patient with t(5;18), CA did not detected at 39.2 months but reappeared at 67.5 months, and this CA had disappeared again at 79.6 months. None of patients developed MDS or AML after SCT.
Summary/Conclusions: This study showed that long-term transplant outcomes in SAA patients with CAs at diagnosis were excellent. Moreover, CAs at diagnosis did not affect the clinical outcome including clonal evolution to other hematologic malignancies after SCT in adult SAA.

E1514

PROGNOSTIC VALUE OF PET/CT PRIOR TO AUTOLOGOUS HCT IN RELAPSED / REFRACTORY LYMPHOMA

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Background: Positron Emission Tomography /Computed Tomography (PET/CT) is emerging as a powerful prognostic tool in the management of Hodgkin Lymphoma (HL) and Non-Hodgkin Lymphoma (NHL). A number of retrospective single center cohorts have reported that a positive PET/CT prior to autologous Hematopoietic Stem Cell Transplantation (HCT) is an adverse factor associated with higher relapse risk. However, important heterogeneity is noted in these studies due to differences in timing of PET/CT prior to HCT as well as different metabolic activity threshold (*i.e.* Deauville ≤2 *vs* ≤3). At our institution, we perform PET/CT within 4 weeks prior to HCT and after all intended salvage therapy is administered.
Aims: We sought to further investigate the prognostic value of PET/CT in relapsed / refractory lymphoma patients prior to HCT.
Methods: After due IRB approval, patients who received autologous HCT at our institution for relapsed / refractory lymphoma between 2010 - 2016 were identified. All variables were retrospectively extracted. PET/CT reports were reviewed and metabolic activity was assigned per Deauville criteria. Patients with primary CNS lymphoma were excluded. Refractory disease indicates disease progression prior to completing planned first line therapy. Categorical and continuous variables were compared using Chi-squared and Wilcoxon tests, respectively. Time to end point analysis was computed using the method of Kaplan and Meier with log ranks. Competing events were computed using Grey's method considering non relapse mortality as a competing event for relapse. Analysis was computed using JMP software, version 11.

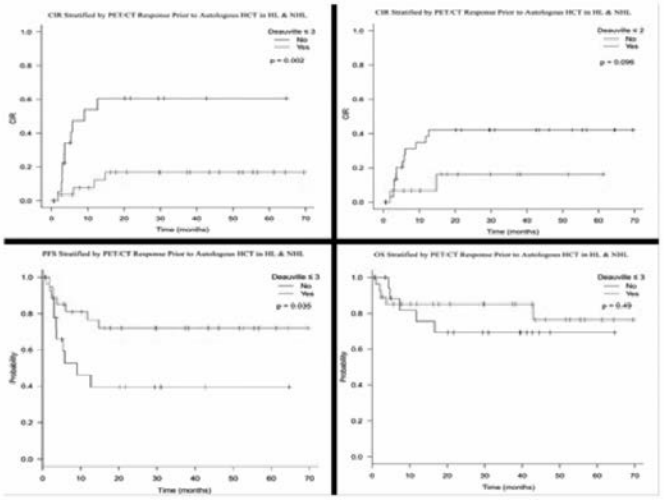

Figure 1.

Results: A total of 53 patients underwent HCT for relapsed / refractory lymphoma with 80% of the cohort having HL. Median follow up of the entire cohort was 26.8 months (0.6-70.5). Cumulative incidence of relapse (CIR), progression free survival (PFS) and overall survival (OS) at 2 years was 37.9%, 56.1% and 74.8%, respectively. A. PET/CT status pre-HCT: A total of 47 patients had pre-HCT PET/CT and were evaluable for further analysis. Median time from PET to HCT was 17 days (6-59). There were no significant differences between the cohorts based on age at HCT, gender, underlying diagnosis, relapsed/refractory status, time to relapse, number of salvage regimens, number of salvage cycles, use of immunotherapy as part of salvage and post HCT immunotherapy use as maintenance. Considering Deauville ≤3 as complete metabolic response (CMR), 2-year CIR was 16.7% *vs* 60.5% for PET negative *vs* PET positive patients (p=0.0021). 2-year PFS was significantly higher in PET negative *vs* PET positive patients at 72% *vs* 39.5%, respectively (p=0.035). 2-year OS was similar irrespective of PET status (p=0.49). Considering Deauville ≤2 as CMR, there was only a trend towards decreased CIR for metabolically negative scans (p=0.096). Significance of these results remained unchanged after excluding NHL cases. B. Relapse post HCT: Median time to relapse post HCT for patients

was 109 days (55-395) *vs* 271 days (55-449) for PET positive *vs* PET negative patients, respectively. Mortality post relapse was 50% with the remaining patients achieving long term disease control with immunotherapy alone (57%), allogeneic HCT (29%) and combination chemotherapy (14%). Median follow up of patients with long term disease control was 1093 days (177-1271). Causes of death post HCT relapse was progression of disease in all cases.
Summary/Conclusions: Despite inherent limitations of this analysis, we present a number of important observations: 1. Deauville score ≤3 is an appropriate cutoff for metabolic activity pre-HCT and is associated with significantly decreased relapse and improved PFS. 2. PET positive status will better identify patients who may benefit from maintenance strategies post HCT. 3. Time to relapse in PET positive patients is significantly shorter highlighting the need for early initiation of pre-emptive maintenance therapy. 4. Long term disease control is possible in a high proportion of patients despite relapse post HCT. These important observations require further study.

E1515

COMPARISON OF OUTCOMES AFTER DONOR LYMPHOCYTE INFUSION WITH OR WITHOUT PRIOR CHEMOTHERAPY FOR MINIMAL RESIDUAL DISEASE AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Minimal residual disease (MRD) can predict impeding relapse after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Thus, MRD-directed immunotherapy may be a reasonable option for relapse prophylaxis. Chemotherapy prior to donor lymphocyte infusion (Chemo-DLI) can further decrease the tumor burden, and immunotherapy should preferably be started in patients with leukemia with relatively low tumor burden. However, some patients who are MRD-positive may refuse or are unable to receive chemotherapy prior to DLI. Few studies have compared the clinical outcomes of Chemo-DLI and DLI alone in patients who were MRD-positive after allo-HSCT.
Aims: The efficacy of DLI without chemotherapy was investigated and compared with that of Chemo-DLI in patients who were MRD-positive after allo-HSCT.
Methods: We enrolled 115 consecutive patients who received either DLI (n=20) or Chemo-DLI (n=95) during the same period. For each DLI recipient, three recipients matched for age at the HSCT, underlying diseases, and the year of the HSCT were randomly selected from the Chemo-DLI cohort (n=60).
Results: The 2-year cumulative incidence of severe acute graft-versus-host disease (GVHD) and chronic GVHD was comparable between the groups. Fifteen (75.0%) and 47 (78.3%) patients in the DLI and Chemo-DLI groups turned MRD negative, respectively. The 2-year cumulative incidences of relapse and non-relapse mortality after intervention were 30.7% *versus* 39.6% (*P*=0.582) and 10.3% *versus* 6.0% (*P*=0.508) in the DLI and Chemo-DLI groups, respectively. The 2-year probabilities of disease-free, overall, and GVHD-free/relapse-free survival after preemptive intervention were 58.9% *versus* 54.3% (*P*=0.862), 69.3% *versus* 78.1% (*P*=0.361), and 44.4% *versus* 35.1% (*P*=0.489) in the DLI and Chemo-DLI groups, respectively. In multivariate analysis, the intervention method did not significantly influence the clinical outcomes.
Summary/Conclusions: In summary, preemptive DLI alone may be effective for patients who are MRD-positive and may be a potential alternative for patients who refuse or are unable to receive Chemo-DLI after HSCT.

E1516

DIFFERENTIAL PROGNOSTIC IMPACT OF HEMATOPOIETIC CELL TRANSPLANTATION SPECIFIC COMORBIDITY INDEX (HCT-CI) ON TRANSPLANT OUTCOMES BY STEM CELL SOURCES

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Background: The hematopoietic cell transplantation specific comorbidity index (HCT-CI) has been proposed to predict the probability of nonrelapse mortality (NRM) and overall survival (OS) in allogeneic hematopoietic stem cell transplantation (HSCT). However, the impact of HCT-CI on clinical outcomes in single unit umbilical cord blood transplantation (UCBT) has not been investigated extensively.
Aims: The purpose of this single-center retrospective study was to investigate the validity of HCT-CI in UCBT.
Methods: We retrospectively analyzed a cohort of 144 consecutive adult patients who received first allogeneic HSCT between July 2008 and December 2016 in our hospital. One patient was excluded from this analysis due to inadequate data regarding comorbidities before HSCT. Patients were divided into the UCBT group (n=90) or the non-UCBT group (n=53). Two-year OS and 1-year NRM were defined as the primary endpoints.
Results: Pre-transplant parameters, such as gender, diagnosis, and the phase of disease, were comparable between the two groups. The median follow-up durations were 562 days and 627 days for the non-UCBT group and the UCBT group, respectively. The most frequent comorbidity was mild hepatic comorbidity (22%), followed by mild or severe pulmonary comorbidities and active infections (16%). For the non-UCBT group, 2-year OS rates for HCT-CI scores of 0, 1-2 and ≥3 were 70% (n=43), 63% (n=30), and 31% (n=17), respectively (P=0.014). For the non-UCBT group, 1-year NRM rates for HCT-CI scores of 0, 1-2 and ≥3 were 10%, 17%, and 35%, respectively (P=0.026). For the UCBT group, 2-year OS rates for HCT-CI scores of 0, 1-2 and ≥3 were 78% (n=26), 46% (n=13), and 69% (n=14), respectively (P=0.38). For the UCBT group, 1-year NRM rates for HCT-CI scores of 0, 1-2 and ≥3 were 9.0%, 15%, and 7.1%, respectively (P=0.75). In multivariate analysis, the HCT-CI score of ≥3 was significantly associated with lower OS (p=0.005; hazard ratio=2.8) and higher NRM (p=0.015; hazard ratio=3.1) for the non-UCBT group, but not for the UCBT group. There was no significant difference in the cumulative incidences of grade 2 to 4 acute GVHD between the non-UCBT group (41%) and the UCBT group (33%, P=0.51). Similarly, there was no significant difference in the cumulative incidences of grade 3 to 4 acute GVHD between the non-UCBT group (6.8%) and the UCBT group (6.1%, P=0.80). The cumulative incidence of extensive chronic GVHD was significantly higher in the non-UCBT group compared with the UCBT group. (38% *vs* 3.8%, P<0.001) Although not significant, patients in the non-UCBT group were more likely to have the systemic steroid therapy compared with those in the UCBT group. (54% *vs* 34%, P=0.064).

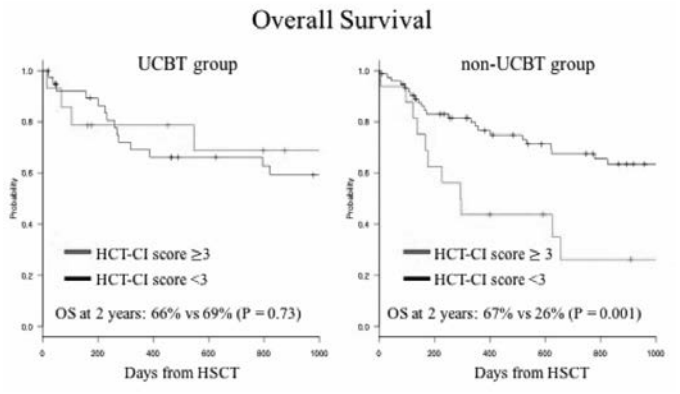

Figure 1.

Summary/Conclusions: UCBT showed good OS with the low incidence of NRM even in patients with high HCT-CI scores. These results indicate that a single unit umbilical cord blood might be a promising stem cell source for patients with multiple comorbidities. Further studies are needed in order to validate these results.

E1517

LOW DOSE POSTTRANSPLANTATION CYCLOPHOSPHAMIDE CAN ENHANCE THE PROTECTIVE EFFECT OF ATG /G-CSF ON GVHD: RESULTS OF A PHASE II PROSPECTIVE TRIAL

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Background: Anti-thymocyte globulin (ATG)/granulocyte colony-stimulating factor (G-CSF)-represented regimen produces essentially universal engraftment with limited relapse and favorable survival, albeit with relatively high rates of graft-versus-host disease (GVHD), especially after HCT from maternal donor or collateral relatives. While use of high-dose, post-transplant cyclophosphamide (PT/Cy) results in low rates of GVHD and favorable immune reconstitution, although with higher rates of relapse and somewhat high rates of graft failure. Thus, novel strategies are needed to refine each approach: under Beijing protocol including ATG and G-CSF, reducing GVHD without abrogating GVL effect is a major priority.
Aims: In order to benefit patients at high risk of developing GVHD without abrogating engraftment and GVL effects, we sought to develop a novel procedure in TCR haplo-HCT with intensified conditioning containing ATG and G-CSF followed by lower-dose of PT/Cy. In addition, the current study attempt to establish a murine model and focus on Treg cells to clarify the immunological mechanisms for GVHD prevention by the new strategy.
Methods: We performed a prospective pilot study of HLA haploidentical cell transplantation (HCT) from maternal or collateral donors with intensified conditioning including G-CSF and ATG, followed by two lower doses of PT/Cy (14.5mg/kgx2 doses; designated as Group A). Outcomes were compared with those of 160 controls from matched-pair analysis who undergone haploidentical HCT from other donors than mother or collateral relatives at the same time period (Group B) as well as with those of 46 historical controls who undergone HCT from mother or collateral relatives at earlier time period (Group C). In addition, the current study attempt to establish a murine model and focus on Treg cells to clarify the immunological mechanisms for GVHD prevention by

the new strategy. Trial registration: The study is registered at www.clinicaltrial.gov as NCT02412423.
Results: We found that low dose PT/Cy combined with ATG could alleviate GVHD in mice and could increase the number of Treg cells while have no effects on CD4+ or CD8+ T cells. A total of 40 patients with myelodysplastic syndrome (MDS) and leukemia undergoing haploidentical HCT from maternal or collateral donors were enrolled in the study. The cumulative, 100-day incidence of acute GVHD, grades II-IV, in Group A (17%; 95% CI, 5%>29%) was significantly lower than both that in Group B (33%; CI, 25%>41%; P=0.04) and that in Group C (56%; CI, 42%>70%; P<0.001). The 1-year probabilities of NRM (5%; CI, 0%>12%), OS (84%; CI, 68%>100%), and LFS (83%; CI, 70%>96%) in Group A were similar to that in Group B, but was significantly lower than that of Group C (28%; CI, 15%>41%; *P*=0.006; 65%; CI, 51%>79%; *P*=0.02; and 65%; CI, 51%>79%; *P*=0.04; respectively).
Summary/Conclusions: Low dose PT/Cy can enhance the protective effect of ATG /G-CSF on GVHD. Conditioning with ATG/G-CSF and low-dose PT/Cy might be a feasible option for patients undergoing HLA haploidentical, T-cell replete HCT, in particular for those with high GVHD risk.

E1518

HEPATITIS B REACTIVATION IN HEMATOPOIETIC STEM CELL TRANSPLANTED PATIENTS: 22 YEARS EXPERIENCE OF A SINGLE CENTRE
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Background: Reactivation of inactive viruses is an important complication of haematopoetic stem cell transplantation (HSCT). Suggestion of strategies to combat this problem will probably decrease transplant related mortality and morbidity.
Aims: Aim of this study is to evaluate the clinical progress and risk factors for reactivation in HSCT patients who were infected with hepatitis B virus (HBV) with the prospect of developing recommendations for a better clinical care.
Methods: Patient files and electronic records of 561 patients who received HSCT between 1994 and 2015 at the Bone Marrow Transplantation Centre of Cerrahpaşa Medical Faculty were retrospectively evaluated. A total of 66 patients with HBsAg (n=15; 12 autologous, 3 allogeneic) and anti HBc IgG positivity (n=51; 29 autologous, 22 allogeneic) were included in the study. Cases were grouped according to transplant types (allogeneic or autologous) and anti-HBS antibody positivity (anti-HBs positive or negative) to calculate relative risks and cumulative incidences of HBV reactivation.
Results: Four (%26) of the 15 patients with HBsAg positivity showed HBV reactivation in an average of 13 months following HSCT. While cumulative incidence of reactivation was 7% at day 60, it went up to 16% and 44% at days 270 and 730 following HSCT, respectively. In Anti HBc IgG positive group, allogeneic HSCT (n=22) was a higher risk factor for reactivation (31.8%) than autologous HSCT (n=29, 6.8%). Relative risk of reactivation in the allo-transplanted patients who were anti-HBc IgG positive and anti-HBs negative was 6,8 when compared to anti-HBs positive patients (n=9, 55% *vs* n=13, 15%) (95% CI: 1,3-46,5). Cumulative incidence of reactivation in anti-HBc IgG positive anti-HBs negative patients (isolated anti HBc IgG positivity) was 11% at day 10 day, 33% at day 133, 50% at day 400 and going up as high as 75% at day 940.
Summary/Conclusions: The results of our study indicate that HBsAg positive patients undergoing autologous or allogeneic HSCT should receive antiviral prophylaxis at least one year posttransplant. Anti-HBc IgG positive patients carry the risk of reverse seroconversion, with receivers of allogeneic HSCT having higher risk than those of autologous HSCT. Patients who are anti-HBc IgG positive and anti-HBs negative should receive prophylaxis for HBV if allogeneic HSCT is to be performed. However, close follow-up seems to be acceptable rather than a prophylactic treatment for anti-HBc IgG positive patients undergoing autologous HSCT.

E1519

ALLOGENEIC HEMATOPOIETIC STEM-CELL TRANSPLANTATION FROM HAPLOIDENTICAL DONOR WITH POST-TRANSPLANT CYCLOPHOSPHAMIDE WAS RELATED TO LESS INPATIENT COST COMPARED TO CORD BLOOD TRANSPLANTATION
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Background: The number of allogeneic HSCT from alternative donors such as cord blood (CB) and haploidentical donor (haplo) is increasing especially after introduction of post-transplant cyclophosphamide (PT/CY) as GvHD prophylaxis for haplo. Although comparison of the survival benefit between CB and haplo with PT/CY has been made by several groups, there is little information about the medical cost and the hospitalization period of HSCT from alternative donors.
Aims: We evaluated the medical costs and the hospitalization period related to allogeneic HSCT in order to clarify the impact of donor sources and other clinical factors on these outcomes.
Methods: Patients (n=134) with hematological malignancies who underwent allogeneic HSCT between January 2013 and December 2016 in University of Tsukuba Hospital were included. The days of the initial hospitalization (from the beginning of the conditioning regimens to discharge), the whole initial inpatient costs and the costs of transfusion during the initial hospitalization was retrospectively analyzed.
Results: The median age of the patients was 46 (range, 16-67) years. The diagnoses were AML (n=66), ALL (n=31), MDS (n=17), lymphoma (n=11), and others (n=9). Twenty-seven patients were transplanted from MRD, 37 from MUD, 22 from haplo with PT/CY, and 48 with single-unit CB. The median initial inpatient cost was €49179 (IQR, 37030-66923), the median transfusion cost was €11500 (IQR, 9500-15250), and the median length of initial hospitalization was 55 (IQR, 44-75) days. CB showed significantly higher inpatient cost (median, €66852) than haplo (median, €49085, *P*=0.008 *vs* CB), MRD (median, €36998, *P*<0.001 *vs* CB), and MUD (median, €39262, *P*<0.001 *vs* CB) (Figure). Also, the transfusion cost was highest in CB (median, €22750) compared with haplo (median, €12866, *P*<0.001 *vs* CB), MRD (median, €12699, *P*<0.001 *vs* CB), and MUD (median, €13118, *P*<0.001 *vs* CB). The median hospitalization days were 67 in CB, 61 in haplo (*P*=1.0 *vs* CB), 46 in MRD (*P*=0.001 *vs* CB), and 49 in MUD (*P*=0.01 *vs* CB). Among the clinical variables such as diagnoses (acute leukemia or others), refined disease-risk index (low/int or high/very high), donor source (MRD, MUD, haplo, or CB), age, first or second HSCT, intensity of conditioning (RIC or MAC), with or without comorbidity, graft failure, GvHD III-IV, and admission to the intensive care unit (ICU), multiple regression models revealed CB (*P*<0.001), haplo (*P*=0.003), graft failure (*P*<0.001), admission to ICU (*P*<0.001), and MAC (*P*=0.05) were the factors that increased the initial inpatient cost. The transfusion cost was increased by CB (*P*<0.001), graft failure (*P*<0.001), admission to ICU (*P*<0.001), and MAC (*P*<0.001). CB (*P*<0.001), haplo (*P*=0.003), and GvHD III-IV (*P*=0.01) were selected as factors associated with longer hospitalization period.

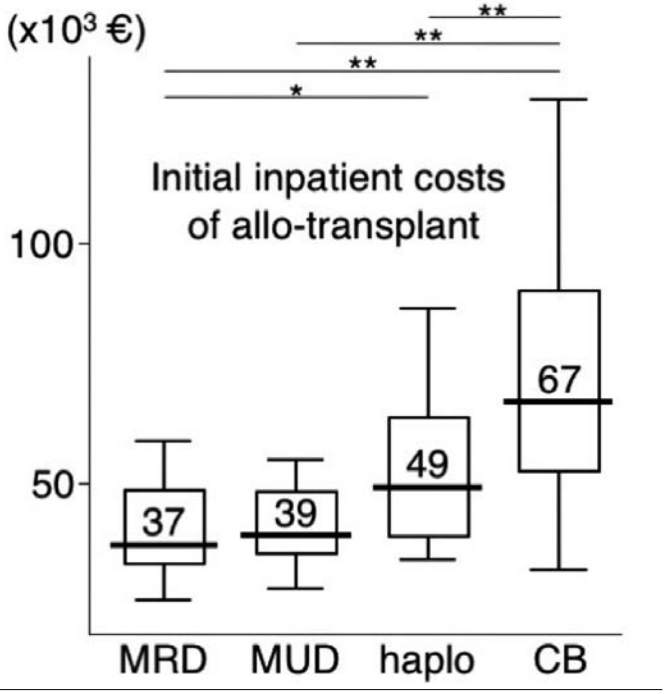

Figure 1.

Summary/Conclusions: Although HSCT from alternative donors was related to the higher initial inpatient cost and longer hospitalization, the impact on those outcomes was more significant in CB than haplo with PT/CY. The higher inpatient cost of CB was partly attributed to delayed hematological recovery which lead to its larger demand for transfusion. The strategy to improve hematological recovery will be needed to reduce the medical cost especially in CB. The larger scale investigation is necessary for better cost-effectiveness in HSCT.

E1520

THE ROLE OF PPARΓ EXPRESSION IN PATIENTS WITH AGVHD FOLLOWING ALLO-HSCT
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Background: The acute graft *versus* host disease (aGVHD) is the main com-

plication after allogenic hematopoietic stem cell transplantation (allo-HSCT). Peroxisome proliferator-activated receptor (PPAR)-gamma (γ), a potent anti-inflammatory agent□is a transcription factor belonging to the nuclear hormone receptor super family which may be participating in aGVHD.
Aims: To explore the role of PPARγin aGVHD after allo-HSCT.
Methods: 65 patients under allo-HSCT and 10 healthy controls were enrolled in study. Peripheral blood (PB) of patients was collected at 15 days, 30 days, 60 days, and 90 days after allo-HSCT. The mRNA expression of PPARγ, IFNγ, T-bet was detected by the real-time PCR. Furthermore, we conducted mixed lymphocyte reaction (MLR) to detect the proliferation of active lymphocytes under different concentration of PPARγ agonist.
Results: Among 65 patients after HSCT, aGVHD occurred in 45 patients. Expression of PPARγ mRNA in healthy controls were significant lower than that in patients after allo-HSCT within 90 days (P<0.05). The expression of PPARγ mRNA hold steady in non-GVHD patients within 90 days after allo-HSCT, and was significantly lower in GVHD group than in non-GVHD group (P<0.05). PPARγ expression in severe aGVHD (grade 3 to 4) was lower than mild aGVHD (grade 1 to 2) patients (P<0.05). The expression of IFNγ and T-bet increased in aGVHD patients and were negatively correlated with PPARγ mRNA expression (P<0.05). The experiment of MLR shows that PPARγ agonist rosiglitazone above concentration of 25μM had dose-dependent inhibition effect to proliferation of lymphocytes.
Summary/Conclusions: Low expression of PPARγ is associated with aGVHD occurrence and degree. PPARγ agonist can inhibit the proliferation of lymphocytes, which may be a new way to treat aGVHD.

E1521

HAPLOIDENTICAL TRANSPLANTATION WITH MYELOABLATIVE CONDITIONING REGIMEN COULD SERVE AS AN OPTIONAL SALVAGE THERAPY FOR YOUNGER PATIENTS WITH REFRACTORY OR RELAPSED NON-HODGKIN LYMPHOMA

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Background: Allogeneic hematopoietic stem-cell transplantation (allo-HSCT) has a well-established role in the treatment of refractory or relapsed (R/R) aggressive non-Hodgkin lymphoma (NHL). However, whether patients with R/R aggressive NHL, in the absence of appropriate HLA-matched donors, can benefit from haploidentical hematopoietic stem cell transplantation (haplo-HSCT) is yet to be elucidated. Herein, we evaluated clinical outcomes of haplo-HSCT in patients with R/R aggressive NHL.
Aims: To evaluated clinical outcomes of haplo-HSCT in patients with R/R aggressive NHL.

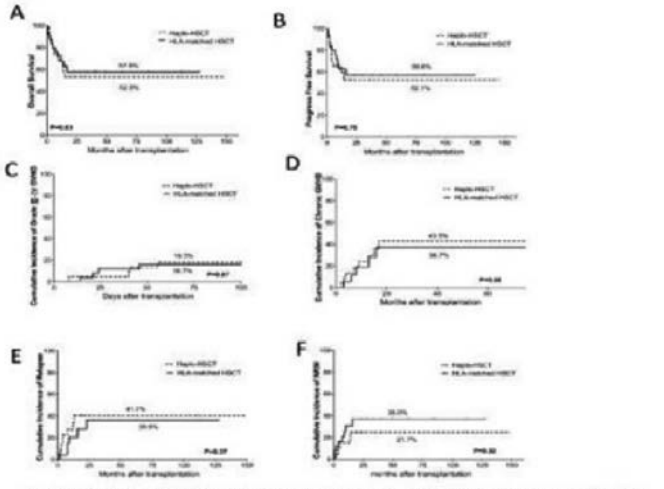

Figure legend: Comparison of outcomes after haplo-SCT and HLA-matched SCT. (a) Overall survival, (b) Progression-free survival, (c) Cumulative incidences of grade III-IV acute GVHD, (d) cumulative incidences of chronic GVHD, (e) cumulative incidences of relapse, (f) cumulative incidences of non-relapse mortality.

Figure 1.

Methods: 23 patients with R/R aggressive NHL who had undergone haplo-HSCT in our center between January 2004 and December 2015 were included, and data were retrospectively analyzed. 25 patients with R/R aggressive NHL who received HLA-matched HSCT during the same period constituted the control group for this analysis. All patients received myeloablative conditioning (MAC) regimen. Antithymocyte globulin (ATG) was administered to prevent graft-versus-host disease (GVHD). The median age of patients who underwent allo-HSCT was 33 years (range, 16-58). Twenty-three patients had received transplant from haploidentical donors, while twenty-five patients received transplant from HLA-matched donors, of which included 13 ISD and 12 MUD. Chemoresistant disease at transplantation was more common in the haplo-HSCT group as compared to that in the HLA-matched HSCT cohort (*P*= 0.005). No significant between-group differences were observed with respect to distribution of age and sex, histological subtype, bone marrow involvement, aaIPI score, chemotherapy regimen and relapse after ASCT.
Results: Median age of patients at allo-HSCT was 33 years (16-58). Over a median follow-up of 23 months, 27 out of the 48 patients (56%) were alive. Progression free survival (PFS) rate at 2-years in the haplo-HSCT and HLA-matched HSCT groups was 52.1% and 56.6%, respectively (P=.75); 2-year-overall survival (OS) rate was 52.8% and 57.8%, respectively (P=.83). Cumulative incidence of relapse (RI) was 41.7% and 35.5% (P=.37), while non-relapse-mortality (NRM) was 21.7% and 35.0%, respectively (P=.32). Collectively, these results showed no significant difference with respect to major allo-HSCT endpoints between the haplo-HSCT and HLA-matched-HSCT groups. On multivariate analyses, older age (> 45 years), primary chemorefractory disease, and occurrence of grade III-IV aGVHD were associated with poor prognosis in both groups. Likewise, the most important factors that influenced the overall survival rate in the haplo-HSCT group were age and occurrence of grade III-IV aGVHD.
Summary/Conclusions: Haplo-HSCT with MAC regimen could serve as an optional salvage therapy, with outcomes comparable to those of HLA-matched HSCT, particularly in younger patients with R/R NHL without appropriate donors.

E1522

OUTCOMES OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH ACUTE MYELOID LEUKEMIA HARBORING INV(3)/(Q21;Q26.2)/T(3;3)(Q21;Q26.2)

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Background: Acute myeloid leukemia (AML) with inv(3)/(q21;q26.2)/t(3;3)(q21;q26.2) [inv(3)/t(3;3)] is categorized as AML with recurrent genetic abnormality in the WHO classification, accounts for approximately 1%-2% of AML, and is characterized by resistance to chemotherapy and poor outcomes. Therefore, the presence of this chromosomal abnormality in AML is an indication for allogeneic hematopoietic stem cell transplantation (allo-HSCT). However, outcomes of AML with inv(3)/t(3;3) remain unclear.
Aims: We retrospectively examined the impact of inv(3)/t(3;3) on the outcomes of allo-HSCT in patients with AML.
Methods: Clinical data were collected from the registry database of the Japan Society for Hematopoietic Cell Transplantation. We selected patients with AML harboring inv(3) /t(3;3), who were aged ≥16 years and underwent their first transplantation between January 2000 and December 2014. We analyzed outcomes such as overall survival (OS), relapse, and nonrelapse mortality (NRM) for the patients underwent allo-HSCT. OS was estimated using the Kaplan-Meier method and compared using the log-rank test. Relapse and NRM were considered as competing risk and were compared using the Gray's test. In a multivariate analysis, the Cox proportional hazard model was used to analyze OS, using the following variables: age, sex, disease status at allo-HSCT, time taken for allo-HSCT from diagnosis, donor source, conditioning regimen, additional monosomy of chromosome 7 or partial depletion of long arm of chromosome 7 and type of 3q abnormality.
Results: Of 15025 patients with AML who were aged ≥16 years and who underwent their first transplantation, inv(3)/(q21;q26.2)/t(3;3)(q21;q26.2) was identified in 66 patients. The median age was 46 years (range, 16-72 years). Of the 66 patients, 10 (15.2%) were in first complete remission (CR1) at allo-HSCT, 54 (81.8%) were in non-CR, and the disease status of two patients was unknown. The probabilities of 2-year OS, relapse, and NRM were 27.8% (95% CI, 16.8-40.0), 64.2% (50.4–75.0), and 21.1% (11.8-32.3), respectively. Multivariate analysis revealed that an age of ≥50 years (HR, 2.05; 95% CI, 1.06-3.99; P =0.03) was significant risk factors for poor OS. Non-CR at transplantation (HR, 2.55; 95% CI, 0.94-6.93; P=0.07), and reduced conditioning intensity

(HR, 2.03; 95% CI, 0.99-4.14; P=0.05) were risk factor with marginal significance for poor OS.
Summary/Conclusions: These findings revealed that AML with inv(3)/t(3;3) had dismal outcome even after allo-HSCT. Multivariate analysis suggested that a myeloablative conditioning regimen might improve the transplantation outcome.

E1523

PHARMACOKINETICS (PK) OF PROPYLENE GLYCOL-FREE MELPHALAN HCL (PG-FREE MEL) IN MULTIPLE MYELOMA (MM) PATIENTS UNDERGOING AUTOLOGOUS TRANSPLANTATION (AHCT)

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Background: Melphalan (MEL) is the most commonly used conditioning agent in AHCT for MM and exhibits a dose response relationship (Nath CE Br J Clin Pharmacol. 2010 May; 69(5):484). PG-free MEL (Evomela^{TM}) has longer stability in solution, results in a slightly higher systemic exposure compared with standard MEL and eliminates propylene glycol administration during high dose melphalan-based conditioning. This agent was shown to be bioequivalent to conventional melphalan leading to successful myeloablation and engraftment in MM pts receiving AHCT with no transplant related mortality or unexpected toxicity leading to its FDA approval (Hari P Biol Blood Marrow Transplant. 2015 Dec; 21(12):2100). Published studies thus far have used PG-free MEL in 2 consecutive daily doses of 100mg/m^2/day while a single daily conditioning dose of 200mg/m^2 (MEL200) is most commonly used in clinical practice.
Aims: Determine the safety and PK variability of high dose PG-free MEL 200mg/m^2 in patients undergoing AHCT for MM
Methods: Open-label phase II study in which 10 serial blood samples at specific time points for the PK evaluation of melphalan were collected immediately prior to and after receiving single 200mg/m^2 dose of PG-free MEL on day -2 as a 2mg/ml solution. The primary objective was a descriptive analysis of melphalan PK while secondary objectives included the response rates, engraftment and the toxicity and safety profile of PG-free MEL conditioning.
Results: As of Feb 2017, a total of 24 pts. were enrolled (63% male) with a median age of 67 years (range 46-72), including 23 (96%) who received upfront AHCT and 1 (4%) after relapse (Figure 1). High-risk cytogenetics was present in 6(25%) pts 25% were in ISS stage 3. Disease status at transplant was complete remission (CR) in 4 (17%), very good partial remission (VGPR) in 12 (50%) and PR in 8 (33%). AHCT was performed entirely as outpatient in 25%. PK data are available for the first 12 pts at this time. Wide variability in MEL exposure was noted with maximum plasma concentration (Cmax) of 10,100 ng/ml, median Cmax 7750ng/ml (range, 5220-10,100) and median area under the concentration- time curve (AUC) of 561500 ng.min/ml (range, 771000-254000). Mean AUC was 549000 (±155000). No grade 4 non-hematologic toxicities or gastrointestinal toxicities were observed including in patients with Cmax >10,000 (upper quartile of distribution) or AUC>625000. All patients are alive and post-transplant responses in those with at least 100 days of follow up indicate sCR/CR in 60% and VGPR in 30%.

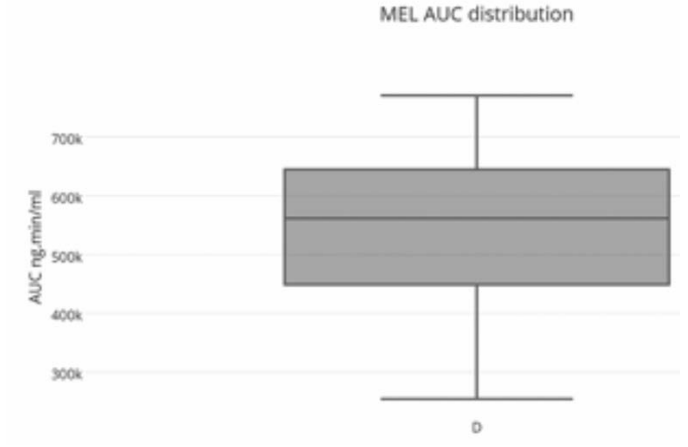

Figure 1.

Summary/Conclusions: PG-Free MEL can be safely administered as a single 200mg/m^2 in conditioning with a favorable toxicity profile. Considerable variability in the PK parameters of high dose MEL indicate that PK directed MEL dosing could be used to optimize MEL exposure. The safety profile of PG-free MEL indicates no increase in mucosal toxicity or adverse events seen even in subjects with highest levels of MEL exposure. For patients in the lowest quartile of AUC, increased PG-free MEL doses up to 20 to 40% over 200mg/m^2 may be safely attempted without additional toxicity if PK directed dosing is used to ensure adequate MEL exposure and utilize the dose response effect of MEL.

E1524

IMPAIRED LYMPHOCYTE RECONSTITUTION AFTER AUTOLOGOUS TRANSPLANT IS ASSOCIATED WITH APOPTOSIS OF CD8+ T CELLS AND PREDICTS ADVERSE CLINICAL OUTCOME

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Background: In patients undergoing autologous stem cell transplantation (ASCT), faster recovery of the lymphocyte counts has been associated with longer disease-free survival (DFS) and longer overall survival (OS). We noticed that the post-transplant lymphocyte counts fluctuated significantly during the first post-transplant weeks and wondered what the clinical significance of this observation is, and what dictates the lymphocyte counts over time.
Aims: Describe the kinetics of post-ASCT lymphocyte reconstitution in a single patient and across patients. Determine whether activation of anti-apoptotic pathways are associated with faster recovery of the lymphocyte counts.
Methods: We reviewed the medical records of 105 consecutive patients with lymphoma (Non-Hodgkin's' lymphoma and Hodgkin disease) or multiple myeloma who underwent ASCT at Tel-Aviv Sourasky Medical Center and were alive 24 weeks after the transplant. In each patient we documented the absolute lymphocyte counts (ALC) starting 2 weeks after the transplant until the 24th post-transplant week. We used flow cytometry to characterize the lymphocyte sub-populations in lymphocytes derived from 20 randomly selected patients, assayed apoptosis by DiO6 binding and used fluorescence anti-MO2 monoclonal antibody to detect the MO2 epitope by flow cytometry. The probability of OS and of DFS was estimated by the Kaplan-Meier method. The log-rank test was used to compare survival distributions.
Results: The ALC was recorded at least once-weekly between the 2nd and 24th post-transplant weeks for each of the 105 study participants. The median ALC during the first 2- 16 weeks was 1.4 X10^3/μL (range: 0.3 to 4.1) and varied considerably in a single patient. After the 16th week, the ALC stabilized and divided the cohort into those with high (n=54, median =1.9 x10^3/μL, range: 1.3 to 3.1) and low (n=51, median=0.9 x10^3/μL, range 0.15 to 1.25) ALCs. Patients with low ALCs were slightly younger, but in all other patient or disease characteristics there were no differences between the two groups. Remarkably, the CD4+ subpopulation was low across all patients, and the difference in ALCs was primarily in the CD8+ subpopulation which remained low in half of the patients and normal or above normal in others. Interestingly, patients with prolonged lymphopenia had higher rates of apoptosis in freshly obtained lymphocytes and the expression levels of MO2, a CD14-derived epitope that protects the cells from apoptosis correlated with lymphocyte counts. Patients with high ALCs during 16-24 post-transplant weeks had longer DFS (P=.07) and OS (P=.04) compared to patients with low ALCs. In a multivariable analysis low ALC at 16 to 24 post-transplant weeks was the strongest predictor for shorter OS.

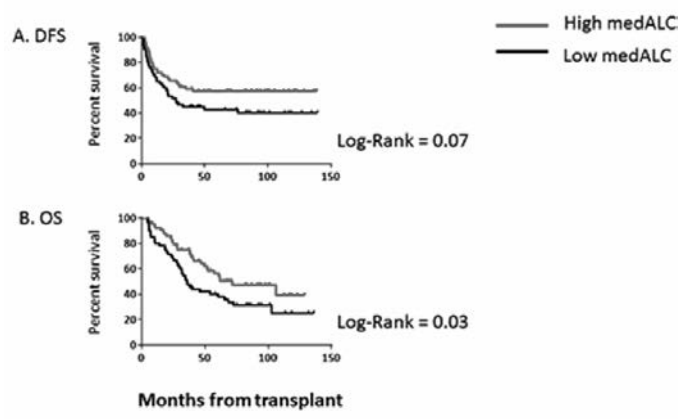

Figure 1.

Summary/Conclusions: The analysis of post-ASCT lymphocyte counts revealed a unique pattern. It fluctuates during the first 4 post-transplant months and stabilizes thereafter, dichotomizing the patients into two groups. In all patients the CD4 subpopulation remained low for at least 6 post-transplant months. However, in half of the patients upregulation of intracellular anti-apoptotic signals was associated with recovery of the CD8+ subpopulation. In the

remaining, both CD4+ and CD8+ subpopulations remained low and these patients were prone to develop relapse. These findings underscore a putative function of CD8+ T-cells in eliminating post-transplant residual disease and maintaining the patients disease free.

E1525

COMPARISON OF TECAM AND BEAM HIGH-DOSE CHEMOTHERAPY FOLLOWED BY AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN LYMPHOMA: EFFICACY AND TOXICITY

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Background: High-dose chemotherapy conditioning regimens followed by autologous hematopoetic stem cell transplantation (AHSCT) generally provide good results in relapsed and refractory lymphomas.
Aims: Limited data are available to guide the choice of conditioning regimen before AHSCT for patients with lymphoma. We evaluated the efficacy and safety of TECAM and BEAM regimens as conditioning with autologous stem cell support in patients with relapsed/refractory lymphomas.
Methods: From July 2011 to October 2016, 64 pathologically confirmed lymphoma patients underwent AHSCT with BEAM (n=32) or TECAM (n=32) regimens in Hematology Division of Ege University Faculty of Medicine. Patients considered as high risk at diagnosis or with relapsed or refractory diseases were eligible for AHSCT. The two groups were well matched in terms of age, gender, histology. Patients were conditioned with TECAM (thiotepa [40mg/m^2 x four days], etoposide [200mg/m^2 x four days], cyclophosphamide [60mg/kg x one day], cytarabine [200mg/m^2 x four days] and melphalan [60mg/m^2 x two days]) or BEAM (carmustine [300mg/m^2 x one day], etoposide [200mg/m^2 x four days], cytarabine [200mg/m^2 x four days], melphalan [140mg/m^2 x one day]) regimens.
Results: The estimated 22-months overall survival for the TECAM and BEAM groups were 53% and 63%, respectively (p=0.41). The estimated 22-months progression-free survival in the BEAM group (59%) was relatively inferior to the TECAM (74%) group, but the differences were not significant (p=0.98). Cardiotoxicities were relatively more common in the BEAM group. No differences were observed in the time to hematopoietic recovery, the duration of hospitalization, hematological and nonhematological toxicities.
Summary/Conclusions: We conducted a single-center retrospective on lymphoma patients undergoing AHSCT, comparing efficacy and toxicity of TECAM and BEAM conditioning regimens. These two regimens are all optional high-dose chemotherapy with favorable efficacy and acceptable toxicity.

E1526

GENETIC MARKERS OF THE NEUTROPENIA DURATION AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH MULTIPLE MYELOMA

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Background: The successes achieved in the treatment of multiple myeloma (MM) in the past few years, associated with the use of high-dose chemotherapy, and with the use of new drugs. Using high-regimes with subsequent autologous hematopoietic stem cells (auto-HSCT) has increased both overall and progression-free survival of patients with MM, as well as improved quality of life. In most cases, patients in the early post-transplant period have severe toxic and infectious complications of varying severity that requires resource-intensive supportive care. The duration of the period of hematopoiesis hypoplasia is dependent on many factors, and an average of 14-16 days. In turn, the attachment of infectious complications in some cases adversely affect the duration of neutropenia.
Aims: To evaluate the possible association of the immune response genes mutation status to the duration of neutropenia after autologous transplantation of peripheral blood stem cells in patients with multiple myeloma.
Methods: The study included 19 patients with multiple myeloma at the age of 32 to 67 years (median - 52 years) who underwent autologous transplantation of hematopoietic stem cells after conditioning regimen with high-dose melphalan. Among surveyed: 8 men and 11 women. In accordance with staging for Durie-Salmon (DSS) system in patients following stages of MM were installed: stage 1A in one patient (5.2%), stage 2A - in 12 patients (63.2%), stage 2B - in two patients (10.5%) and stage 3A - in four patients (21.1%). In the pre-transplantation period partial remission of the disease was achieved in seven patients (36.8%), very good partial remission - in eight patients (42.1%) and complete response in four patients (21.1%). Genotyping of polymorphisms of the innate immune response genes *TLR2* (rs5743708), *TLR3* (rs3775291), *TLR6* (rs5743810), *TLR9* (rs5743836), *IL1β* (rs2856841), *IL2* (rs2069762), *IL4* (rs2243250), *IL6* (rs1800795), *IL10* (rs1800871), *IL17A* (rs2275913), *CD14* (rs34424920), *TNFα* (rs1800629), *FCGR2A* (rs1801274) was performed by polymerase chain reaction with allele-specific primers (Liteh, Russia) at the time of diagnosis.
Results: Depending on the duration of the neutropenia period all examined are divided into two groups. The first group included 10 patients with MM who have early observed recovery (within the first 13 days, 11-13 days), the number of leukocytes ≥1000 cells per ml after auto-HSCT. The second group consisted of nine patients with agranulocytosis held more than two weeks (≥14 days, 14-19 days). When comparing the genotyping data found that a longer period of neutropenia after autologous HSCT was significantly associated with the presence in genotype of MM patients homozygous wild-type allele A gene *IL17A* at position -197 (OR 13.15, 95%CI: 0.60-288.34, *p*=0.03) and with a predominance of heterozygous mutant allele C of the gene *IL1β* at position -31 (OR 8.17, 95%CI: 1.03-67.94, *p*=0.04).
Summary/Conclusions: Our findings point to immune response genes involved in the rate of recovery of hematopoiesis in MM patients after autologous HSCT. Identification of the wild-type allele in intron gene *IL17A (G-197A)* and mutant allele in intron gene *IL1β* (T-31C) will predict the risk of prolonging the period of agranulocytosis and, consequently, the risk of post-transplant complications, and develop a personalized strategy of managing them.

E1527

SUCCESSFUL TREATMENT WITH GRANULOCYTE TRANSFUSION AND EARLY NEUTROPHIL ENGRAFTMENT IN ALLOGENEIC TRANSPLANT PATIENTS WITH FEBRILE NEUTROPENIA

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Background: Febrile Neutropenia is very severe and urgent early complication after bone marrow transplantation before engraftment. Infection delays engraftments. In this study we retrospectively evaluated the effect and outcome of Granulocyte transfusion on febrile neutropenia and neutrophil engraftment in patients receiving allogeneic transplantation.
Aims: Between 2015-2016, five patients receiving allogeneic bone marrow transplantation (BMT) were treated with granulocyte transfusion at the time of febrile neutropenia before engraftment. The reasons for the use of the granulocyte transfusion were prolonged febrile neutropenia episode.

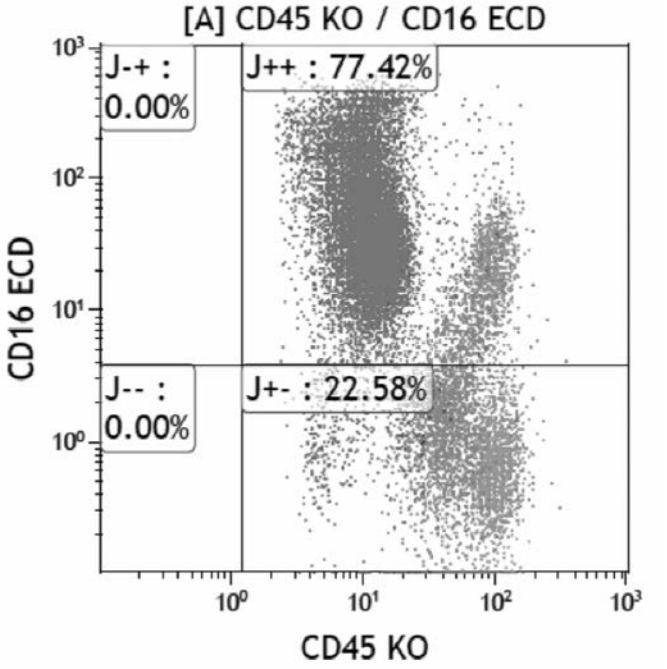

Figure 1.

Methods: Five AML patients underwent allogeneic transplantation. Three of them transplanted from match sibling donors, one from unrelated donor, and one from (7/10) mismatch mother (haploidentic transplant). They had febrile neutropenia after transplantation, before engraftment. They were given antibiotics. Before the granulocyte transfusion, on the 13th-18th days of transplantation, their neutrophil counts were 0.03-0.08x10^3/dl.
Results: We started Granulocyte transfusion for three days. Granulocyte was collected from unrelated and same blood groups donors. Mean infused gran-

ulocyte counts were 3.6x10^10 (1.3-4.6x10^10)/day. Twenty-four hours after granulocyte transfusion, mean neutrophil counts were 0.6x10^3/dl (0.4-0.8x10^3/dl). Neutrophil counts were 2.6 x 10^3/dl, (1.7-2.6x10^3/dl), after 48 hour. After 72 hours, neutrophil counts were 3.4x10^3/dl. (2.1-4.5x10^3/dl). After 4th days of granulocyte transfusion, neutrophil counts were normal levels (>0.5x10^3/dl.).
Summary/Conclusions: Granulocyte transfusions during the febrile neutropenia, helped to better-overcome febrile neutropenia periods in allogeneic transplant patients before engraftment. In addition, granulocytes transfusion also may help early neutrophil engraftments.

E1528

DEFIBROTIDE FOR THE PREVENTION AND TREATMENT OF HEPATIC VENO-OCCLUSIVE DISEASE AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION; A SINGLE CENTER EXPERIENCE

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Background: Hepatic veno-occlusive disease (VOD) is a common and serious complication of hemotopoietic stem cell transplantation (HSCT) in children. We aimed to assess prospectively the use of prophylactic defibrotide in pediatric patients undergoing HSCT.
Aims: We aimed to assess prospectively the use of prophylactic defibrotide in pediatric patients undergoing HSCT.
Methods: In this study, 113 patients who underwent HSCT were given defibrotide prophylaxis as 25mg/kg per day in four divided intravenous infusions over 2h, starting on the same day as the pretransplantation conditioning regimen. The mean duration of use of defibrotide is 25 days as a prophylaxis.
Results: In this study, 113 patients were recruited, 66 male patients and 47 female patients, with the average of 9.1 years, range 1-20; 8% infants, 55% children and 37% adolescent. There were 50 patients with thalassemia major, 41 patients with leukemia, 11 patients with aplastic anemia, one patient with Diamond Blackfan anemia, two patients with congenitale dyserythropoetic anemia, one patient with osteopetrosis, four patients with famial hemophagocytic lymphohistiocytosis, two patienrs with severe immune deficiency and one patient with Kostman syndrome. All transplants were allogeneic. No serious side effects were seen. In eight patients developed clinical VOD (Seattle criteria). In these patients, defibrotide dose was increased to a treatment dose of 40-60mg/kg per day. One infant patient with Kostman syndrome died due to hepatic and pulmonary veno-occlusive disease. After 36 months of follow up, 7 patients who developed VOD are being well and no patient have transplant related complications.
Summary/Conclusions: Hepatic veno-occlusive disease, which is caused by hepatocyte and sinusoidal vessel endothelium damage, can ocur early after HSCT, and in its severe form, may lead tol iver faillure, hepatorenal syndrom, portal hypertension, and eventually death from multiorgan faillure. In this prospective study, we demonstrated that the use of defibrotide is safe and effective in preventing and treating VOD in pediatric patients at high risk.

E1529

ACUTE RENAL IMPAIRMENT IN ALLOGENEIC STEM CELL TRANSPLANT RECIPIENTS, A PREDICTOR OF MORTALITY

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Background: Allogeneic stem cell transplant (ASCT) remains the only curative option in many malignant and non-malignant conditions. There remains however a risk of significant morbidity and mortality. One risk, acute kidney injury (AKI), can result from drug toxicity and/or haemodynamic instability from sepsis and/or graft *vs* host disease (GvHD). Existing reports on the impact of AKI have concentrated on patients undergoing mainly myeloablative (MA) conditioning alone, whilst those undergoing reduced intensity conditioning (RIC) transplants have reported outcomes from limited patient numbers.
Aims: To investigate the incidence, causes and consequences of AKI in patients undergoing ASCT, including survival.
Methods: The prospectively maintained database of the South Wales Blood and Marrow Transplant programme which serves 77% of the Welsh population, was interrogated to identify patients undergoing ASCT from January 2010 to December 2015. Patients received ciclosporin as GvHD prophylaxis to 100 days post ASCT and weaned thereafter in the absence of GvHD. Serum creatinine and derived estimated glomerular filtration rate (eGFR) acted as the main assessment of renal function. The Acute Kidney Injury Network classification was used to grade AKI. Causes of AKI were assigned after independent review of clinical notes and relevant laboratory data. Patients undergoing second ASCT were excluded. Statistical analysis was carried out using SPSS, version 23 including COX regression and Kaplan-Meier survival analysis.
Results: A total of 229 patients were identified (MA-n=35, 15%; RIC-n=194, 85%). Acute myeloid leukaemia was the most common indication (n=103, 45%). Mean age at ASCT was 51 years (18-72 years). Median follow up after ASCT was 2.19 years (range 9 days-6.6 years). Overall survival to 100 and 365 days was 93% and 74% respectively. Pre-existing renal impairment was uncommon (mean eGFR 92ml/min, range 45-143ml/min). During the first 100 days, no difference was seen in mean eGFR in survival *vs* non-survival groups (75 and 80ml/min respectively, p=0.23). Amongst all patients, AKI incidence in the first 100 days was greater in the non-survival group (93.2% *vs* 80.6%, p=0.02). On multivariate analysis, AKI event in the first 100 days and HLA mismatch (<8/8) were independent factors predicting mortality (p=0.02 and p=0.04 respectively). Recipient age and gender, ASCT indication, history of hypertension, CMV status, donor sex, stem cell source and conditioning regimen (MA *vs* RIC) were not statistically significant (p>0.05). Within the first year of ASCT, pre-terminal AKI was noted in 29% (n=23) of all patients dying (n=59) with sepsis accounting for the major non-relapse cause of death (n=15). Of the patients alive, only 11 (8%) had chronic renal impairment. Chronic GvHD was associated with these patients (73%) one of whom was dialysis dependent.
Summary/Conclusions: AKI is very common post ASCT. Chronic renal failure is uncommon in long-term survivors. AKI is however a prominent event preceding death. Consistent with other reports AKI and HLA mismatch conferred inferior outcomes. Poor survival from AKI probably reflects physiological strain from other complications (e.g. sepsis and GvHD). Early recognition and treatment of AKI are important measures in the supportive care of patients with AKI.

E1530

PREDICTIVE INDEXES FOR ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION, A SINGLE-CENTER EXPERIENCE

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Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is often associated with complications such as graft-versus-host disease (GVHD), resulting in poor outcome, relapse and death. Introduction of reduced intensity conditioning (RIC) regimens and improvements in supportive care, have allowed offering allo-HSCT to more and older patients (pts). A balanced risk-benefit approach of candidates for allo-HSCT is the key for maximized chances of cure with acceptable quality of life.
Aims: Compare the potential utility of two pretransplant predictive models: PAM (pretransplant assessment of mortality; Parimon et al, AIM 2006) and HCT-CI (HCT comorbidity index; Sorror et at, Blood 2005), in our cohort of pts.
Methods: We retrospectively studied 154 pts, 86 (55.8%) were males with a median age of 51 years (range: 15-68), who underwent allo-HSCT in our center between May 2005 and December 2014. Patients' baseline diseases were: acute myeloblastic leukemia (24%), multiple myeloma (22.7%), non Hodgkin lymphoma (11.7%), acute lymphoblastic leukemia (11%), myelodisplasic syndrome (9.1%), chronic lymphocytic leukemia (5.2%), Hodgkin lymphoma (3.9%), aplastic anemia (3.9%), myelofibrosis (3.9%), chronic myeloid leukemia (1.3%), Waldenström macroglobulinemia (1.3%) and others (1.9%). Eighty (51.9%) pts received cells frommatched siblings, seventy (45.5%) from unrelated donor and the remainder haploidentical-HSCT (2.6%). Only 43 (27.9%) pts received RIC regimens. Stem cell source were: peripheral blood (n=86), bone marrow (n=63) and umbilical cord (n=5). Median and maximum follow-up were 31 and 228 months, respectively.

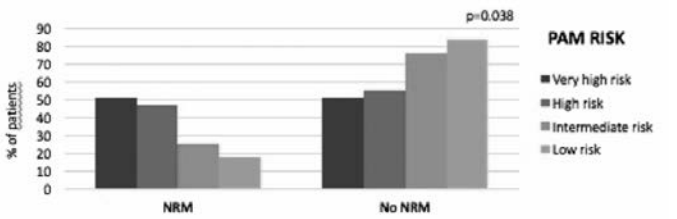

Figure 1.

Results: After allografting, 57.1% pts had complications, the most frequent were: infections (45.5%), followed by nephrotoxicity (25.3%), hepatotoxicity (12.3%), pulmonary toxicityes (9.7%) and cardiotoxicity (3.9%). Eighty-two pertent of pts with high/very high risk group of PAM score presented complications *versus* 46% of pts included in low/intermediate risk (p<0.001). Regarding GVHD, 41.6% and 31.2% of pts developed aGVHD (grades II to IV) and cGVHD, respectively. PAM score was a good predictor for aGVHD risk: 38.1% of pts with low/intermediate risk had aGVHD *versus* 59.3% of pts with high/very high risk (p=0.043). Non-relapse mortality (NRM) was 26%. Causes of NRM included infections (45.8%), hemorrhage (10%), pulmonary toxicities (16%), second neoplasia (14.6%), GVHD (6.25%), cardiotoxicity (2%) and hepatic toxicity (2%). PAM score effectively risk-stratified pts for NRM: 17%, 24.7%, 45.8%, and 50%

in the low, intermediate, high and very high risk groups, respectively, showing a clear distinction by categories (p=0.038) (figure 1). Refarding relase, 44 (28.6%) pts relapsed. Neither PAM nor HCT-CI were good predictors for relapse. However, HCT-CI was not good predicting complications, GVHD, NRM or relapse.
Summary/Conclusions: In our series of pts, risk-groups based on PAM score provided much better discrimination of post-HSCT complications, aGVHD (II-IV) and NRM than HCT-CI model. None of the indixes were acceptable predictors of relapse. Furthermore, correlation between both indexes was poor.

E1531

ROLE AND TIMING OF HEMATOPOIETIC CELL TRANSPLANTATION FOR HIGH-RISK PERIPHERAL T-CELL LYMPHOMAS

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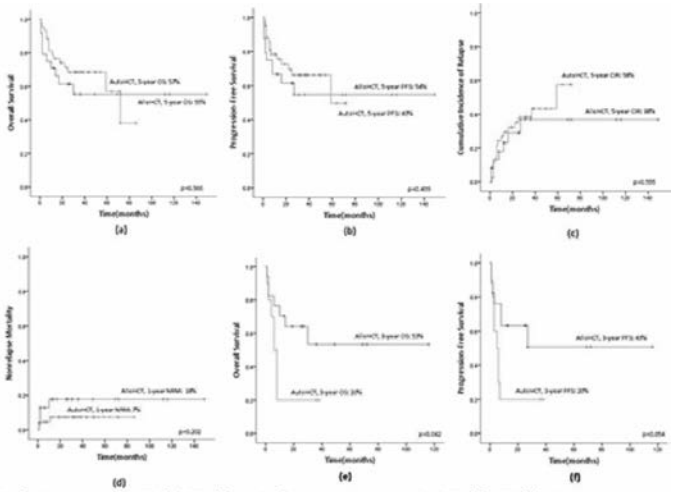
Background: Peripheral T-cell lymphomas (PTCLs) often carry poor outcomes with conventional chemotherapy, and hematopoietic cell transplantation (HCT) can benefit patients with PTCL. Recent retrospective studies have reported that autoHCT as consolidation can offer a durable survival benefit in high-risk patients with first complete or partial response, and alloHCT could result in long-term disease control for relapsed and refractory patients.
Aims: To explore questions about the optimal timing for stem cell transplantation and relative efficacy of auto-HCT *versus* alloHCT.
Methods: We conducted a retrospective review of 67 patients with peripheral T-cell lymphoma who underwent autologous HCT (autoHCT, n=43, median age 40 years) or allogeneic HCT (alloHCT, n=24, median age 36.5 years) from 2004 to 2016.
Results: With a median follow-up of 27 months, 5-year PFS and OS of auto-HCT patients were 49% and 57%, respectively. Among alloHCT recipients, the 5-year PFS and OS were 54% and 55%, respectively. When considering incidence of disease relapse or progression (CIR) and nonrelapse mortality (NRM), the 5-year CIR and 1-year NRM of alloHCT recipients were 38% and 18%, respectively, and 58% and 7% of autoHCT patients, respectively. There were no differences between autoHCT and alloHCT on 5-year PFS (*P*=0.499), OS (*P*=0.566), CIR (*P*=0.555) and NRM (*P*=0.202). When specifically examining recipients in primary refractory disease, 3-year PFS rates of autoHCT and alloHCT were 20% and 49% (*P*=0.054), 3-year OS rates were 20% and 53% (*P*=0.042), respectively.



Kaplan-Meier curves for OS (a), PFS (b) and Cumulative incidence curves for CIR (c), NRM (d) for patients who underwent autoHCT versus alloHCT. Kaplan-Meier curves for OS (e), PFS (f) for patients in primary refractory disease who underwent autoHCT versus alloHCT.

Figure 1.

Summary/Conclusions: This analysis shows that HCT can benefit patients with high-risk PTCL in both remission and primary refractory setting. The outcomes did not differ significantly between autoHCT and alloHCT approaches, but alloHCT recipients in primary refractory disease resulted in significantly better outcomes than autoHCT patients. So, we favor proceeding to alloHCT if patients with PTCL in primary refractory disease.

E1532

IMPACT OF BASELINE BILIRUBIN ON SURVIVAL IN PATIENTS WITH HEPATIC VENO-OCCLUSIVE DISEASE/SINUSOIDAL OBSTRUCTION SYNDROME RECEIVING DEFIBROTIDE: POST-HOC ANALYSIS OF EXPANDED-ACCESS PROTOCOL FINAL DATA

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Background: Veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS) is an unpredictable, potentially life-threatening complication of hematopoietic stem cell transplantation (HSCT) conditioning. VOD/SOS with multi-organ dysfunction (MOD) may be associated with >80% mortality. Defibrotide is approved in the European Union to treat severe hepatic VOD/SOS post-HSCT and in the United States to treat hepatic VOD/SOS with renal or pulmonary dysfunction post-HSCT. Prior to approval, defibrotide had been available in the United States via an expanded-access program.
Aims: A *post-hoc* analysis of final data from the defibrotide expanded-access program was used to explore Day +100 survival post-HSCT based on bilirubin-level categories at the time of study entry.
Methods: Patients in the defibrotide expanded-access program had VOD/SOS diagnosed by investigators using Baltimore criteria (bilirubin ≥2mg/dL and ≥2 of: hepatomegaly, ascites, ≥5% weight gain), modified Seattle criteria (≥2 of: bilirubin >2mg/dL, hepatomegaly, or ascites and/or ≥5% weight gain), or biopsy; bilirubin >2 was not required for modified Seattle criteria or biopsy. MOD (renal/pulmonary) was permitted. After informed consent, defibrotide treatment (25mg/kg/day) was recommended for ≥21 days. Here, D*ay* +100 survival was explored by bilirubin level at study entry using categories that are part of the European Society for Blood and Marrow Transplantation (EBMT) proposed grading scale for adults (≥2 to <3mg/dL, ≥3 to <5, ≥5 to <8, and ≥8), as well as bilirubin <2mg/dL, which is not part of the scale but has been reported in children with VOD/SOS.
Results: There were 1000 HSCT patients enrolled, between December 2007 and September 2016, with a confirmed diagnosis of VOD/SOS and receiving ≥1 dose of defibrotide, 512 patients had MOD. Median age was lowest in patients with bilirubin <2 (4.5 years; 19% of patients). Median ages were 16 years in the bilirubin ≥2 to <3 group (53.5% of patients) and 13.5 in the ≥3 to <5 group (20.4% of patients); median age in other groups ranged from 15 to 17 years. Kaplan-Meier estimated Day +100 survival in all HSCT patients was 58.9%, with 85.6% in patients with BR <2; other bilirubin groups were older and survival estimates decreased (**Table 1**). In the pediatric (aged ≤16 years) and adult (aged >16 years) patients, patterns were similar (**Table 1**). Estimated survival rates were lower for patients with MOD across all groups. Of all 1000 HSCT patients with confirmed VOD/SOS, 210 (21%) had treatment-related AEs (TRAEs). The TRAEs in ≥2% of patients were pulmonary hemorrhage (4.6%), gastrointestinal hemorrhage (3.0%), epistaxis (2.3%), and hypotension (2.0%).

Table 1. Day +100 Survival (Kaplan-Meier, N=1000).

Bilirubin (mg/dL)	All HSCT Patients		Age ≤16 Years		Age >16 Years	
	n	Survival	n	Survival	n	Survival
<2*	190	85.6%	133	90.9%	57	73.7%
≥2 to <3	535	55.2%	278	63.7%	257	46.2%
≥3 to <5	204	47.2%	120	58.5%	84	31.4%
≥5 to <8	39	53.7%	22	54.2%	17	52.9%
≥8	23	23.9%	11	13.6%	12	33.3%

Summary/Conclusions: This post-hoc analysis found that higher bilirubin levels were generally associated with lower Day +100 survival. These results should be interpreted with caution, as only 1 EBMT criterion was analyzed. MOD was also associated with lower Day +100 survival. The results suggest that diagnosis and treatment of VOD/SOS, before bilirubin becomes markedly elevated, may be associated with improved outcome.

Support: Jazz Pharmaceuticals.

E1533

LONG-TERM FOLLOW-UP OF A PROSPECTIVE TRIAL OF INTENSIFIED CHEMO-IMMUNOTHERAPY WITH AUTOLOGOUS OR ALLOGENEIC STEM CELL TRANSPLANTATION IN PATIENTS AFFECTED BY PERIPHERAL T-CELL LYMPHOMA

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Background: The prognosis of patients with newly diagnosed peripheral T-cell lymphomas (PTCLs) is very poor following conventional therapy alone with 5-years PFS ranging from 20% to 30%. From 2006 to 2010, we conducted a multicenter prospective phase II trial to evaluate the efficacy of upfront consolidation of clinical response with autologous (auto) or allogeneic (allo) stem cell transplantation (SCT) in patients at diagnosis. The results were previously reported (4 year PFS of 70% and 69% for auto and allo SCT, respectively) (Corradini P. 2014).
Aims: In this analysis, we extended the follow-up of our trial.
Methods: Sixty-one patients were enrolled after central review pathology [Peripheral T-cell Non Hodgkin Lymphomas (PTCL-NOS) n=33, Anaplastic lymphoma kinase-negative anaplastic large cell lymphomas (ALCL) n=12, Angioimmunoblastic lymphomas (AITL) n=14, enteropathy-associated T-cell lymphomas (EATL) n=2]. The induction therapy consisted of 2 courses of CHOP and alemtuzumab followed by 2 courses of high-dose methotrexate, cytarabine and cyclophosphamide. Patients in clinical remission with HLA identical donors received allo SCT whereas those without a suitable donor received auto SCT. Three patients relapsed after auto SCT and were subsequently allografted (data of last follow-up was censored at date of second transplantation procedure).
Results: Only 37 patients underwent transplantation [autologous SCT (n=14), allogeneic SCT (n=23] whereas 24 did not for toxicity (n=5), progressive disease (n=18) or clinical decision (n=1). In intention to treat analysis, at a median follow-up of 78 months, the estimated 7-years progression-free survival (PFS) and overall survival (OS) for all the patients were 42% (95% CI, 29% >54%) and 41% (95% CI, 28% >53%), respectively. Despite auto or allo SCT consolidation was chosen based on donor availability, the majority of patients allografted had a diagnosis of PTCL-NOS [19 of 23 (83%) *versus* 6 of 14 (43%) autografted, (p=0.02)]. The PFS and OS were not significant different in patients transplanted with a diagnosis of PTCL-NOS as compared to others subtypes but numbers are too small for definitive conclusions [PFS: 48% (95%CI, 32% >62%) *versus* 33% (16% > 51%) (p=0.26); OS: 50% (95%CI, 33% >64%) *versus* 30% (13% >49%) (p=0.36)]. Considering only the patients who underwent a consolidation with any type of transplant (n=37), the PFS were 48% (95% CI, 18% >73%) and 62% (95% CI, 46% >83%) (p=0.40) in patients autografted and allografted, respectively. We did not observe a significant difference in OS between auto or allo consolidation 69% (95% CI ,31% -88%) *versus* 63% (38% >79%) (p=0.51)], but 3 patients in relapse after auto SCT were allografted. The main cause of failure after auto SCT was relapse (6 of 14, last relapse occurring at 81 months after auto). The Crude Cumulative Incidence of non-relapse mortality and relapse after allo SCT were 19% (n=4 deaths, one patient died of cardiac complication 62 months after allo SCT) and 17% (n=4 deaths), respectively.
Summary/Conclusions: The long-term outcome of patients receiving any transplantation strategy remains satisfactory. In the future, biological markers could help physician to select the better therapeutic option for the patients.

E1534

UNRELATED DONOR ATTRITION AT A LATE STAGE: THE BRITISH BONE MARROW REGISTRY EXPERIENCE

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Background: The success of searches for unrelated stem cell donors (UDs) relies on the existence of large international donor registries and the availability and reliability of donors on the register. Donor attrition at the verification typing (VT) or later stage results in delay of transplant and can adversely affect patient outcomes. The British Bone Marrow Registry (BBMR) provides UDs to international transplant centres (TCs) and to UK TCs via the Anthony Nolan registry. Data reported by international registries on donor attrition is scarce and mainly focused on attrition at the VT stage. BBMR donors are recruited from blood donors and may differ in their reliability from non-blood donors included in existing reports.
Aims: To investigate donor attrition rates and causes of cancellation among finally selected or backup BBMR donors at the post-VT stage.
Methods: Data on requests for work-ups from April 2002 to December 2016 were extracted from BBMR databases and donor notes and were analysed retrospectively. The reasons for cancellation were categorised: cancellation initiated by TCs, donor reason or mixed reasons. Within donor reasons we distinguished 3 categories: donor medical reasons, donor withdrawal on personal grounds and inability to contact the donor. We examined associations between cancellations for donor-related reasons and the following factors: donor sex, age at time of donation/cancellation, time on the register and donor reliability score. The reliability score relates to blood donation and runs from 1 (best) to 5 (worst), increasing if a donor fails to attend appointments for blood donation.
Results: A BBMR final/backup donor was selected for 3184 stem cell or lymphocyte collections. 82% of the requests (n=2613) were completed. Out of the 571 (18%) cancelled cases the reason for cancellation was not available for 5 cases. Overall more than half of the cancellations (n=302, 53%) were activated by TCs mainly due to patient death, deterioration or alternative donor choice. Donor reasons accounted for 38% of cancellations (n=216, 6.8% of requested donors), of which 69% (n=148) happened for medical reasons, 27% (n=59) for donor pull-out on personal grounds and 4% (n=9) due to uncontactable donors. The medical reasons for withdrawal were varied but the most frequent health issues were obesity and cardiovascular disease. Analyses of factors affecting donor reasons showed that donor sex and time on the register were not associated with donor fitness or withdrawal rate. Age had no impact on donor pull-out, but it was significantly associated with medical eligibility and donors who failed medically were older (p=0.005). Donor pull-out showed significant association with blood donor reliability score (p=0.029, score 5 *vs* others). In 48 cases (8%) there were mixed reasons where TCs had other donor options and pursued them because of issues such as donor availability for ideal dates or CMV mismatches.
Summary/Conclusions: In our registry patient-related issues accounted for more than half of cancellations at a late stage in the stem cell donor pathway. Cancellations for donor reasons were unusual (6.8% of requested donors), which figure compares favourably with international data (12.4% of requested donors, WMDA Annual Report 2015). This is likely due to the fact that most BBMR donors are regular blood donors: few donors withdrew for personal reasons and very few were uncontactable. Medical conditions were the most frequent cause of cancellation for donor reasons. Further work is underway to allow earlier or reduced deferral of medically unsuitable donors such as control of high blood pressure and to explore personal reasons which cause donors to withdraw. This study should provide reassurance to TCs that BBMR provide reliable and accessible stem cell donors.

E1535

POLIMORPHISM IN TGFB1 GENE PREDISPOSES TO RELAPSE AND DEVELOPMENT OF ACUTE GRAFT-VERSUS-HOST DISEASE GRADES III-IV

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Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the most effective treatment option for certain hematological malignancies. Cytokines play a well established role in the mechanism of acute GvHD (aGvHD), which is one of the most significant complications of allo-HSCT. Transforming growth factor B1 (TGFB1) is one of the inflammatory cytokines, which play a pivotal role in the development of aGvHD.
Aims: The aim of this study was to investigate the role of *TGFB1* -1347C>T polymorphism in the outcome of HSCT.
Methods: We examined the association of recipient and donor TGFB1 -1347C>T and allo-HSCT outcome in a cohort of 419 adult patients who underwent first allo-HSCT between January 2007 and December 2013 at our single center. 217 patients received stem cells from their siblings, 202 patients from matched unrelated donors (MUD). For identification of *TGFB1* rs1800469 from genomic DNA LightCycler melting curve analysis (LightCycler 480II, Roche Diagnostics) was performed.
Results: We did not find any association between recipients' *TGFB1* -1347C>T polymorphism and HSCT outcome. However, in patients whose unrelated donors carried homozygous *TGFB1* -1347TT variant, aGvHD grades III-IV occurred more frequently (aGVHD grade III-IV: 28.9% *vs* aGVHD grade 0-II: 9.6%, p=0.006). Similar finding was observed on a subgroup of patients with acute leukemia: in aGVHD grade III-IV 37.5%, while in grade 0-II 11.5% of patients had TT genotype (p=0.022). Donor TT genotype did not influence the relapse rate significantly. Patients with MUD carrying TT genotype had lower overall survival (OS) that that of donors bearing at least one C variant, but the difference did not reach the level of significance (OS at 40 month for CC and CT variant donors: 45.3% and for TT donors: 26.2%). In case of sibling donors, we did not find association between recipient or donor genotype and aGVHD, but relapse rate was increased if donor had at least one T variant (n=115, 67.9% *vs* 32.1%, p=0.028). Significant differences in OS between the subgroups with different genotypes were not observed.
Summary/Conclusions: Our findings suggest that TGFB1 -1347C>T polymorphism in HSCT donors might influence the development of aGVHD in unrelated and the relapse rate in related HSCT.

E1536

EARLY AND LATE LOST OF PROTECTIVE ANTIBODY LEVELS AGAINST MEASLES, MUMPS AND RUBELLA IN PATIENTS GIVEN ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

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Background: Live-vaccines should be avoided in the early period following allogeneic hematopoietic cell transplantation (HCT), due to a possible uncontrolled proliferation of the attenuated strains. The post HCT immune system is severely compromised by pharmacological immunosuppression and disruption of lymphoid tissues by conditioning and donor T cell alloreactivity. Patients frequently lost their antibody-based immunity against measles, mumps, and rubella after receiving allogeneic HCT.
Aims: Here, we studied the dynamics of antibody (AB) titers against measles, mumps, and rubella post-HCT.
Methods: We retrospectively analyzed serial AB titers in 240 patients who underwent allogenic HCT from related und unrelated HLA-matched donors from 2002-2014 at our center. AB titers against measles, mumps and rubella were measured prior to HCT, at 6 months (m), and every year (y) post-HCT.
Results: Most patients had protective AB titers (measles 90%, mumps 86%, rubella 92%) prior to HCT. AB protection against mumps was lost in a substantial proportion of patients after HCT (protective AB titers in 72%@1y, 56%@5y, 50%@8y), comparing to AB against measles, which persist more frequently (protective AB titers in 85%@1y, 74%@5y, 73%@8y). We found a faster lost of protective AB in the first years for patients given a rmyeloablative condition (MAC) in comparison to patients with reduced condition (RIC), but the proportion of seropositive patients became more equal over time (Figure 1 displays the percentage of seropositive patients to Measles AB given MAC or RIC during 8 years post-HCT). The proportion of patients who retained protective AB titers at 5y post-HCT was higher in recipients of mobilized peripheral blood compared with bone marrow (BM) grafts (measles p=0.01, mumps=0.06, rubella p=0.08). For rubella, absolute AB titers were available. Patients with lymphoid malignancies, ongoing GVHD and pharmacological immunosuppression had a steeper decline of rubella AB titers as compared to patients with myeloid malignancies.

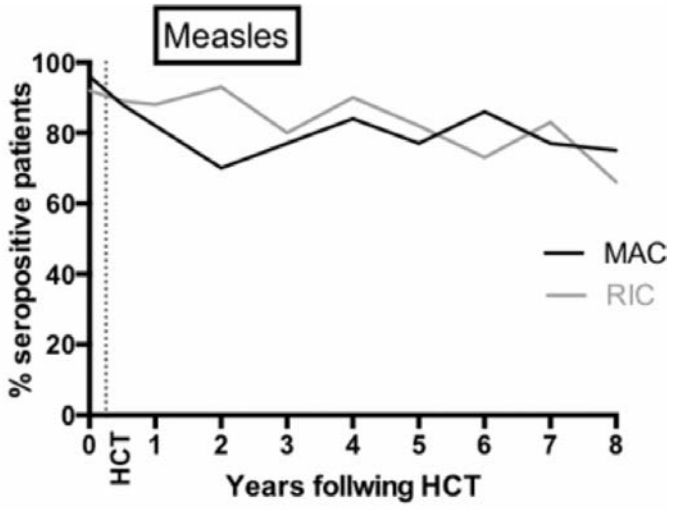

Figure 1.

Summary/Conclusions: We found a marked decline of AB titers post-HCT with loss of protection in a substantial proportion of patients. Surprisingly, BM grafts did not provide better AB protection post-HCT, despite their higher content of (donor) plasma cells. Together with the observations that (i) patients with lymphoid malignancies (who have received (B-) lymphocyte targeted therapies prior to HCT) had lower AB levels, while (ii) those given reduced intensity conditioning have a higher percentage of protective AB levels in the first years, our data suggest, that residual host plasma cells significantly contribute to AB production during the first years post-HCT. In opposite, the loss of protective AB levels in later years after transplantation was independent of the toxicity of the conditioning regime and may be a effect of weakening signaling for host plasma cells or late donor alloreactivity.

E1537

MICA AND NKG2D POLYMORPHISMS HAVE A SIGNIFICANT IMPACT ON GRAFT *VERSUS* HOST DISEASE AFTER HLA-MATCHED HEMATOPOÏETIC STEM CELL TRANSPLANTATION.

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Background: MICA (MHC class I polypeptide-related sequence A) is a highly polymorphic gene closely linked to the HLA-B locus. It encodes a cell stress inducible glycoprotein, which mediates an activatory signal towards the NKG2D receptor expressed on NK-cells, CD8+ T-cells and NKT-cells. MICA polymorphisms have been shown to influence NKG2D signaling. Indeed, a methionine to valine change at position 129 in exon 3 categorized the MICA alleles into strong (MICA-129 met) and weak (MICA-129 val) binders of NKG2D receptor. 5 repetitions of GCT with 1 additional nucleotide insertion (G) in exon 5 designed the MICA A5.1 alleles with a premature stop codon. Moreover, NKG2D polymorphisms identified alleles associated with a low (NKC3 C/C and NKC4 C/C) or high cytotoxic activity (NKC3 G/G and NKC4 T/T).
Aims: In this study, we hypothesized that polymorphisms at the MICA and NKG2D loci are associated with adverse outcomes in HSCT.
Methods: Here, we evaluated whether recipient MICA and donor NKG2D polymorphisms (respectively MICA-129, MICA A5.1 and NKC3, NKC4) could influence the incidence of acute and chronic graft versus-host disease (GVH), overall survival (OS) and relapse free survival (RFS) on 124 patients undergoing allogenic hematopoietic stem cell transplantation using an HLA-matched donor (10/10).
Results: In an univariate model, recipient MICA A5.1 heterozygosity (p=0.030) and donor NKC4 C/C polymorphism (p=0.013) are associated with the increase of incidence of acute GVH (grade I to IV). Recipient MICA A5.1 heterozygosity is also associated with chronic GVH (p=0.04) while Recipient MICA-129 val/val tends to be a risk factor of chronic GVH without being statistically significant. These polymorphisms have no significant impact on OS and RFS in our study (median of follow up=15 months; range 0.2-49 months).
Summary/Conclusions: Our data suggest that a MICA or NKG2D low activity status can be related to an increase of acute GVH according to a mechanism that remains to be elucidated, maybe by a low cytotoxic activity on recipient dendritic cells.

E1538

STEM CELL TRANSPLANTATION WITH MYELOABLATIVE CONDITIONING USING TIMED SEQUENTIAL BUSULFAN IMPROVES OUTCOMES IN OLDER AML AND MDS PATIENTS

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Background: We previously reported 6% 100 day NRM with a MA fludarabine (Flu) and busulfan (Bu) in older patients with a median age of 60 years. MA dose of Bu in this timed sequential (TS) regimen was administered over a longer period of time. To assess its impact on survival, we compared the outcomes of older patients treated with the TS Bu (TS cohort) or the RIC Flu/Bu regimen, which is used as standard (ST) for older patients at our center ST cohort.
Aims: To assess its impact on survival, we compared the outcomes of older patients treated with the TS Bu (TS cohort) and the reduced intensity conditioning with Flu/Bu regimen, which is used as standard (RIC cohort) for older patientsat our center.
Methods: Patients in the TS cohort received IV Bu 80mg/m^2/d on day -13 and -12 and Flu 40mg/m^2/d followed by IV Bu on day -6 to -3, dose adjusted to achieve a total Bu course AUC of 20,000µmol-min based on PK studies. Patients in the ST cohort received Flu 40mg/m^2 day followed by IV Bu daily for 4 days (day -6 to -3) dosed to achieve AUC of 16,000µmol-min. Patients with AML or MDS were eligible for the study if they had adequate organ function, had matched related or unrelated donor and were treated between Jan 2012 and Se.
Results: Patient characteristics including age, sex, disease status, cytogenetic risk group, donor type, graft source CMV status and comorbidity were similarly distributed between the two cohorts. Median age was 66 and 65 years in TS-MAC and RIC cohorts respectively. Overall survival (OS) and progression free survival(PFS) were significantly better in the TS -MAC cohort. This was due to a reduction in the disease progression without any increase in the TRM. After adjusting for other covariates, the multivariate analysis for PFS confirmed longer PFS with TS-MAC regimen (HR: 0.36; P=0.003). The benefit was mainly seen in patients with a comorbidity score ≤3.

Table 1.

	RIC cohort	TS-MAC cohort	RIC vs. TS-MAC	
	Estimate	Estimate	HR	p
2 year OS	31%	51%	0.6	0.01
2 year PFS	24%	45%	0.6	0.004
2 year progression	59%	34%	0.5	0.003
2 year TRM	12%	15%	1.3	0.5

Summary/Conclusions: The myeloablative timed sequential Bu regimen improves survival and appears promising in older patients with AML/MDS. The myeloablative timed sequential Bu regimen improves survival and appears promising in olderpatients with AML/MDS.

E1539

HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION WITH DEPLETION OF TCR AB (+) IN CHILDREN: ERCIYES PEDIATRIC BMT CENTER

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Background: Recently, haploidentical hematopoietic stem cell transplantation (HSCT) posses an alternative option for patients without a suitable donor. Erciyes Pediatric BMT Center is the first pediatric center for haploidentical HSCT with depletion of TcR αβ **(+)** in Turkey.
Aims: We would like to share our pediatric experience with a follow up period of more than four years.
Methods: All children who underwent haploidentical HSCT in our center from December 2012 to February 2017 were included in the study. Total 51 haploidentical HSCT in 44 children (17 relapsed/refractory AML, 9 relapsed/refractory ALL, 4 SAA, 4 HLH, 2 Fanconi aplastic anemia, 2 Griscelli syndrome, 1 JMML, and 5 SCID) were performed. Transplantation-related mortality (TRM) was 13.7%. The regimen included ATG, Fludarabine, Thiotepa, Melphalan. Mycophenolate mofetil (MMF) was given as GvHD prophylaxis if the graft contained>5 x10^4/kg TcR αβ **(+)**.
Results: The mean of collected CD34 cells were 18,60 (range 3,98-43,66) x 10^6/kg. The graft had a purity of 99.9% TCRαβ depletion with a median of 0,257 (range 0.003 to 1,47) x 10^5 TCRαβ cells. The median engraftment days for myeloid and platelet were both 12th day of HSCT (range 7 to 28, 9 to 33 day) respectively. Grade II skin GvHD was detected in 8 patients, and treated with steroids without any further complications. However grade III, and grade IV gastrointestinal GvHD were observed in three patients. Although the patients with gastrointestinal GvHD were treated with steroid, budenosid, cyclosporine, MSC; one patient did not respond and died. MMF was given as GvHD prophylaxis in 36 patients and 15 patients did not receive any immune suppressive drug. The mean day of discharge was 34th day of HSCT. The long term follow up including immunological reconstructions were performed in 18 patients. The analysis of the immune reconstitution of the patients transplanted in haploidentical HSCT group showed a rapid immune reconstitution for CD3+ T cells 732 (range 126-2432)/mm3; for CD4+ helper T cells 92 (range 1–419)/mm3; CD8+ T cytotoxic cells 310 (range 95-2235)/mm3 at 28th day of HSCT. Twenty nine patients are currently alive, with a median follow up of 22 months (range 1 to 49 months). Overall survival was 65,9% in these group.
Summary/Conclusions: Our primary results underline that haploidentical HSCT with depletion of TcR αβ (+) can be an option in experienced center in countries which unrelated donor programs are not satisfactory, as in Turkey. The availability of a haploidentical donor in most families is a potential advantage. Moreover probably more potent graft-versus tumor effect can be induced with haploidentical HSCT.

E1540

SECONDARY MYELODYSPLASTIC SYNDROME AND/OR ACUTE LEUKEMIA INCIDENCE AFTER AUTOLOGOUS TRANSPLANTATION FOR LYMPHOMA PATIENTS IS CONNECTED WITH DECREASE OF HEMATOPOIETIC RESERVE

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Background: Secondary myelodysplastic syndrome and acute myelogenous leukemia (sMDS/AML) is one of the most important long term complication of high dose therapy (HDS) with autologous stem cell transplantation (ASCT). The factors usually described to be associated with sMDS/AML development are pretreatment, HDS itself, radiotherapy, age and recently the evidence of TP53 mutations (Wong, Nature 2015) or clonal hematopoiesis (Gibson, JCO 2016) before ASCT.
Aims: The aim of the study was to analyse the incidence and risk factors for sMDS/AML after HDT and ASCT for lymphoma.
Methods: Patients who underwent HDT with ASCT for lymphoma in one centre since 12/1993 till 7/2016 were analysed. Pretreatment characteristic, graft quality, engraftment characteristics were included into analysis. Patients were censored at the time of death or allogeneic stem cell transplant. Pearson, Kaplan Maier, log-rank and cox regression tests were used.
Results: Altogether 728 pts underwent ASCT for lymphoma in given time period. The cohort consists out of 77% B-NHL (n566), 6% T-NHL (n 43) and 16% HL (n 119), 58% were men, age median at the transplant was 49 years (18-71). The median of previous lines was 2 (1-9). The stem cell collection was performed after chemotherapy and G-CSF mobilization in most cases, 19 pts were mobilized by G-CSF only and bone marrow only was used in 4 pts. The target CD34 dose was 3x10^6/kg. The median number of apheresis was 2 (1-12). At the time of ASCT 90.6% of patients had chemosensitive disease (51.1% CR) and 9.4% were transplanted for chemoresistant disease. Tandem HDT and ASCT was used in 36 pts, BEAM was the most frequent HDT regimen (92.5%, 15 pts received ibritumomab tiuxetan and BEAM), the total body irradiation was used only in 4 pts, the rest of the patients received other chemotherapy regimens (CPB, thiotepa based, ICE and others). All pts except 4 received peripheral blood progenitor cells (PBPC) with median CD34 dose 8.6x10^6/kg (0.4-115.5). BM was used in 22 cases (in 18 together with PBPC). G-CSF was administered from day +7. Involved or extended field radiotherapy either during previous therapy or in the period after ASCT was used in 37.7% of pts. With median follow-up 7.2 years there were observed 19 cases of sMDS/AML. The cumulative sMDS/AML incidence was at 5, 10 and 15 years 2.7%, 4.0% and 5.3% (figure A) in all lymphoma pts, 3.3% at 5,10 and 15y in HL pts, and 2.6%, 4.3% and 6.3% in NHL pts (figure B). There was significantly increased sMDS/AML incidence in pts with ³3 previous lines (7.7% *vs* 1.9% at 5y, HR 3.9, p 0.005), in pt's group with chemoresistant disease (8.1% *vs* 2.3%, HR 3.5, p 0.05), in CD34+ dose<3.0x10^6/kg (14.3% *vs* 2.5% at 5y, HR 4.9, p 0.05), in BM reinfused group (13.7% *vs* 2.5% at 5y, HR 4.7, p 0.05), in patients with prolonged platelet engraftment above 20x10^9/l - ³15 days *vs* 11-14 days *vs* £10 days (5.4% *vs* 3.0% *vs* 0.9%, p 0.05). There was no difference between groups of NHL and HL, with and without radiotherapy, according to the apheresis number or neutrophil engraftment. In multivariate analysis in the whole cohort the independent risk factors were number of previous therapy lines, disease status at ASCT and the speed of platelet engraftment (p <0.05). For NHL only number of previous therapy lines (p<0.05), for HL number CD34+cell reinfused, use of BM as the progenitor cell source and disease status (p<0.05).

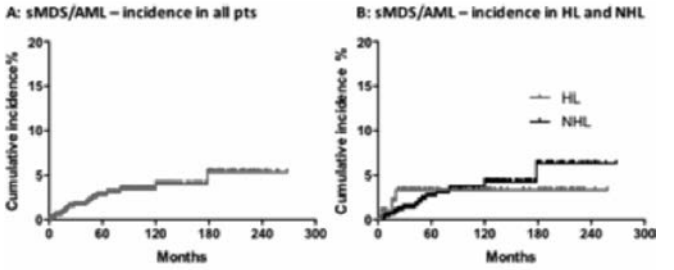

Figure 1.

Summary/Conclusions: The risk of sMDS/AML was 4.0% at 10y after ASCT and was connected with heavier pretreatment, which leads to the decrease of BM reserve, hematopoietic clonal development. The lower dose of CD34+ cell, the necessity to use BM progenitor cell and prolonged platelet engraftment could be considered as clinical markers of these biological processes.

E1541

USE OF DEFIBROTIDE TO TREAT TRANSPLANT-ASSOCIATED THROMBOTIC MICROANGIOPATHY

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Background: Transplant-associated thrombotic microangiopathy (TA-TMA) is a severe early transplant complication which results from endothelial injury and it exhibits characteristics of an atypical hemolytic uremic syndrome. Beyond removal or treatment of precipitating factors and, more recently, treatment with eculizumab, TA-TMA remains a therapeutic challenge. Defibrotide, with marked protective effects on the endothelium and the potential to restore thrombotic-fibrinolytic homeostasis in small vessels, may be considered a therapeutic option for TA-TAM.
Aims: To analyze our center's experience in the treatment of TA-TMA with defibrotide.
Methods: We reviewed all cases of TA-TMA treated with defibrotide in our allogeneic transplant recipients between October 2008 and November 2016. All cases had non-inmune hemolytic anemia with high LDH, low haptoglobin and negative Coombs test, >2 schistocytes per high-power field and thrombocytopenia (<50x10^9/L or <50% of normal baseline). Cases without signs of renal or neurological dysfunction were classified as probable TA-TMA [Uderzo C, 2014].
Results: We identified 17 TA-TMA episodes treated with defibrotide in 16 allogeneic transplant recipients: 9 men; median age 38 years old (16-57); 10 single-cord blood plus third-party donor cells [Bautista G, 2009], 3 HLA-identical siblings and 3 unrelated donors; 13 myeloablative conditioning regimen, 10 with total body irradiation (Table 1). Concomitant risk factors at the time of TA-TMA onset were: calcineurin inhibitor treatment in all cases (13 cyclosporin, 4 tacrolimus), acute GVHD grade ≥II/IV in 8 cases, 3 CMV reactivations and 3 severe fungal (1 pulmonary aspergillosis, 1 *Scedosporium Prolificans* septicemia) or bacterial (1 *E Coli* sepsis) infections. Median onset of TA-TAM was on day +43 after transplant (2-556); 11 cases of early onset (<2 months) and 6 of late onset. Nine episodes were probable TA-TMA without organ dysfunction, 8 had renal failure and 2 presented with concomitant diffuse alveolar hemorrhage. First line replacement of calcineurin-inhibitors for basiliximab or other

drugs for GVHD was performed in all cases. Defibrotide was subsequently administered as monotherapy in 5 cases, in combination with rituximab and/or plasma exchange in 7, and with other agents in 5 others (2 vincristine; 1 eculizumab; 1 bevacizumab; 1 mesenchymal stromal cells). Complete resolution of TA-TMA (CR) was achieved in 11 episodes (65%), and associated with a reduced all-cause mortality: 18% of CR cases (2/11: 1 multilobar pneumonia and 1 toxic encephalopathy) *versus* 83% of cases without CR (5/6, *P*=0.035; 1 TA-TAM-related diffuse alveolar hemorrhage, 1 lymphocytic encephalitis and 3 severe infections). TA-TMA cases triggered by severe infections had early (days 4, 11 and 15 after onset) and higher mortality rates (3/3, 100% *vs* 4/14, 29%; *P*=0.023). Rates of CR were higher in cases of probable TA-TAM without renal failure (8/9, 89% *vs* 3/8, 38%; *P*=0.027) and early-onset TA-TAM (9/11, 82% *vs* 2/6, 33%; *P*=0.046).

Table 1.

Table 1. TA-TMA episodes overall and per response to defibrotide treatment.

	All TMA episodes	Resolved TMA episodes	Unresolved TMA episodes
Number of episodes	17	11	6
Age at HCT (years)	38 (16-57)	40 (16-57)	33 (21-48)
Sex			
- Male	9	7	2
- Female	8	5	3
Indication for HCT			
- ALL	8	5	3
- AML/MDS	4	3	1
- Lymphoma	3	2	1
- Multiple Myeloma	1	1	0
- Aplastic Anemia	1	1	0
Potential Risk Factors			
- GVHD	8	6	2
- CMV reactivation	3	2	1
- Infection	3	0	3
- CsA / Tacrolimus	13/4	9/3	4/1
- CB / SD / UD	11/3/3	8/1/3	3/2/0
- MAC / RIC	14 / 3	9 / 3	5 / 0
- MAC TBI	11	7	4
- MAC No TBI	3	2	1
TMA by organ dysfunction			
- Probable TMA	9	8	1
- With organ dysfunction	8	4	4
Day of TMA onset	+43 (2-556)	+36 (2-308)	+117 (3-556)
- Early onset (<+60)	11	10	1
- Late onset (>+60)	6	2	4
TMA treatment			
- DF monotherapy	5	3	2
- DF in combination	12	9	3
All cause mortality	7	2	5

Abbreviations: ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; CB: Cord Blood; CMV: cytomegalovirus; CsA: cyclosporin A; DF: defibrotide; GVHD: graft-versus-host disease; HCT: hematopoietic cell transplant; MAC: Myeloablative Conditioning; RIC: reduced intensity conditioning; SD: Matched Sibling Donor; TA-TAM: transplant-associated thrombotic microangiopathy; TBI: Total Body Irradiation; UD: Matched Unrelated Donor;

Summary/Conclusions: TA-TMA is a severe endothelial dysfunction syndrome for which, beyond the complement inhibitor eculizumab, treatment is not well established. Defibrotide has proven to be safe and effective in sinusoidal obstruction syndrome. Here, we provide encouraging evidence suggesting that defibrotide, as monotherapy or in combination with other agents, may also have a role in the treatment of TA-TMA. Our data show complete resolution of TA-TMA in two thirds of cases, and even higher in those with early treatment and early onset forms of the disease. Validation of single-center experience in prospective controlled studies should be warranted.

E1542

PRE-TRANSPLANT COMORBIDITY AS AN OUTCOME PREDICTOR IN HEMATOPOIETIC CELL TRANSPLANTATION FOR SEVERE APLASTIC ANEMIA

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Background: In the context of allogeneic hematopoietic cell transplantation (allo-HSCT), comorbidites are an important risk factor. Use of the hematopoietic cell transplantation-specific comordigity index (HSCT-CI), which was modeled to effectively capture comorbidity and predict post-transplant outcomes. HSCT-CI had been evaluated in a chort of patients with a variety of hamtologic malignancies. However, it was not validated in a cohort of adult patients with non-hematologic malignancies.
Aims: We performed multi-center retrospective study to validate the prognostic impact of HSCT-CI on transplant outcomes in a cohort of aplastic anemia patients undergoing allo-HSCT.
Methods: In this study, we applied the HCT-CI to 140 patients with severe aplastic anemia (SAA) who underwent allogeneic HCT at the Asan Medical Center, Seoul, and Haeundae Paik Hospital, Busan, Korea between April 1995 and March 2013. Required data were retrieved from Asan medical center and Haeundae Paik Hospital BMT Registry Database. We stratified the patients based on comorbidities, as assessed by HCT-CI. Post-transplant outcomes were evaluated in terms of overall survival (OS) and event-free survival (EFS). Event was defined as graft failure including primary and secondary, relapse, donor lymphocyte infusion, and death.
Results: The median age of including patients was 31 year-old (range, 31-61 year-old) and male was 81 patients (58%). HCT-CI score was 0 in 92 patients (65.0%), 1-2 in 34 (24.3%), and ≥3 in 14 (10.2%). The most prevalent comorbidity captured by the HCT-CI was infection (n=20, 14%) followed by moderate/severe hepatic comorbidity (n=10, 7%). During a median surviving post-HCT follow-up period of 45.5 months (range, 4.1-178.4 months), 32 patients (24%) died and 20 (14%) experienced primary or secondary graft failure. The 10-year probability of OS and EFS was 73.4% and 68.3%, respectively. OS and EFS was significantly different according to HCT-CI score; the OS for HCT-CI 0, 1-2, and ≥3 at 4 years was 84.1%, 68.6%, and 60.6%, respectively (*P*=0.007). The EFS for HCT-CI 0, 1-2, and ≥3 at 4 years was 76.5%, 60.0%, and 56.3%, respectively (*P*=0.019). Multivariate analysis after adjustment for other variables demonstrated that higher HCT-CI score were associated with increased OS and EFS as judged by increasing hazard ratio compared to patients with HCT-CI score of 0 (Table 1).

Table 1.

Prognostic factors		EFS		OS	
		HR (95% CI)	P	HR (95% CI)	P
HCT-CI scores (compared to low risk)	Intermediate risk	2.764 (1.388-5.486)	0.004	3.369 (1.629-7.422)	0.003
	High risk	2.923 (1.090-7.837)	0.033	3.418 (1.102-10.582)	0.033
Age (years)	<40 vs ≥40	1.995 (1.014-3.917)	0.046	2.052 (1.194-6.692)	0.006
Duration from Dx to HCT	≤8 vs >8 (month)	2.715 (1.098-6.710)	0.031	1.928 (0.648-5.748)	0.238
HLA matching (serum)	Full matched vs one or more mismatched	2.154 (0.853-5.439)	0.105	2.905 (1.086-7.778)	0.034

Table 1. Multivariate analysis of prognostic factors for event free survival (EFS) and overall survival (OS) among patients with severe aplastic anemia with allogeneic hematopoietic cell transplantation (HCT). CsA: cyclosporin, Dx: diagnosis, HLA: human leukocyte antigen, HCT-CI: hematopoietic cell transplantation comorbidity index, IST: immune suppressive treatment

Summary/Conclusions: In conclusion, our data indicate that the presence of pre-transplant comorgicity assessed by HSCT-CI may predict worse outcomes after allo-HSCT in severe aplastic anemia.

E1543

EFFICACY AND SAFETY OF FILGRASTIM BIOSIMILAR COMPARED TO FILGRASTIM ORIGINATOR IN THE STEM CELL MOBILIZATION AND HEMATOPOIETIC ENGRAFTMENT IN PATIENTS UNDERGOING STEM CELL TRANSPLANTATION

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Background: Neupogen® is the original Filgrastim used for peripheral blood stem cell mobilization (PBSC) in patients and donors selected for stem cell transplantation (SCT). Nivestim® is a Filgrastim biosimilar approved for the same indications as Neupogen®.
Aims: To evaluate the efficacy and safety of Nivestim® in the PBSC mobilization for harvesting and hematopoietic SCT.
Methods: Retrospective, controlled, observational study conducted at the University Hospital of Salamanca (Spain) between JAN08 and DEC15.
Results: The study included 365 patients candidates for ASCT and 217 healthy sibling donors for Allo-SCT who underwent PBSCs mobilization. Neupogen® (Amgen Europe BV, Breda, NL) was administered for mobilization at standard doses until SEP2012, while Nivestim® (Hospira, Maidenhead, UK) was used from that date. Among PATIENTS, 145 were mobilized with Nivestim® and 220 the originator Neupogen®. Patient characteristics between groups were similar, although lenalidomide was more frequently used in the Nivestim® group, as it corresponds to more recent transplants. The mean number of $CD34^+$ cells/µl in the peripheral blood after 4 days of mobilization treatment was not significantly different (Neupogen®73.2, SD=113.0; Nivestim® 94.5, SD=166; p=0.15), but the mean of the total $CD34^+$ collected cells was 4.75, SD=4.41 in the Neupogen® and 6.35±6.42 in Nivestim® group (p=0.01), with a larger number of apheresis procedures needed in the Neupogen® group (1.39, SD=0.65 *vs* 1.24, SD=0.45; p=0.02). The mobilization failure rate was slightly higher with Nivestim® (22%) than with Neupogen®(13%, p=0.04), although it was attributed to a more frequent use of lenalidomide. Most patients underwent ASCT: 87% and 92% patients in the Neupogen® and biosimilar groups, respectively. There were no statistically significant differences in hematopoietic recovery and trans-

plant-related toxicity. The median hospitalization time (20, range 14-70 *vs* 20, range 14-53, p=0,72) and the consecutive number of re-admissions after discharge (27% *vs* 35% p=0,35) were also similar between Neupogen® and Nivestim® groups. In the group of HEALTHY DONORS, 95 were mobilized with Neupogen® and 122 with Nivestim®. Donor characteristics were equivalent between groups, and no severe adverse events were registered in any of them. Mean of $CD34^+$ cells collected/kg of recipient body weight was $7.62x10^6$, SD=$3.45x10^6$ for Nivestim® *vs* $6.26 x10^6$, SD=$2.71x10^6$ Neupogen®(p=0.002), but the minimal target cell dose ($2x10^6$/kg) was collected in all donors. 8.5% of donors mobilized with Nivestim® failed to achieve the optimal cell dose ($4x10^6$/kg) compared with 13% in the Neupogen® group (p=0.25). All recipients were successfully transplanted. All donors for haploidentical transplants (N=25) were mobilized with Nivestim®; none with Neupogen®. There were no other transplant differences. Platelet and neutrophil engraftment were comparable between the two groups, as well as transfusion requirements and infectious complications after transplant. The incidence of grade 1 to 4 acute graft-versus-host disease was not different (Nivestim®65.5% *vs* Neupogen® 67.7%; p=0,7). The hospitalization period was similar in Neupogen® and Nivestim® groups, (30 days, range 16-102; 30 days, 16-136, respectively).

Table 1.

Characteristics and main results comparison in patients who underwent autologous stem cell transplantation

Results	Nivestim® (N=145)	Neupogen® (N=230)	P value
CD34+cells/μL in peripheral blood, median (range)	35.07 (1-1151)	43.96 (1.5-1019)	0.18
Mobilization failure, n (%)	31 (21%)	29 (13%)	**0.026**
Lenalidomide therapy n (%)	8 (26%)	1 (3%)	**0.018**
Total CD34 collection x 10^6/kg. median (range)	3.48 (0,44-42)	3.1 (0.03-32)	0.053
Apheresis procedures			
median (range)	1 (1-3)	1 (1-4)	**0.023**
mean±SD	1.24±0.45	1.39±0.65	
ASCT procedure, n, %	130 (89.7%)	184 (83.6%)	0.1
Haematological recovery, days (range)			
ANC>0.5 x10^9/L	11 (8-19)	11 (6-27)	0.53
PLT>20 x10^9/L	12 (8-29)	11 (4-47)	0.59
Primary engraftment failure, n	1	0	0.23
Secondary engraftment failure, n	2	1	0.63
G-CSF injections, days, median (range)	6.39±2.83	6.88±2.45	0.10
Neutropenia febrile episodes, n (%)	117 (90%)	154 (84.2%)	0.09
Neutropenia febrile, days, median (range)	2 (1-16)	2 (1-10)	0.16
Documented infections n (%)	33 (28%)	34 (22%)	0.25
Number of RBC transfusions, mean± SD	1.23±1.84	0.87 ±1.57	0.06
Number of PLT transfusions, mean± SD	3.98±6.03	3.05±3.67	0.09
Hospitalization duration, days median (range)	20 (8-71)	20(14-53)	0.37
TRM (%)	3 (2.3%)	3 (1.6%)	0.67

RBC, red blood cells; PLT, platelet count; ANC, absolute neutrophil count; TRM, transplant-related mortality; SD, standard deviation. Bold values indicate statistically significant p values.

Summary/Conclusions: Although prospective data are still required, our study supports that the use of the Filgrastim biosimilar Nivestim® has a similar efficacy and safety as mobilization agent compared with the originator Neupogen®.

E1544

PERIPHERAL BLOOD STEM CELL DONATION IN OLDER SIBLING DONORS: IS IT SAFE?

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Background: The introduction of reduced intensity conditioning regimens has led to an increase in allogeneic haematopoietic stem cell transplantation (HSCT) in older patients with a consequent increase in age of family members who are asked to donate HSCs for them. Such donors are expected to have more co-morbidities than younger donors and careful assessment of their suitability to donate is required.
Aims: Our aim was to assess the frequency and nature of issues concerning the eligibility of related peripheral blood stem cell donors seen at Churchill Hospital, Oxford between 2012 and 2016. We wished to examine the influence of age and the nature of any extra interventions required to establish donor suitability.
Methods: For clinical data collection donors' notes were reviewed and analysed retrospectively. At our centre the same template was used in all cases for sibling donor selection and screening.
Results: During the study period 90 related donors were screened, of whom 1 declined to proceed because of his concerns regarding G-CSF safety, 2 were excluded due to pre-existing medical conditions and 2 were defined medically ineligible during work-up, and finally 85 donors donated PBSCs to their relatives (36% of allogeneic HSCT performed at our centre). The median donor age was 51 years (range 25-71, n=17 over 60). Nearly half of the donors (44%) took regular medications. Two thirds (67%) suffered from at least one significant comorbidity (25% hypertension, 24% back problems, 16% asthma, 9% cardiovascular conditions, 9% diabetes mellitus, 8% autoimmune disease). The presence of comorbidities was significantly associated with age (p=0.033). 59% travelled abroad, of whom 14% visited a malarial area within a year of donation. Based on donors' history or examination findings, 47% needed extra blood tests on top of the mandatory tests before the clearance, including malaria (31%) and haemoglobinopathy screening (13%). 6% underwent specific haematology investigations *e.g.* BMA, molecular studies. Additional imaging studies were performed in 13%. In 16% specialist opinion was sought from other specialties with concerns regarding donor fitness or safety. 13 out of 85 cases were handled as planned deviation from our standard eligibility criteria. Two donors needed central venous access for stem cell collection. The collected median CD34+ dose was $5.73x10^6$/kg (range 1.76-22.45). Collection was completed in one day in 54%, in two in 44% and in three in 2%. Male (p=0.017) and younger donors (p=0.041) were more likely to achieve stem cell yield in one day collection. The stem cell dose was higher for collections being successful in one day (median 6.5 *vs* 5.03, p<0.001). Citrate related toxicity was the most common complication of the apheresis procedure (52%). The only documented serious complication affected a 69-year old donor who was hospitalized on 3rd day of G-CSF treatment with chest and abdominal pain and troponin rise, but investigations excluded acute coronary syndrome or other significant acute pathology and she managed to donate successfully with no further issues.
Summary/Conclusions: Peripheral blood stem cell collection seems to be safe among sibling donors, who are significantly older than unrelated donors. With careful assessment and planning even individuals with significant co-morbidities can donate successfully. The demographic trend and its implications should be considered when planning resources in HSCT programmes.

E1545

LONG-TERM RESULTS OF DONOR LYMPHOCYTE INFUSIONS IN RELAPSED AND MIXED CHIMERISM PATIENTS AFTER ALLOGENEIC STEM CELLS TRANSPLANTATION

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Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a potentially curative treatment for patients with hematological malignancies. However, relapse remains the major cause of treatment failure after allo-HSCT. Mixed chimerism (MC) can induce immunologic tolerance and lead to relapse. One of the most effective approaches to treat these patients is donor lymphocyte infusion (DLI) with or without chemotherapy.
Aims: To analyze long-term results DLI in early posttransplant MC and in relapsed patients after allo-HSCT.
Methods: The study included 61 patients of in whom DLI with interleukin 2 (IL-2) was administered at the National research center for Hematology from 2011 till 2016. DLI with IL-2 was administered for patients with MC, more than 10-15% recipient DNA (n=26). A median age was 33 years old (19-54 years). Eight were males, 20 – females. There were AML (n=17), ALL (n=4), MDS (n=2), CML/MPN (n=3). Before allo-HSCT complete remission had in 20 patients and 6 had relapse/progression disease. Patients received allo-HSCT from related (n=20) or unrelated (n=6) donor. The intensity of conditioning was mainly reduced intensity (n=15) rather than myeloablative conditioning (n=11). Bone marrow (BM) as a graft source was used in 20, PBSC – 6. DLI was started at low dose $1*10^7$CD 3+ per kg. Every following dose of infusion CD 3+ increased until $5*10^7$CD 3+ per kg. Number of infusions depended on achievement 100% donor chimerism. Thirty five patients with relapse after allo-HSCT (AML, n=27, ALL, n=5, MDS, n=2, CML, n=1) were administered DLI with IL-2. Number DLI was 1 or 5 in different causes. Complete remission before allo-HSCT had in 25 patients and 10 had relapse/progression disease. 33 patients received chemotherapy and after chemotherapy on 7 days DLI was using an escalating dose following infusions. Two patients received DLI with IL-2 without chemotherapy. A median age was 33 years old (18-60 years). 14 were males, 21 - females. Stem cell source was BM and (PBSC) in 22 and 13 of the cases, respectively. Patients were transplanted from related (n=17) and from an unrelated donor (n=18). Condition regimen was MAC (n=7), RIC (n=28). Bone marrow as a graft source was used in 22, PBSC – 13.

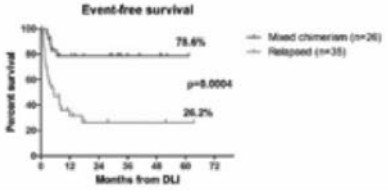

Figure 1.

Results: A median follow up was 5 months (0,3-63). A median time between allo-HCST and DLI was 3 months (1,5-64). 100% donor chimerism was achieved in 17 patients with MC from 26 (65%). A median number of infusions

was 2 (1-5). There were 5 (19%) graft failures. Acute GVHD appeared in 8 (32%), all of them as grade 3; chronic GVHD occurred in 7 (27%). Patients with a MC had better overall survival 77,6% than patients with relapse after allo-HSCT (22%). Remission was achieved in 16 (48%) patients with relapses. However, 5 patients relapsed again. Acute GVHD was developed in 8 cases (22%). Nineteen patients died from relapse and 1 patient died from aGVHD in remission. Event free survival in patients with MC and in patients with relapses was 78,6% and 26,2%, respectively.
Summary/Conclusions: The prognosis of hematological malignancies is poor if relapse is established after allo-HSCT. DLI protocol as preventive therapy must be created for improving long-term results in high risk patients. "Prevention is better than cure."

E1546

MEMORY T CELLS DONOR LYMPHOCYTE INFUSIONS AFTER HAPLOIDENTICAL STEM CELL TRANSPLANTATION AS A SAFE PROCEDURE TO IMPROVE T-CELL RECONSTITUTION

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Background: Hematopoietic stem cell transplantation (HSCT) is a potential curative treatment for patients with hematologic malignant diseases. Haploidentical transplantation with extensive *ex vivo* T cell depletion of the graft, has demonstrated to prevent graft *versus* host disease (GVHD), but the major disadvantage has been the development of graft failure, relapse and infections due to delayed immune reconstitution. A selective T cell depletion method that removes T naïve cells that express CD45RA+ in haploidentical donor lymphocytes, which are responsible for GVHD, as well as preservation of memory T cells CD45RO, is a novel therapy that may provide functional T cells with anti-infection, anti-leukemia and anti-rejection properties.
Aims: We describe the outcome of CD45RA+ cell depletion of donor lymphocytes infusions, in patients with hematologic diseases with mixed chimerism, severe infections and high risk of relapse after hematopoietic stem cell transplantation.
Methods: Patients with hematologic diseases with poor prognosis who lacked an HLA matched donor were included. The recipients received a CD45RA-depleted haploidentical transplantation, on day 0 they received a first graft with a median CD34+ cell dose of $6.44x10^6$/Kg (range $5x10^6$/Kg-$9x10^6$/Kg), on day +1 they received a CD45RA-depleted graft. After transplantation studies of chimerism, quantification of lymphocyte subsets as well as control for viral infections were made to all patients.
Results: We present the results of six patients with a median age of 11 years (range 4 to 15 years), diagnosis included B-Cell acute lymphoblastic leukemia (n=2), T cell acute lymphoblastic leukemia (n=1), acute myeloblastic leukemia (n=2), aplastic anemia (n=1), these patients received a selective CD45RA-depleted haploidentical transplantation. During the follow up after HSCT, three patients had persistent lymphopenia, four patients developed infections caused by CMV, norovirus, HHV-6, BK virus and toxoplasma, one patient had increasing levels of mixed chimerism and one had graft failure. These patients were treated with infusions of 16 aliquots of cryopreserved CD45RO+ haploidentical donor lymphocytes, the CD45RA+ cells depletion was made using the cliniMACS system. The median dose of CD45RO+ cells was $1,02x10^7$/Kg, starting at a dose of $1x10e^5$/Kg until a maximal dose of $2x10e^8$/Kg, infused every 21 days. The CD45RA+ cell dose was a median of $0.0045x10^3$/Kg (range: 0-$1,6x10^3$/Kg). All the procedures were well tolerated, neither adverse events nor GVHD were noticed. After the DLI, a progressive increase in T cells count were observed.
Summary/Conclusions: In our experience DLI enriched for CD45RO+ memory T Cell is a promising and safe strategy for patients with severe viral infections and risk of relapse after haploidentical HSCT, these cells has demonstrated to trigger the CD4 and CD8 T-cell reconstitution, which will help reduce risk infection with a low risk of GVHD. However further studies are needed in order to support this therapy.

E1547

FLAG REGIMEN WITH IDARUBICINE AS CYTOREDUCTION THERAPY BEFORE ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH REFRACTORY ACUTE MYELOID LEUKEMIA

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Background: Allogeneic hematopoietic stem cell transplantation (Allo-HSCT) is the only curative option for patients with refractory acute myeloid leukemia (AML). However, allo-HSCT with standard conditioning regimen could merely achieve a long-term survival of 20% and the key problem is the high relapse rate even after transplantation.
Aims: We have evaluated the safety and efficacy of new conditioning regimen with sequential intensive chemotherapy (FLAG-IDA) followed by conditioning of Flu-Bu(3).
Methods: The study was designed and developed in two separate transplantation centers in Rui Jin Hospital (RJH, Shanghai) and Institut Paoli-Calmettes (IPC, Marseille) respectively. A total of 47 refractory AML patients with median bone marrow blast of 35% (1~90%) and median age at 42y (16~62) were enrolled. Thirteen patients received transplantation with mobilized peripheral blood stem cells (PBSC) from HLA-matched sibling donor while 18 and 16 with matched unrelated or haplo-identical donors. All patients received FLAG + 3-days idarubicine ($12mg/m^2$ in RJH or $10mg/m^2$ in IPC) and then received Fludarabine (5 days) with IV Busulfan (3-days) with a 7-day interval. The GVHD prophylaxis regimens were CsA+MMF±ATG (RJH) or post-cyclophosphamide (IPC).
Results: With a median follow-up of 8 months (1~70m), a total of 14 patients relapsed with a median time of relapse at 4.8 months (2.1~18.1) and most of them (11/14) relapsed within first 6 months after transplantation. A total of 24 patients died due to relapse (n=12) or non-relapsed mortality (NRM, n=12). The estimated 3-year relapse rate (RR) and NRM were 42.0±9.2% and 25.9±6.5% respectively. The estimated 3-year OS and DFS were 43.6±7.8% and 42.2±7.8%. In the primary multivariate analysis (including age, cycles of pre-transplantation chemotherapy, bone marrow blasts, cytogenetics and treatment center), only bone marrow blast ≥35% and age over 40 were associated with disease-free survival and relapse respectively while there was no significant difference between RJH and IPC in terms of transplantation outcome in uni- and multivariate analysis.
Summary/Conclusions: Our primary data demonstrated a promising outcome with FLAG-IDA chemotherapy as debulking therapy sequential with Flu-Bu3 conditioning regimen in patients with refractory AML and clinical trial with larger patients cohort is warranted.

E1548

STUTTER PCR PRODUCTS MAY NOT INTERFERE WITH STR BASED CHIMERISM MONITORING AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION

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Background: Chimerism analysis is one of the main methods to monitor the bone marrow engraftment or disease relapse after allogeneic bone marrow transplantation. Routine test is based on differences in the length of short tandem repeats (STR) from the donor and the recipient. However, chimerism estimation is complicated by stutter PCR peaks appearing due to irregular DNA polymerase activity. Generally, these sequences are 4 nucleotides shorter than a specific marker and may concur with a specific sequence of recipient's DNA hindering chimerism estimation based on that locus. This problem seems to be especially serious in case of a sex-matched sibling BMT when most of the alleles for donor and recipient are the same. One may suggest to limit the use of these markers for the cases with stutter-bands comparable with donor allele peak height. Thereby, the absence of "stutter-peaks free" markers hinders mixed chimerism estimation at the point of low recipient hematopoiesis output.
Aims: To identify the contribution of stutter-bands to the total amount of PCR-product and to derive universal formulas for the chimerism calculation excluding stutter percentage.

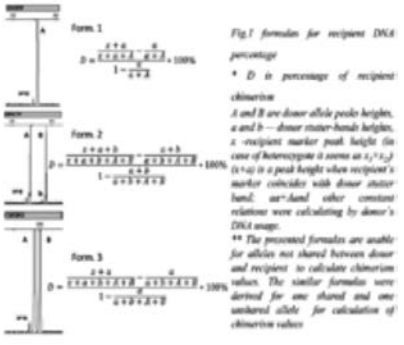

Figure 1.

Methods: Genomic DNAs of donors and patients were isolated from bone marrow samples. Chimerism was assessed by the STR-PCR analysis (polymerase chain reaction with a panel of primers for loci of short tandem repeats) with COrDIS Plus multiplex kit for amplification of 19 polymorphic STR-markers and amelogenin loci. The fragment analysis was performed on a 3130 Genetic Analyzer. The data processing was accomplished using GeneMapper v.4-0 software. Informative loci were chosen beforehand comparing pretransplant

patient DNA and donor DNA. The percentage of donor chimerism as well as stutter percentage was calculated using standard formula.
Results: Fifty transplant cases with stutter peaks were evaluated: 18 homozygous; 15 heterozygous with both alleles showing detectable stutter; 17 heterozygous with one stutter visible only. Stutter percentage and standard deviation were calculated in each case for donor DNA sample and for four bone marrow DNA samples from recipient with established complete donor chimerism taken during the time. It was found that the contribution of the stutter-peaks into the total amount of product ranges from 1.2% to 11% (SD was no more than 1.5% for each locus) for markers with appreciable stutter-bands and seems to be locus-specific constant for each patient. Assuming the stutter percentage as a locus- and patient-specific constant (for the same PCR conditions) we derived a formulae for recipient DNA percentage: *Actual recipient's%=(apparent rec./total DNA ratio - stutter/total DNA ratio):(1-stutter/total DNA ratio)*100%* (special formulas for hetero- and homozygous on fig. 1). To test these formulae the panel of DNA samples with mixed chimerism from 50 to 97% estimated by independent "stutter free" assay was used. The results of chimerism estimation based on "stutter-complicated" markers (using proposed formulae) conventional "stutter-free" markers appeared to be the same (SD<1%).
Summary/Conclusions: The use of formulae described may circumvent the absence of the "stutter-free" informative markers for mixed chimerism estimation.

E1549

INTRODUCING PLERIXAFOR TO IMPROVE MOBILIZATION IN MULTIPLE MYELOMA PATIENTS WHO BEHAVE AS POOR-MOBILIZERS IS COST-EFFECTIVE CONSIDERING THE WHOLE MOBILIZATION AND TRANSPLANT PROCEDURE

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Background: Plerixafor, a CXCR4-antagonist, is efficient to improve CD34$^+$ cell mobilization and collection in candidates for autologous transplantation who behave as poor-mobilizers. The cost of the drug is however of concern. Published medico-economics studies were mostly conducted in the US, and few including detailed and comprehensive micro-costing of the collection and transplantation process; conclusions may thus not apply to European countries where cost structures are different.
Aims: To compare costs and effectiveness of plerixafor-free and plerixafor-replete management strategies for multiple myeloma patients who behaved as poor-mobilizers after adequate administration of a standard rhG-CSF mobilization regimen.
Methods: Sixty patients diagnosed with multiple myeloma were consecutively identified during years 2009-2011, immediately before and after EMA granted marketing authorization for plerixafor. Poor-mobilizers were defined as having circulating CD34+ cell counts below 20/µL. Plerixafor was introduced or not as a result of the attending physician's decision, reflecting progressive changes in medical practices over this transitional period. The historical and study groups were matched over four criteria: disease stage at diagnosis, age, gender and number of chemotherapy treatments received before mobilization. Two cost-effectiveness analyses (CEA) were conducted; the primary CEA looked at the criterion "collecting at least $2x10^6$ CD34+ cells"; a secondary CEA looked at the criterion "successful autologous transplant administered". Detailed micro-costing evaluations (2015 figures) did not or did include transplantation costs for the first and second CEA respectively.
Results: The two groups were similar in terms of age, sex distribution, disease characteristics or previous treatments. 27/30 and 26/30 patients proceeded to high-dose melphalan and autologous transplantation in the study and historical groups, respectively. There was a trend to a higher number of collected CD34$^+$cells in the control group; however, the proportion of patients who met the minimal target number of $2x10^6$ collected CD34$^+$ cells/kg was identical (28/30).Length of hospitalization, times to neutrophil and platelet recoveries, numbers of PRBC and platelet transfusions were identical in the two groups. Mobilization and collection costs per patients were more important in the plerixafor group that in the historical group (8.757 *vs* 5.460 €, p<0.0001), and proportionally higher in patients who received plerixafor as part of a remobilization treatment rather than pre-emptively (10.401 *vs* 8.162€ respectively). The main CEA concluded to a 3.237€ increase in costs for the same number of patients achieving a minimal target number of $2x10^6$ collected CD34$^+$ cells/kg. The second CEA found a decrease in the cost of transplant, with 12.724€ in the study group *vs* 13.634€ in the historical group (NS). In total, the 2.035€ increase for the complete procedure cost (22.866€ per successfully autografted patient in the study group *vs* 20.831€ in the historical group) was not statistically different.
Summary/Conclusions: Cost-effectiveness arguments should not been used against the administration of plerixafor in multiple myeloma patients in the European context. Future prospective researches looking at patients reported outcome criteria and labour organization in apheresis facilities are needed.

This work was supported by a grant from SANOFI S.A.

E1550

PERIPHERAL BLOOD STEM CELL (PBSC) HAPLOIDENTICAL TRANSPLANTATION *VERSUS* MISMATCHED UNRELATED DONOR TRANSPLANTATION: A SINGLE UK CENTRE EXPERIENCE

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Background: Haploidentical (Haplo) and mismatched unrelated donor transplantation (MMUD) are potential alternatives for those without a fully matched available donor. Recent collaborative and single centre studies suggest that haploidentical donor outcomes are comparable to unrelated donor outcomes in the T cell-replete setting.
Aims: In this single centre review, we aimed to compare outcomes of T cell-replete haploidentical allogeneic stem cell transplantation with mismatched unrelated donor allogeneic stem cell transplantation.
Methods: From January 2010 to December 2015, 38 patients underwent T cell-replete HLA-matched haploidentical transplantation with post transplantation cyclophosphamide given on days +3 and +4 given as graft *versus* host disease (GvHD) prophylaxis. These were retrospectively compared with 45 patients underwent single HLA-locus mismatched unrelated donor transplantation with alemtuzumab as GvHD prophylaxis. Data was censored at time of last contact in 2016. Analysis was performed using SPSS v.23.0 and R 3.3.2 statistical software.
Results: The median recipient age was similar in both groups; 51 (19-69) years in Haplo and 59 (28-74) years in MMUD transplants, p=0.012; 68.7% of all patients were male. Non-Caucasian ethnicity comprised 63.2% of Haplo *versus* (vs.) 15.6% of MMUD transplants, p<0.001. Myelodysplasia (MDS)/acute myeloid leukaemia (AML) was the commonest transplant indication in both groups (60.5% of Haplo and 93.6% of MMUD transplants). The disease risk index (DRI) in this subgroup was overall low/intermediate in 69.2% and high/very high in 26.2% (unknown in 4.6%). Reduced intensity conditioning was used in all but two Haplo (4.6%) and 4 MMUD transplants. Patients were followed up for a median of 544 days with a similar 2-year overall survival of 61.5% (95% confidence interval, CI, 52.4 – 69.3%) and 58.1% (95% CI 48.8-66%) and 3-year overall survival of 56.4% (95% CI 45.8 – 65.6%) and 48.9% (95% CI 41 – 56.2%) in Haplo and MMUD transplants respectively, p=0.67. Overall progression free survival (PFS) at 2 years was 53.3% (95% CI 44-61%) and 40.1% (95% CI 34-46%) in Haplo and MMUD transplants respectively, p=0.31. In those with MDS/AML, the 2-year progression-free survival was 64.2% (95% CI 49-75%) in Haplo *vs* 38.5% (95% CI 33-43%) in MMUD transplants, p=0.1. In Haplo and MMUD transplants, the 3-year cumulative incidences of non-relapse mortality were 25.5% (95% CI 12-41%) and 31.2% (95% CI 18-45%) respectively, p=0.61 and of relapse were 25.6% (95% CI 12-41%) and 34.8% (95% CI 20-49%) respectively, p=0.51. Median time to neutrophil engraftment was 18 and 12 days and for platelet engraftment 21 and 12 days in the Haplo and MMUD transplants respectively. Engraftment was successful in 89.4% (Haplo) and 95.5% (MMUD) of patients. The incidence of acute GVHD was 42.1% in Haplo and 35.6% in MMUD transplants but severe (grade 3/4) acute GvHD only occurred in 7.9% (Haplo) and 8.9% (MMUD). Chronic GvHD occurred in 15.8% of Haplo and 33.3% of MMUD transplants, p=0.067. Chronic GvHD did not impact overall or progression free survival in either transplant group.
Summary/Conclusions: T cell-replete haploidentical transplantation when compared with T cell-deplete mismatched unrelated donor transplantation showed high engraftment rates, low rates of severe acute and chronic GVHD and comparable overall survival, non-relapse mortality and relapse rates. We suggest that T cell-replete haploidentical transplantation is a safe and acceptable alternative when a matched unrelated donor is unavailable.

E1551

IMPACT OF ABO BLOOD GROUP INCOMPATIBILITY ON THE OUTCOME OF RECIPIENTS UNDERGOING ALLOGENIC TRASPLANTATION: EXPERIENCE IN OUR CENTER BETWEEN 2013 AND 2016

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Background: ABO blood group compatibility is not an essential requirement or priority in the selection of the allogenic bone marrow donor, unlike what happens in solid organ transplant; thereby, up to 30-50% of allogeneic transpantation shows ABO incompatibility[1], but its clinical impact is controversial. It's accepted that it may provoke hemolitic reactions and delayed erytrhocyte engraftment. Nevertheless, its influence on leukocyte and platelet engraftment, graft-versus-host disease (GvHD), and overall survival is not fully elucidated yet[2].
Aims: To describe the experience in our center in allogenic transplantation with ABO mismatching and its relation with hemolytic events (HE), red blood cell

(RBC) recovery, neutrophil and platelet engraftment, pure red cell aplasia (PRCA), acute GvHD, relapse and overall survival (OS).
Methods: We retrospectively studied allogeneic transplants performed from January 1, 2013 to December 31, 2016. We collected the baseline variables reflected in Table 1 and analyzed the incidence of HE, neutrophil and platelet engraftments, RBC recovery, PRCA (defined as anemia with transfusional requirement and reticulocytes <1% in day +60 without other cytopenias), acute GvHD, relapse of the background disease and survival (at 6, 12 and 24 months) in the ABO compatible groups (ABOc) and in the incompatible (ABOi), the latest divided into major, minor and bidirectional disparity.
Results: A total of 133 transplants were included, with a mean follow-up time of 16.4 months. The median age was 52 years and there were 79 males and 54 females. Diagnoses were mainly AML (n=72), ALL (n=19) and NHL (n=11) (see Table 1). 60 received low intensity and 73 myeloablative regimens. They were HLA identical (n=44), unrelated donor (n=50), haploidentic (n=38) and cord (n=1) and, in most cases, hematopoietic progenitors were obtained from mobilized peripheral blood (90.2%). 44.3% (n=59) presented some type of ABOi: major (n=26), minor (n=25) and bidirectional (n=8). The product was processed in order to prevent hemolysis in only 7 cases (red cell depletion in 4 and deplasmatization in 3). There were 23 hemolytic (18 immediate and 5 delayed) -mostly mild- events, which appeared predominantly in patients with ABO-incompatibility (38.98%)- 50% in major disparity, 28% in minor and 37.5% in bidirectional- *vs* ABOc (2.7%) and this difference was statistically significant (p<0.0001). No differences were observed in the neutrophil graft between the ABOc group and the ABOi group, nor in the platelet engraftment; in contrast, we found a statistically significant effect on the time to erythrocyte recovery (mean: 49.94 days in ABOi *vs* 24.69 in ABOc; p=0.032). Only 6 cases of PRCA were documented (all in ABOi). The occurrence of acute GvHD did not differ significantly among the groups (52% in ABOc *vs* 53.5% in incompatibles) nor in its severity. We have not found differences either in the rate of relapse (24.6% *vs* 19.6%) nor in the survivals at 6, 12 or 24 months (66.1% *vs* 78.8%, 48.2 *vs* 47.2% and 38.4 *vs* 39.4%, respectively).

Table 1.

Table 1

	ABOc (n=74)	ABOi (Major) (n=26)	ABOi (bidirectional) (n=8)	ABOi (minor) (n= 25)
Gender (M: F)	45: 29	17:9	3:5	14:11
Age (median)	52	52	30	54
Background disease				
-AML/MSD	45	12	4	11
-ALL	9	4	2	4
-NHL	5	2	-	4
-HL	2	1	1	-
-MM	5	1	-	1
-AA/PNH	1	1	1	2
-Other	7	5	-	3
Donor thype				
-Related HLA-identical	30	6	2	6
-Unrelated	20	11	4	15
-Haploidentical	24	9	2	4
Source (PB: BM: Cord)	69: 5:0	21: 5: 0	6: 1: 1	24: 1: 0
Intensity (MA: RIC)	40:34	15: 11	7: 1	11: 14
Product processation	0	3	3	1

** M=male, F=female; AML= acute myeloid leukemia, MSD: Myelodisplastic syndrome, ALL=acute lymphoblastic leukemia, NHL= non hodgkin lymphoma, MM= multiple myeloma, AA= aplastic anemia, PNH= paroxysmal nocturnal hemoglobinuria; PB= peripheral blood, BM= bone marrow; MA= myeloablative, RIC= reduced intensity conditioning.*

Summary/Conclusions: In our study ABO-mismatched transplants have shown a greater number of hemolytic events and red cell aplasia, as well as a greater delay in achieving erythrocye recovery. However, we have not found an association with delayed neutrophil and platelet recoveries, increased acute GvHD, relapse or worse OS in the ABO incompatible group, in keeping with most previous reports**, although the absence of effect might be as well be related to an insufficient study power due to low sample size.

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E1552

LOW BLOOD CONCENTRATION OF TACROLIMUS CAN BE A RISK OF GRAFT FAILURE AFTER CORD BLOOD TRANSPLANTATION

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Background: Cord blood transplantation (CBT) has recently emerged as an attractive alternative donor. However, graft failure still remains potential threats for morbidity and mortality.
Aims: Several biological mechanisms may contribute to graft failure. Immunological rejection of the graft is known as a major cause of graft failure. Graft failure may also be caused by septicemia, viral infections, drug toxicity and so on.These events have been frequently occurred just before engraftment, and we often experience fluctuation of blood levels of immunosuppressive drugs. Here, we analyzed an association between blood levels of Tacrolimus (Tac) before neutrophil engraftment and neutrophil engraftment.
Methods: Between January 2011 and Jury 2016, 76 patients received single-unit CBT at our institutions. We analyzed 59 patients for whom Tac was used for GVHD prophylaxis including Tac and Mycophenolate mofetil (MMF) combination (n=26) and Tac with an additional short Methotrexate (sMTX) (n=33). Sixteen patients who underwent second or third CBT and a patient for whom Tac was not used for GVHD prophylaxis were excluded. We also excluded a patient whose Tac concentration we didn't check more than two times a week. Tac was started at a dose of 0.02mg/kg/day by continuous i.v. infusion. Tac blood concentrations were monitored at least three times a week before engraftment, and dosages were adjusted to maintain serum levels about 10-20 ng/ml.
Results: Of the 59 patients, 48 patients achieved neutrophil recovery at a median of 22 (range 13-35) days. Two patients died before engraftment from severe PIR and active infection. Nine patients (18.6%) experienced graft failure. Patients who could maintain Tac level above 12ng/ml during the second week after CBT (Tac high group) had an incidence of graft failure of 4.8%, which was significantly lower than the 26.3% seen in the other patients (Tac low group) (p<0.01). Patients for whom Tac and MMF were used (MMF group) had an incidence of graft failure of 3.8%, which was significantly lower than the 36.4% seen in the other patients for whom Tac with an additional sMTX (MTX group) for GVHD prophylaxis (p<0.01). Combined of these factors, the patients of Tac low group and MTX group had had an incidence of graft failure 40.9%, which was significantly highest than the 5.4% seen in the other patient including Tac high group and MMF group even if the patient were included of Tac low group.

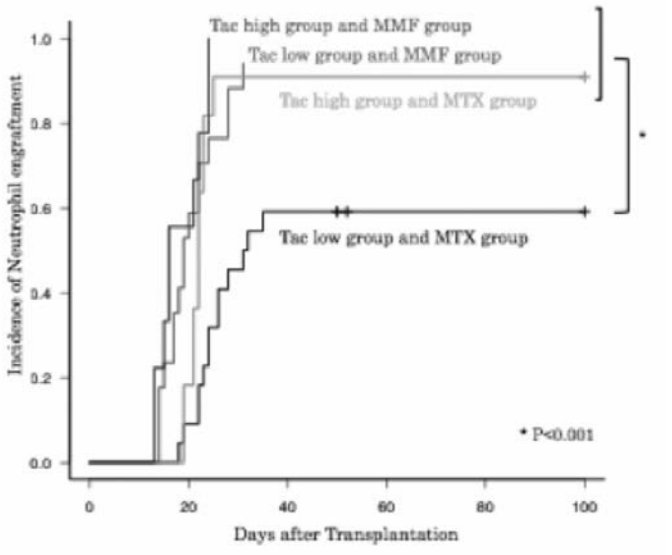

Figure 1.

Summary/Conclusions: Low levels of Tac blood concentration were significantly associated with the incidence of graft failure of the patient for whom Tac with an additional sMTX were used for GVHD prophylaxis. Before engraftment, frequent checks of the Tac blood concentration and maintaining the drug level should be considered for these patients.

E1553

THE EXPRESSION OF TOLL-LIKE RECEPTORS GENES IN PATIENTS WITH LYMPHOID MALIGNANCIES AFTER AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

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Background: Peripheral blood stem cell transplantation (PBSCT) is one of the main strategies for the treatment of malignant hematological diseases. Toll like receptors (TLRs) are present on various immune cells including natural killer cells, monocytes, macrophages, T lymphocytes and B lymphocytes. Ten different TLRs have been evaluated in humans. TLRs play a central role in immune surveillance and in the initiation of the inflammatory response. The expression of TLRs genes and their association with outcome in patients treated with PBSCT remains unclear.
Aims: The objective of the current study was to investigate association between expression of TLRs genes and hematopoietic recovery and rate of infections in patients treated with PBSCT.

Methods: The evaluation of TLRs expression genes were performed in 40 patients who underwent PBSCT. The median age of patients was 54 years (range: 25-65 years). There were 15 patients with multiple myeloma (MM), 20 patients with non-Hodgkin lymphomas (nHLs) and 5 patients with Hodgkin lymphoma (HL). Peripheral blood samples were taken before megachemotherapy with autologous stem cell transplantation and at time of hematopoietic recovery in patients after PBSCT. Relative expression of Toll-like receptors was assessed by real-time PCR using inventoried TaqMan® Assays from Life Technologies/ThermoFisher. Beta glucoronidase (GUSB) served as endogenous control. Reaction was performed in 7500 Real Time PCR instrument (LifeTechnologies) using Gene Expression MasterMix (LifeTechnologies/ThermoFisher). Comparative C_T method (**) was used to compare expression among patients and with healthy controls. Statistical analysis was conducted using STATISTICA 12 software (StatSoft, Polska). For quantitative variables arithmetic means (X) and standard deviations (SD) of estimated parameters were calculated in the analysed groups. Distribution of variables was examined using tests of Lilliefors and W-Shapiro-Wilk. In cases of independent quantitative variables with the normal distribution the statistical analysis took advantage of t test for unlinked variables. In cases of variables manifesting distribution distinct than the normal one, for independent quantitative variables U test of Mann-Whitney was used. For dependent quantitative variables of the normal distribution, the t test for linked variables was applied. In cases of quantitative dependent variables with the distribution distinct from normal, the pair sequence test of Wilcoxon was applied. In order to define a relationships between the studied variables, correlation analysis was performed. Results at the level of p<0,05 were assumed to be of statistical significance.
Results: The mRNA expression of TLR2 and TLR9 was significant higher in patients after PBSCT than before PBSCT procedure (ΔCt TLR2 1,4209±1,0461 *vs* 1,7877±1,4974 and *Δ*Ct TLR9 117,853±141,0870 *vs* 289,788±271,98) (p<0,05). We observed that expression of TLR9 was significant higher in patients with bacterial and fungal infection after PBSCT in comparison to group without infection after PBSCT (ΔCt TLR9 117,853±141,087 *vs* 289,788±271,98) (p<0,05). Moreover we found significant positive correlation between expression of mRNA of TLR9 and neutrophil recovery after PBSCT (r=0,4075; p=0,023).
Summary/Conclusions: In conclusion our findings suggest that TLRs could be useful markers in outcome in patients treated with PBSCT.
This observation should be validated by larger study.

E1554

TIMING OF DEFIBROTIDE INITIATION POST-DIAGNOSIS OF HEPATIC VENO-OCCLUSIVE DISEASE/SINUSOIDAL OBSTRUCTION SYNDROME AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION: EXPANDED ACCESS PROGRAM FINAL DATA

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Background: Hepatic veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS) is an unpredictable, potentially life-threatening complication of hematopoietic stem cell transplantation (HSCT). VOD/SOS with multi-organ dysfunction (MOD) may be associated with >80% mortality. Defibrotide is approved to treat severe hepatic VOD/SOS post-HSCT in the European Union and to treat hepatic VOD/SOS with renal and/or pulmonary dysfunction post-HSCT in the United States. Prior to approval in the United States, defibrotide had been available via an expanded-access program.
Aims: To perform an exploratory *post hoc* analysis of final data from the expanded-access program on the impact on Day +100 survival of timing of initiation of defibrotide after diagnosis of VOD/SOS in HSCT patients.
Methods: In an expanded-access study, patients diagnosed with VOD/SOS (per Baltimore criteria, modified Seattle criteria or biopsy) with or without renal/pulmonary MOD after HSCT or chemotherapy received defibrotide 25mg/kg/d in 4 divided doses for a recommended ≥21 days after patients provided informed consent. For these exploratory analyses, D*ay +100* survival rates in HSCT patients were examined *post hoc* by time from VOD/SOS diagnosis to start of defibrotide for (1) all patients before/after days 1, 2, 3, 4, 7, and 14, using Fisher's exact test and (2) patients starting defibrotide on a particular day: 0, 1, 2, 3, 4, 5, 6, 7, 8–14, and ≥15, by Cochran-Armitage test for trend across days. Causes of treatment delay were not assessed.
Results: In the final dataset, timing of initiation date was available for 1000 HSCT patients (512 with MOD) who received ≥1 dose of defibrotide. In 31.0% of all HSCT patients, defibrotide was started the day of diagnosis; in 92.9%, by Day 7. In the population-wide analysis of initiation before/after days 1, 2, 3, 4, 7, and 14 post-diagnosis in both the overall group and MOD subgroup (**Figure 1**), earlier initiation was associated with significantly higher Day +100 survival rates for all days (*P*≤.001), except Day 14 (2.6% of patients started defibrotide after Day 14). The trend test for particular initiation days also showed a significant trend over time for higher Day +100 survival with earlier defibrotide initiation post-diagnosis in both the overall HSCT group and MOD subgroup (*P*<.001). Adverse events (AEs) and serious AEs occurred in 70.8% and 53.4% of patients, respectively. Other than VOD/SOS and MOD, the most common AE was hypotension (11.7%) and most common serious AE was respiratory failure (7.3%).

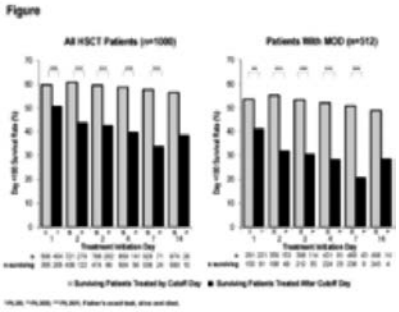

Figure 1.

Summary/Conclusions: In this exploratory analysis of final study data, earlier defibrotide initiation post-VOD/SOS diagnosis significantly improved Day +100 survival, confirmed by the Cochran-Armitage test (*P*<.001). No specific day provides a clinically meaningful cutoff for better Day +100 survival, suggesting that later intervention retains value if treatment must be delayed.

Support: Jazz Pharmaceuticals.

E1555

RED BLOOD CELL DISTRIBUTION WIDTH (RDW) AS AN ACUTE GRAFT *VERSUS* HOST DISEASE PREDICTOR MARKER IN ALLOGENIC STEM CELL TRANSPLANTATION

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Background: The red blood cell distribution width (RDW) is a common parameter for measuring anisocytosis in the study of anemia. Recently it has been regarded as a surrogate marker of inflammation and adverse outcome in several diseases. Acute graft-versus-host disease (GVHD) is a common complication of allogeneic hematopoietic cell transplant (allo-HSCT) which is related to inflammation in the context of damage of the host tissue and the release of inflammatory cytokines. We decided to study the utility of this potential inflammatory marker in the setting of GVHD in the allo-HSCT.

Table 1.

N:	103
GENDER:	
Male	59
Female	44
MEDIAN YEARS OLD:	43.7 (2 - 70)
HAEMATOLOGICAL DISEASE	
AML:	41
MDS:	15
Lymphoproliferative syndrome:	
CML:	30
APLASTIC ANEMIA:	5
MM:	6
cMPS:	3
	3
aGVHD:	
YES	62
NO	41
MEDIAN RDW:	16.4 (11.2 - 38.5)
RDW DISTRIBUTION:	
<14.7:	27
14.7-16.3	26
16.4-18.4	26
> 18.4	24
MEDIAN OS:	41 months
MEDIAN FOLLOW UP:	12.8 months
RDW 18.4	aGVHD
<18.4 (N 72)	51% (37)
>18.4 (N 31)	80% (25)
Correlation of high RDW and GVHD	
<14 vs >14	P= (0.604)
<16 vs >16	P= (0.828)
<18.4 vs >18.4	**P= (0.009)**
Haplo-HSCT subgroup	N 13
Male	9
Female	4
RDW>16	5
RDW<16	8

Aims: RDW values were evaluated at the day of infusion (RDW 0), we choose this point in time to evaluate the tissue injury and inflammation secondary to the conditioning regimen, in order to evaluate if there is a major incidence of GVHD.
Methods: We retrospectively evaluated 103 patients who had underwent allo-HSCT for different indications at our center, with a median follow up of 12.8 months (0-235) at our center. The population consisted of 59 males and 44 females, the median age was 43.7 years. The RDW was collected from the hemogram at the day of the HSCT cell infusion, before it was performed (table 1). The IBM SPSS STADISTICS program was used for all statistical analyses. Differences were considered statistically significant when p<005. The median of RDW values in our study was of 16.4 (11.2-38.5). The areas under the receiver operating characteristic (ROC) curves of RDW were ≤18.4 and >18.4 for the selection of the increased RDW cutoff. We evaluated the association of increased RDW (>18.4) with the development of GHVD. A survival analysis of the association of different levels of increased RDW was performed. A subgroup analysis of the Haploidentical HSCT patients (N=13) was also evaluated.

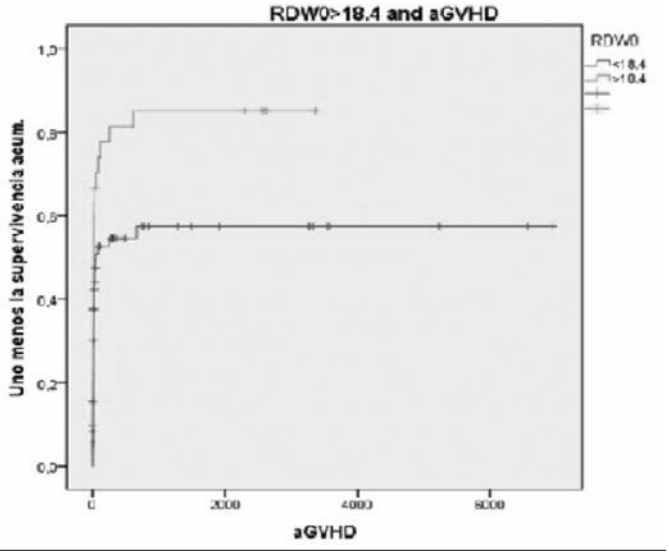

Figure 1.

Results: The presence of increased RDW >18.4 was strongly associated with an increased risk of developing acute GVHD (p=0.009) being present in 80% of the patients. In the haploidentical HSCT subgroup an increased RDW >16 was associated with acute GVHD. (p=0.044). There was no association of chronic GVHD with elevated RDW at day 0 (p=0.563). The survival analysis didn't found an association of high RDW levels with mortality or survival (p=0.301) but a tendency to an increased survival was show between the RDW level subgroups. (figure2). Where a higher RDW seems to have a better survival, but this should be evaluated in a wider sample.
Summary/Conclusions: RDW at day 0 is a feasible predictor factor of Acute GHVD, most likely as a secondary surrogate marker of inflammation secondary to the conditioning regimen. The presence of other factors contributing to the RDW increase (secondary to other comorbidities) cannot be ruled out; but by itself RDW it's an easy and affordable prognosis marker for aGVHD that should be further evaluated.

E1556

COMPARISON OF THE BEEAM CONDITIONING REGIMEN AND THE BEAM CONDITIONING REGIMEN IN THE AUTOLOGOUS TRANSPLANATAION FOR HL AND NHL

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Background: The BEAM has established itself as a standard of care conditioning regimen in the autologous lymphoma HSCT setting for most transplant centres in Europe. Yet however various other regimens are being compared with it in order to achieved better safety profile, better OS and DFS, in order to improve results with chemoresistant and unfavourable patients. One such regimen is BeEAM (bendamustine, etoposide, cytarabine, melphalan).
Aims: We aimed to compare the efficacy of the BEAM and BeEAM conditioning regimens and to compare there myelotoxicity profile.
Methods: We evaluated retrospectively 114 patients, receiving auto-HSCT at the National Specialized Hospital for Active Treatment of Hematological Diseases in Sofia for relapsed/refractory HL or NHL for the period from 1.01.2013 to 1.07.2016 with a follow-up of patients up to 1.11.2016. 92 of the patients received BEAM and 22 received BeEAM. 2 and 3 year OS and DFS were compared, CR rates and the average time periods to hematological recovery.

Results: The OS at 2 and 3 years respectively was 86.1%, 86.1%, for BeEAM and 78%, 71% for BEAM, the DFS at 3 years was 76.4% in BeEAM and 73.2% BEAM, provided that the differences did not have statistical significance. The CR rate was 63.63% in the BeEAM group *versus* 50% in the BCNU group. 22.72% of the patients receiving BeEAM in SD or in diseases progression achieved CR *versus* 10.86% respectively for the BEAM group. The mean time to hematological recovery for neutrophils was 11.27 days (BeEAM) *versus* 10.24 days (BEAM) and 12.64 days (BeEAM) *versus* 11.12 days (BEAM) for platelets.

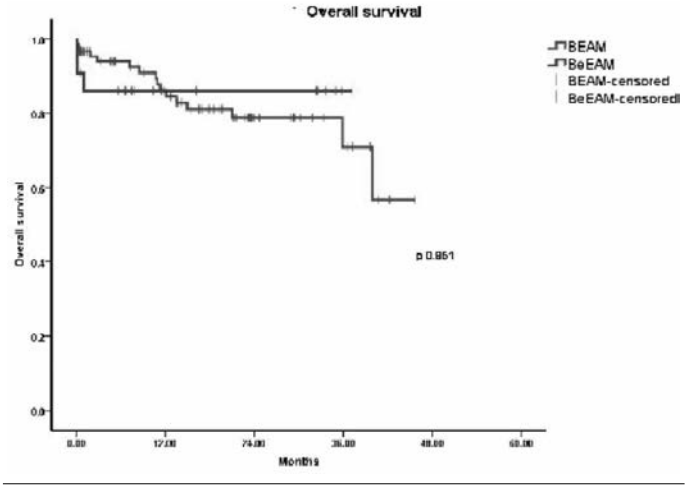

Figure 1.

Summary/Conclusions: BeEAM appears to be a non-inferior alternative conditioning regimen to the standard BEAM, it shows a trend towards higher myelotoxicity, but also a trend towards better short-term results in chemoresistant patients.

E1557

DOUBLE UMBILICAL CORD BLOOD TRANSPLANTATION IN ADULTS: CORRELATION OF ALLELE-LEVEL HLA MATCHING WITH OUTCOME AND WHICH CORD BLOOD UNIT WILL BECOME DOMINANT

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Background: Umbilical cord blood (UCB) has been used for alternative donor transplantation for the past 3 decades. Graft failure is not uncommon due to higher degrees of histoincompatibility between recipient and UCB units and fewer hematopoietic precursors in the product. To improve engraftment rates, especially in larger (*i.e.* adult) patients (pts), two UCB units can be used. Double UCB transplantation (DUCBT) is being utilized at many centres although it has been noted that, while both units may contribute to engraftment, only one unit becomes "dominant" – *i.e.* persists to provide long-term hematopoiesis. A variety of predictors of which unit will become dominant have been suggested, primarily the unit that is more closely HLA-matched or the unit with the highest total nucleated cell (TNC) count.
Aims: To determine the likelihood of engraftment, incidence of GVHD, influence of TNC count and HLA mismatch on survival and selection of the dominant cord following DUCBT in adults with high-risk hematologic disorders.
Methods: A retrospective review was performed of adult pts undergoing DUCBT at the referral centre for British Columbia. Recipients signed informed consents for all clinical trials in which they participated. HLA typing at A, B, C and DRB1 loci was done on all pts using high-resolution allele-level testing (HRT). HRT was available at these 8 loci for both UCB units in 25/31 pts; for the remaining units, class I typing was done by serology. UCB units selected had to be ≥4/6 match at A, B (serologically) and DRB1 (by HRT). Combined TNC count for the units had to be ≥30x10^6/kg recipient weight. Conditioning was Fludarabine 40mg/m^2 x4 and TBI 150 cGy x9; GVHD prophylaxis was Tacrolimus/Mycophenolate. Pts received G-CSF 300 mcg s.c. daily from day +1. Outcomes were compared using Fisher's exact test.
Results: Between 06/09 and 09/16, 31 pts underwent DUCBT - 11 males, 20 females with median age 50 years (range 19-59). Diagnosis was acute myeloid leukemia (AML; n=12), acute lymphoid/mixed phenotype leukemia (n=7), chronic lymphoproliferative disease (n=5), MDS (n=4) or other (n=3). All 31 pts recovered ANC>0.5x10^9/L at median of 20 days (range 14-72). Platelet count reached >20x10^9/L in 26/31 pts at median of 38 days (range 24-188). Acute GVHD developed in 26/31 pts (84%) and chronic GVHD in 17 of the 26 pts (65%) that survived to day +100. Seventeen pts (55%) remain alive, in contin-

uous remission at median follow-up of 3 years (range 0.5-7.0). Ten pts (32%) experienced non-relapse mortality from GVHD (5 pts), infection (4 pts) or unknown cause (1 pt). Four pts (13%) have relapsed at 3, 5, 10 and 12 months. Outcomes for pts when the best cord unit match was 0-2 antigen-mismatched (Ag-M/M) were superior (8/12 alive and well) to those pts when the best unit was 3 Ag-M/M (3/9 alive and well; p=0.20). Unexpectedly, 6/9 pts whose best unit was ≥4 Ag-M/M are alive and well. Information on the dominant cord was available on 19 pts (Table 1); in 15/19 pts, the dominant cord was the same or a better HLA match compared to 4/19 with a dominant cord that was an inferior HLA match (p<0.001). However, the TNC was of less importance with the lower TNC unit being dominant as frequently as the higher TNC unit for each HLA match category (Table1).

Table 1.

HLA Match	Higher TNC	Same TNC	Lower TNC
Better n=7 (37%)	3(43%)	1(14%)	3(43%)
Same n=8 (42%)	4(50%)	2(25%)	2(25%)
Worse n=4 (21%)	1(25%)	2(50%)	1(25%)

Summary/Conclusions: DUCBT is effective in adults with life-threatening hematologic disorders. With current UCB inventories, conditioning therapies and supportive care, graft failure is rare - even in adults. HLA disparity between the UCB unit and the patient is a better predictor than the TNC regarding which unit will become dominant. Pts receiving well-matched UCB units (0-2 Ag-M/M) may have better outcomes than pts receiving 3 Ag-M/M units although successful outcomes can be seen even with a high degree (≥4 Ag-M/M) of HLA incompatibility.

E1558

CLINICAL ANALYSIS OF HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR 46 ACTIVE RELAPSED AND REFRACTORY ACUTE PEDIATRIC LEUKEMIA

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Background: Given the dismal prognosis for relapsed and refractory (R/R) acute leukemia, many physicians discourage offering hematopoietic stem cell transplantation (HSCT) to adults patients with bone marrow (BM) blasts over 25%. Therapeutic recommendations for pediatric subjects with a similar situation are not available.
Aims: With no significant alternative managing options for these patients, more data are required to make an informed and patient tailored decision.
Methods: We retrospectively analyzed the preliminary outcome of 46 active R/R acute pediatric leukemia including 30 AML and 16 ALL receiving transplantation between 2012 and 2016. Median age at HSCT was 13 years. Active R/R disease was all confirmed by cytogenetics/molecular genetics and aggressive clinical course. Median bone marrow blasts was 46.4% (5-99%). Of note, 27 patients had over 50% blasts in BM. The earliest 13 transplants were conditioned with conventional Bu/Cy or TBI/Cy regimen, thereafter, all received intensified conditioning including FLAG/TBI (N=21), FLAG/Bu/CY (N=2) and CLAG/Bu/Cy (N=10). Immuno-suppressive agents withdrawal started since day 30 if no acute GVHD occurred. Variety of post-HSCT intervention including donor lymphocytes infusion and interleukine-2 injection were performed to reduce relapse. Median follow-up of the whole cohort is 19 months (3–53 months).
Results: Forty-five (97.8%) achieved CR following HSCT. One died of infection before engraftment. All 3 death occurred before 90 day due to relapse. Transplant-related mortality at 1 year was 15.2%. Acute GVHD incidence was 49.3% (grade III 20.4%), chronic 59.5%. Relapse was the major cause of treatment failure and occurred in 28.3% of patients at a median of 1 year post HSCT. Two-year overall survival and leukemia-free survival were 44.8±9.5% and 44.6±8.9%, respectively. Survival of AML patients was superior to those of ALL. Besides, outcomes in patients with primary refractory disease were equivalent to those with relapsed refractory AML which not seen in ALL. Blast percentage 50% in the BM pre-HSCT, TBI based conditioning and chronic GVHD proved to be favorable prognostic features.
Summary/Conclusions: This may validate decision making on if this special group of patients should receive HSCT as salvage treatment.

E1559

POST-TRANSPLANT HIGH-DOSE CYCLOPHOSPHAMIDE AFFECT T-CELL RECONSTITUTION IN BONE MARROW, BUT NOT IN PERIPHERAL BLOOD STEM CELLS RECIPIENTS

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Background: Hematopoetic stem cell transplantation (HSCT) it the only curative therapy for many patients with hematologic malignancies. Occurrence of complications and mortality after allo HSCT still high and it's strongly associated with immune reconstitution. Despite the wide-spread of Post-Transplant High-Dose Cyclophosphamide (PTCy) immune reconstitution and immunological safety of this method is still poorly understood.
Aims: Evaluated immune reconstitution profile in patients who received HSCT with and without PTCy.
Methods: 62 patients who underwent allogeneic PBSCT in our institution were analyzed in 2 groups; patients with PTCy (n=28) and without PTCy (n=34). The total cohort had 22 males and 40 females, and had median age of 33 years (range 19-61). All patients had hematological malignancy. 21 patients underwent myeloablative conditioning and 41 patients non-myeloalative. In 41 patients received bone marrow transplant. The GVHD prophylaxis consisted of a combinations of ATG-PTCy-CsA-MMF (n=10), ATG-PTCy (n=5), Mono-PTCy (n=1), ATG-CsA-MMF-MTX (n=20), CsA-MTX (n=2), ATG-CsA-MTX (n=2), CsA-MMF-MTX(n=1). In 21 patients received PBSCT. The GVHD prophylaxis consisted of a combinations of ATG-PTCy-CsA-MMF (n=4), ATG-PTCy (n=8), ATG-CsA-MMF-MTX (n=8), ATG-CsA-MTX (n=1). 21 patients had progression disease before transplantation, this could affect the results. Immune reconstitution profile was tested via serial flow cytometry analysis of peripheral blood on day +14, +30, +60 and +90 after allo-HSCT. Anti-CD3 FITC, anti-CD16PE, anti-CD56 PE, anti-CD45 Per-CP-CY^{TM}5.5, anti-CD4 PE-Cy^{TM}7, anti-CD19 APC, anti-CD8 APC-Cy7; anti-CD62L FITC (BD Biosciences, USA); anti-CD14PE, anti-CD16PE, anti-HLA-DR APC (eBiosciences, USA) were used to defined white blood cells subsets.
Results: In a bone marrow recipients the number of CD4$^+$ cells was significantly lower when using PTCy (see Figure 1). On day 14 CD4$^+$ cells count for bone marrow recipients was 42,62±9,99; on day 30 - 114,29±42,36; on day 60 – 140,81±42,53; on day 90 - 126,83±26,12. On day 14 CD4$^+$ cells count for PBSC recipients was 47,47±19,99; on day 30 - 131,49±83,26; on day 60 – 148,08±58,22; on day 90 - 162,93±62,94. At the same time when using the PBSC transplant number of CD4$^+$ cells was not significantly different.

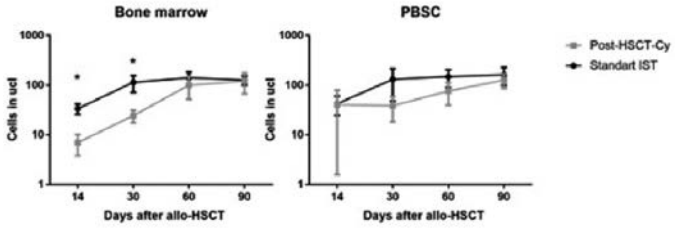

Figure 1. Short-term reconstitution in BM and PBSC recipients with and without Post-HSCT-Cy.

Summary/Conclusions: Lymphocyte recovery was immpared for the PTCy groups in the immediate post-HSCT period but quicly recovered. The mechanism of tolerance induction using PTCy on the + 3, + 4 day not limited to deletion of alloreactive T-cell clones, but also affects other leukocyte subpopulations (B cells, monocytes, granulocytes). The use of PTCy at + 3, + 4 a day is immunologically safe method for prevention of GVHD.

E1560

OUTCOMES OF PATIENTS RELAPSING FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION FOR AML IN FIRST CR: SINGLE CENTER EXPERIENCE

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Background: Allogeneic stem-cell transplantation (SCT) is a curative therapy for patients with AML but disease relapse continues to be the most common reason for treatment failure. There is no standard therapy for relapse after SCT and treatment results are very poor. Treatment options range from supportive care through chemotherapy and donor lymphocyte infusion (DLI) up to a second SCT from the same or a different donor.
Aims: We report a retrospective study of 36 patients AML relapsed patients following allogeneic stem cell transplantation in first CR.
Methods: Between 2000 and 2016, 130 adults with AML in first CR underwent allo-SCT. We identified 36/130 patients (27%) who had relapsed and proceeded to review the management and outcomes of these patients; the incidence of relapse was 20% and 54% after myeloablative and reduced intensity conditioning, respectively. The median time to disease relapse after allo-SCT was 11 months (range 5-48); 15/36 (41%) of relapsed patients suffered aGvHD grade II-IV or extensive cGvHD. At time of relapse 15/36 (41%) patients were still taking immunosuppressive treatment, which was immediately suspended.

Results: The patients was subdivided into three groups according to the salvage treatment received palliative /supportive care (PSC group, n=9, 25%), intensive chemotherapy alone(CHT group, n=18, 50%) and chemotherapy with immunotherapy (donor lymphocyte infusion or second SCT)(IT groups, n=9, 25%). Median age at the start of treatment from relapse was 10, 20 and 25 days in the PSC, CHT and IT groups, respectively. In the CHT group, 3 patients (16%) achieved a second CR and 4 (22%) died during reinduction chemotherapy. In the IT group, 6 (66%) pts achieved a second CR after chemotherapy and DLI/second allo-SCT and 3 (34%) died of treatment toxicity. In the whole patients sample, median overall survival (OS) was 7 months (range 2-74), being 4, 5, 13 months in the PSC, CHT and IT group, respectively. Estimated 1-year and 2-years overall survival was 10%, 15%, 40% and 0%, 0%, 12% in the PSC, CHT and IT groups, respectively. In our experience, 3 independent factors for a longer OS after chemotherapy and immunotherapy have be identified: the absence of previous acute o chronic GvHD (HR=2.7,p<0.001), a longer interval between the allo-SCT and relapse than 12 months (HR=1.2, p<0.005) and age less than 40 years (HR=1.3, p<0.005).
Summary/Conclusions: This study shows that salvage chemotherapy (with DLI or second allo-SCT) provides the best results and should be offered, whenever possible, to patients with AML who relapse after allo-SCT performed in first CR. Patients undergoing chemotherapy alone had a poorer outcome. Our results emphasize the need to schedule a prospective protocol combining cytoreductive treatments and immunotherapy in patients with AML relapsing after allo-SCT.

E1561

ALLOGENEIC STEM CELL TRANSPLANTATION FOR PATIENTS WITH CHEMOREFRACTORY HODGKIN LYMPHOMAS: A RETROSPECTIVE MULTICENTER EXPERIENCE OF THE RETE EMATOLOGICA PUGLIESE (REP)

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Background: Second-line salvage high-dose chemotherapy and autologous stem cell transplantation (SCT) have become the standard of care for refractory/relapsed Hodgkin's lymphomas (HL), leading to durable responses in approximately 50% of relapsed patients and a minority of refractory patients. Patients with refractory HL after autologous SCT generally have poor clinical outcomes with available therapies and by far, allogeneic SCT represents the only strategy with a curative potential.
Aims: We examined allogeneic transplantation outcomes patients with HL chemorefractory following last salvage treatment.
Methods: 39 patients with HL who received allogeneic SCT in chemorefractory disease, from 2000 to 2016 were retrospectively studied. The median age was 34 years (range 16-57 years) and 23 (59%) were male. The majority of patients (80%) had had a prior autologous SCT. Most (90%) patients received reduced-intensity conditioning, 59% received matched sibling donor and 41% matched-unrelated donor grafts.
Results: 36 patients survived beyond 100 days and were evaluable for chronic GVHD of whom 22 (61%) remained free of cGVHD and 14 (39%) developed cGVHD. The disease status at day 100 post-transplant was reported in 36 out of 39 evaluable patients. 7 (19%) achieved a CR, 11 (31%) had a PR, 15 (42%) a stable disease and 3 (8%) had progressive disease. Following transplantation 30 (77%) patients have relapsed or progressed at a median time of 12.7 months (range 1- 39 months) post-transplant. With a median follow-up of 28 months (range 3-95 months) 7 patients remain alive in complete remission, 2 are in stable disease and 26 have died. The Kaplan-Meier estimates PFS at five years was 18%. 6 patients (18%) died of non-relapse mortality (NRM) at a median of 300 days (range 28 days- 40 months) following transplantation. The causes of death included infection (n=2), GVHD (n=3), multi-organ failure (n=1).
Summary/Conclusions: Allogeneic SCT could also be a viable option for patients who are refractory to salvage chemotherapy, especially because better results are obtained when this treatment is applied earlier. Despite the reduction of NRM and GVHD, disease relapse still represents the major issue in the setting of allogeneic SCT failure. The availability of novel agents resulting in objective responses may eventually result in increased eligibility for allogeneic SCT.

E1562

RESULTS OF THE IMPLEMENTATION OF CRYOTHERAPY IN PROTOCOLS OF ORAL MUCOSITIS PROPHYLAXIS IN PATIENTS SUBJECT TO A TRASPLANT OF HEMATOPOYETIC PROGENITORS. EXPERIENCE OF ONE CENTER

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Background: Oral mucositis (OM) is one of the main complication during stem cell trasplantation (HSCT). It has an incidence varies between 47-100%. Numerous prevention strategies have been studied. However, the recommendations of the international guidelines have low evidence to back them up. Cryotherapy is used to reduce OM in conditions that use Melphalan. In our center, we have the cryotherapy implemented in our OM prevention protocol since 2012.
Aims: The main aim is to compare the results in terms of incidence and severity of OM (measured according to World Health Organization scale) in patients in whom cryotherapy was applied and in whom it was not applied as well as the necessity of using morphine and parenteral nutrition. The secondary endpoint is to analyze the occurrence and duration of fever and documentation of infection. Also the length of hospital stay and mortality on day 100.
Methods: We used a cohort of patients with plasma cell dyscrasias who underwent autologous stem-cell transplant with conditioning melphalan 200, Busulfan-Melphalan 140 or melphalan 100 in hemodialysis regimen in which cryotherapy was not applied (2007-2011) and another cohort in which was applied (2014-2016). We did not collect 2012 or 2013 because the measure was being implemented. It consists of administering ice poles to the patient who must chew before, during and after the infusion of melphalan. The t-Studen and Chi square method was used to estimate the rates of incidence and the baseline characteristics. The regression logistic method was used to the multivariant and univaritan analysis. Hazard ratios and 95% were estimated with the use of logistic regression model.
Results: The baselines characteristics can be seen in table 1. All patients in both groups had OM. In the cryotherapy and non-cryotherapy groups, the distribution was respectively: grade I 20% *vs* 16%, grade II 40% *vs* 10.8%, grade III 31.4% *vs* 59.4% and grade IV 8.5% *vs* 13.5%. We observed a reduction in the incidence of severe mucositis (grade III and IV) in the group in which cryotherapy was used against the cohort in which it was not (40% *vs* 72.9%, p=0.005). The need for morphine was also lower in the cryotherapy cohort (54% *vs* 72%, p=0.149). The use of parenteral nutrition was lower in the non-cryotherapy group (8.57% *vs* 13.5%, p=0.7).The prevalence of fever was predominant in the cryotherapy group (51% *vs* 43%, p=0.48), but and infection was documented on more occasions in cryotherapy group (27% *vs* 81%, p=0.04). The median number of days the patients were discharged from the cryotherapy group was lower (+14 *vs* +15 median days, p=0.39) and the mortality at day 100 was higher in the non-cryotherapy group (0% *vs* 8%, p=0.24). Decreased mucositis degree was associated in both univariate and multivariate analysis only with cryotherapy (p= 0.01 and p=0.0003). Hazar ratio was 0.81 (IC 95% 0.06-0.55).

Table 1.

Characteristics	NON-CRYOTHERAPY	CRYOTHERAPY	p
N	37	35	
Gender F/M n (%)	40/60	60/40	0.23
Age Median (Range)	59 (26–70)	57 (38–73)	0.99
Previous lines Median (Range)	2 (1–4)	1 (0 –5)	0.2
Response n (%)			
CR	27	40	
PR	56	14	
VGPR	13	40	
SD/PD	2,7	5,7	
Conditioning n (%)			0.2
MEL200	78	74	
BUMEL140	21	22,8	
MEL100HF	0	2,8	

Summary/Conclusions: In our center, cryotherapy reduces significantly the severity of mucositis. The use of morphine and parenteral nutrition and other complications do not present such a drastic decline, probably because they influence the gastrointestinal mucositis, which is not combatted with cryotherapy. With this results, we are encouraged to continue to include cryotherapy in our protocols.

E1563

REDUCED INCIDENCE OF PRIMARY GRAFT FAILURE IN PATIENTS UNDERGOING HAPLOIDENTICAL STEM CELL TRANSPLANTATION: A SINGLE CENTER EXPERIENCE

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Background: Haploidentical stem cell transplantation (HSCT) is an alternative for patients without HLA matched donors. However, primary graft failure (PGF) and graft *versus* host disease are still limitations derived from alloreactivity due to HLA mismatch. T cell depleting approaches (*in-vivo* with post-transplant cyclophosphamide (PT-Cy) or *ex-vivo* with graft engineering) and surveillance for anti HLA antibodies are strategies intended to reduce these complications. PGF has a high mortality, and treatment with a second graft is not well defined in terms of donor, source, graft engineering or conditioning.
Aims: Our objective is to describe the incidence and risk factors of PGF and treatments if needed.
Methods: We retrospectively analyzed 40 consecutive patients who underwent HSCT from 2014 to 2016: unmanipulated for 20 adults and graft engineering for 20 children (CD34 selection/TCRab depletion, n= 6; and CD34 selection/CD45RA depletion, n=14). The stem cell source was mobilized peripheral blood in all cases. GCSF was sistematically used from day 5 until engraftment. We used descriptive statistical methods for analysis.
Results: Patient characteristics are described in Table 1. Conditioning regimen was Bu-Flu-Cy (n=18, adults), Thio-Bu-Flu (n=2, adults), Flu-Mel-Thio for all pediatric patients. ATG was used in 6 children and TLI in 14 children. All adult patients were given PT-Cy. Only one adult patient had high titer donor specific anti HLA antibodies and was desensitized with plasma exchange, Rituximab and IVIG before transplantation. All patients engrafted before day 28 and no PGF diagnosis was established in our serie. We found that 4 patients (3 children, 1 adult) required a boost of CD34 selected graft from the same donor for secondary GF and poor graft function.

Table 1.

	Adults n=20	Children n=20
Age (years)	50 (22-70)	5 (0-15)
Sex	17 M / 3 F	5 M / 15 F
Diagnosis		
AML	5	6
MDS	3	0
ALL	2	9
Aplastic anemia	0	2
Lymphoma	10	0
Immunodeficiencies	0	3
Conditioning		
Myeloablative	10	0
Reduced intensity	10	20
ATG	0	6
TLI	0	14
PT-Cy	20	0
Graft		
Unmanipulated	20	0
TCRab depleted	0	6
CD45RA depleted	0	14
Cell dose (median)		
CD34 x10^6/Kg	4,4 (2,7-7,5)	6,44 (2,4-9,5)

Summary/Conclusions: PGF incidence described in literature is 5-10%, we did not find any primary graft failure in our serie. Desensitization therapy appeared to be effective in one patient with anti HLA antibodies. All CD34 boosts were performed for secondary graft failure/poor graft function due to treatment toxicities or viral infections. Unfortunately, analysis of causes and risk factors for secondary GF requires a larger number of patients to be determined.

E1564

RESULTS OF HAPLOIDENTIC HEMATOPOIETIC STEM-CELL TRANSPLANTATION IN PATIENTS WITH LYMPHOMA: A SINGLE CENTER EXPERIENCE

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Background: Allogeneic hematopoietic stem-cell transplantation (allo-HSCT) is a potentially curative treatment for a variety of hematologic malignancies and nonmalignant hematologic disorders. However, only about a third of candidates for allo-HSCT have HLA-matched siblings. For patients who lack HLA-matched siblings, partially HLA-mismatched (haploidentical) related donors are good alternative sources of stem cells for allo-HSCT
Aims: In this retrospective, single center study we evaluated safety and efficacy of haploidentical allo-HSCT compared to those of HLA-matched allo-HSCT in patients with lymphoma
Methods: A total of 81 lymphoma patients(Hodgkin and Nonhodgkin) with a mean age of 42 years who underwent allo-HSCT(HLA matched n=46, haploidentic n=35) between July 2010 and July 2016 were analyzed. All patients received Cyclophosphamide (Cy) 50mg/kg i.v. on days +3 and +4. All patients initiated CsA day +5, and then adjusted according to the plasma levels. In addition to CsA, all haploidentical allo-HSCT recipients received MMF until day +35
Results: There were no significant differences in age, sex, diagnosis, disease status up-front HSCT, or transplant characteristics between the groups except a higher median number of stem cells infused in haploidentical group (p=0.004). The median follow-up was 13 months for haploidentical group and 12 months for HLA-matched group. Outcomes of patients are summarized in Table-1
Summary/Conclusions: Our results suggest that haploidentical allo-HSCT is a safe treatment modality in patients with relapsed lymphoma who lack HLA-matched siblings. The major problem are seems to be viral infections. Future challenges remain in improving post-transplant immune reconstitution and finding the best approach to reduce the incidence and severity of viral infections, while preserving graft-versus-lymphoma effect to prevent the recurrence of the underlying disease

E1565

COLLECTION OF PERIPHERAL BLOOD HEMATOPOIETIC PROGENITOR CELLS (PBPC) FROM HEALTHY DONORS: 15 YEARS SINGLE CENTER EXPERIENCE

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Background: hematopoietic stem cell transplantation (HCT) is, nowadays, a consolidated therapy within the treatment of multiple hematological pathologies. In the last two decades, the main method of obtaining hematopoietic progenitor cells is blood leukapheresis after mobilization with granulocytic colony growth factors (G-CSF).
Aims: To describe the experience of our center in apheresis of healthy family donors in the last 15 years. Furthermore, analyze the influence of different variables on the procedure and the yields obtained.
Methods: retrospective analysis was performed on 189 hematopoietic progenitor cell collection (HPCC) from January 2002 to December 2016. The study was carried out at Apheresis Unit, Hospital de La Princesa, Madrid, Spain. Progenitor cells mobilization was performed with G-CSF in all cases at a dose of 10mg/kg b.w. Apheresis device was COBE Spectra in all cases and citrate was the anticoagulant used for all the apheresis procedures. All donors were carefully evaluated and informed on the donation procedure and signed an informed consent for apheresis. The venous access used was mostly peripheral venous access in antecubital veins, and in only 7 cases (3.7%) central venous catheter was required. Donor details studied were age, sex, AB0 group, number of apheresis, number of CD34+ per kilogram collected, and processed volume.
Results: among the 189 donors, 85 were females and 104 were males (45% *vs* 55%). The hematologic pathologies that motivated transplantation were, in order of frequency, Acute Myeloid Leukemia (AML) (40.2%), Myelodysplastic Syndrome (MDS) (13.8%), Acute Lymphoblastic Leukemia (ALL) (10.1%), Hodgkin´s Lymphoma (HL) (8.5%), Non-Hodgkin´s Lymphoma (NHL) (6.3%), Multiple Myeloma (MM) (5.3%), Chronic Myeloid Leukemia (CML) (4.2%), others (11.6%). Donors most frequent AB0 group was A and in 123 of all cases (65%) donor and recipient had the same group. Median weight of donors was 74 Kg and in recipients was 70.5 Kg. Median age of our donors was 50 and median age of recipients was 51 years. Twenty donors were >65 years (10.6%) and 10 were>70 years (5.3%). Median of processed volume was 13 liters, but if we stratify that volume by recipient's weight, in those whose were heavier than 100 kg, median of processed volume was 18 liters. Two apheresis procedures were performed only in ten donors. Of these, 2 were older than 70 years (20% of total donors over 70 years of age) compared to 8 under 70 years of age (4.5% of all patients in that age range). The median of CD34 + / kg collected was 5 x 10^6. Among the age ranges, median yield of CD34+/Kg in patients older than 70 years was 3.55 x 10^6, in patients between 31 and 69 years was 4.96 x 10^6 and in patients younger than 30 years was 5.5 x 10^6. The apheresis procedure was mostly well tolerated, with only mild symptoms of hypocalcemia and disturbances related to venous access in a minority of cases. No significant long term adverse effect has been observed in the blood tests reported to our centers during the five years of follow up after the donation.
Summary/Conclusions: donor age and weight discrepancy with recipient were the factors that significantly affected PBPC yields in our experience in healthy donors. These factors had also an impact in the amount of liters of volemia processed, although in most cases only one apheresis procedure was enough. Adverse effects of apheresis for PBPC collection were the same as for other apheresis procedures such as those related to venous access, almost always peripheral one and citrate toxicity.

Stem cell transplantation - Experimental

E1566

ALLORESPONSES OF HUMAN T-CELLS FROM ADULT PERIPHERAL BLOOD AND UMBILICAL CORD BLOOD ARE DIFFERENTIALLY IMPACTED BY LENALIDOMIDE - IMPLICATIONS FOR AHSCT

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Background: Immunomodulatory drugs (IMiDs), such as lenalidomide provide a tool to enhance both direct anti-tumor and graft-versus-tumor effects after allogeneic haematopoietic stem-cell transplantation (AHSCT). However, early clinical experience with IMiDs after AHSCT using adult peripheral blood (APB) as a stem cell source has been limited by induction of graft-versus-host disease. Characterization of the mechanisms by which IMiDs can modulate alloresponses of T-cells from different cell sources could facilitate more effective use of these drugs in the setting of AHSCT.
Aims: To use *in vitro* modelling to identify changes in alloresponses of APB and umbilical cord blood (UCB) T-cells after exposure to the widely used IMiD lenalidomide.

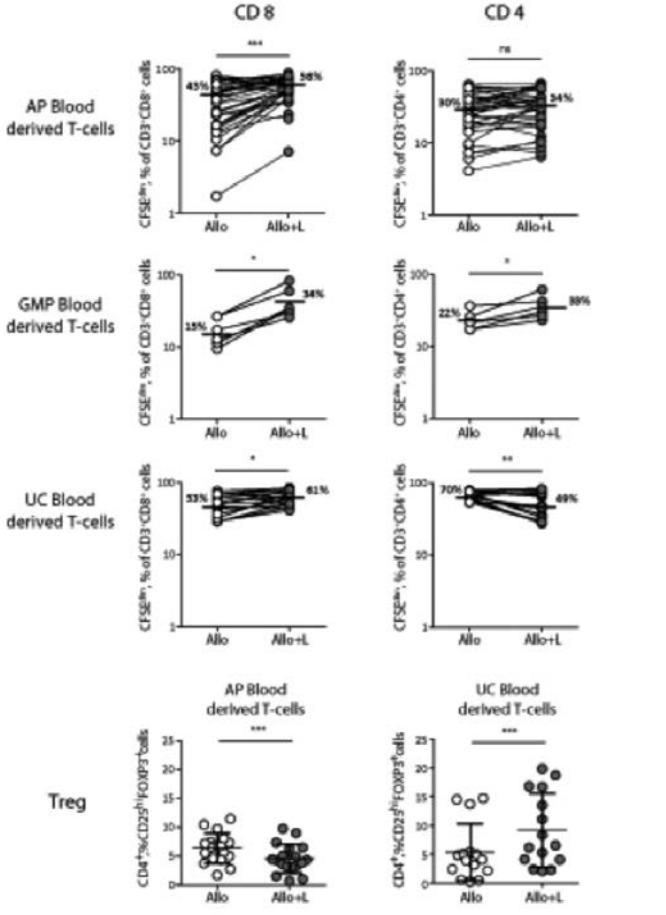

Figure 1.

Methods: We have used multi-parameter flow cytometry and gene expression profiling to perform an in-depth characterisation of the phenotypic and genotypic effects of clinically relevant concentrations of lenalidomide treatment on T-cells during allogeneic co-culture. Using GCSF-mobilised APB (GMPB), steady state APB and UCB PBMC as responder cells in allogeneic co-culture we have been able to compare the differential effect of lenalidomide on these three cell sources. Allogeneic responder cells were labelled with CFSE (carboxyfluorescein diacetate succinimidyl ester) to allow quantification of allo-proliferation. Responder T-cell subsets including naive, memory, activated, cytotoxic and regulatory were interrogated. Functional effects of lenalidomide treatment including cellular capacity to produce cytokines, degranulate and exert direct cytotoxicity was also assessed. RNA was extracted from highly purified proliferative and non-proliferative $CD8^+$ T-cell fractions following a combination of magnetic and flow-sorting and gene expression changes assessed by Affymetrix whole genome array and qRT-PCR.

Results: We demonstrate that lenalidomide increases net alloproliferation of APB T-cells by selectively enhancing allospecific proliferation of $CD8^+$T-cells. These $CD8^+$ T-cells have enhanced effector memory differentiation, are enriched for polyfunctional effectors, and have a distinct gene expression profile with altered expression of key immunoregulatory genes and pathways. This effect on $CD8^+$T-cell proliferation was seen across all 3 cell sources. Importantly a differential effect on $CD4^+$ T-cell responses was observed depending on cell source. Lenalidomide treatment of APB results in no change in CD4+ T cell proliferation overall, but leads to reduced frequencies of $CD4^+$ regulatory T-cells (Treg). In contrast lenalidomide treatment of GMPB resulted in a significant increase in $CD4^+$ T cell proliferation, with no effect on Treg cell frequencies. Most strikingly, although lenalidomide treatment of UCB T-cells during allostimulation results in a similar increase in alloreactive effector $CD8^+$T-cells, it also reduces allospecific proliferation of $CD4^+$T-cells and selectively expands frequencies of Treg, resulting in a net reduction in UCB T-cell alloproliferation.
Summary/Conclusions: Our findings show that lenalidomide has a qualitatively different impact on alloresponses of T-cells from different cell sources, with a potentially tolerogenic effect on UCB T-cells. These findings have important implications for the future use of IMiDs in the setting of AHSCT.

E1567

USING MARKER GENES ANALYSIS INSTEAD OF MLR ASSAY FOR IDENTIFICATION OF FUNCTIONAL CD4+FOXP3+ REGULATORY T CELLS IN GVHD PROPHYLAXIS

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Background: There are two types of CD4+CD25+FoxP3+ regulatory T cells (Tregs), natural Treg cells (nTreg) developing in thymus, and induced Treg cells (iTreg) arising from CD4+ naïve T cells. The iTreg cells have been considered important for maintenance of immunological tolerance and correlate with the occurrence of GVHD in some studies. Establishing a quick method to identify the functional iTreg cells is worthy of focusing. Five to ten percent Tregs could be found in human CD4+ T cells and should be expanded via *in vitro* cell culture.
Aims: In order to improve the efficiency of Treg cells for the prevention of GVHD, we attempt to establish a relatively quick analytic method to identify the functional iTreg cells, and then to curtail the iTreg cells harvest time for clinical use. Therefore, using qPCR for marker genes analysis instead of MLR (mixed lymphocyte reaction) assay is an important issue.
Methods: Mouse splenocytes were prepared from mouse spleen. Human PBSC were prepared from peripheral blood (PB) of healthy donors by Ficoll-Hypaque density gradient centrifugation. All T cells were isolated by negative selection, then CD4+naïve T cells were harvested. CD4+ naïve T cells were activated with anti-CD3/CD28 beads in the presence of IL-2, TGF-β and retinoic acid (RA) containing RPMI1640 medium. The protocol is showed in Fig. 1.

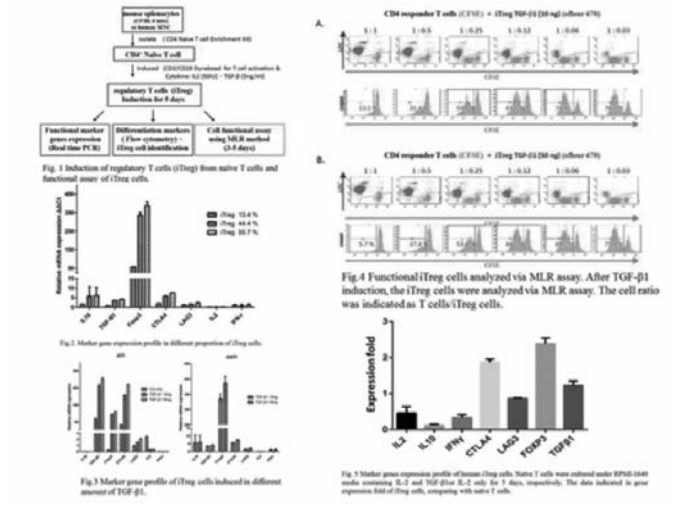

Figure 1.

Results: Seven genes for qPCR analysis were used to identify the functional iTreg cells. We used the different proportions of iTreg cells in total naïve T cells for 7 genes expression analysis and MLR assay to investigate the relationship between gene expression profile and iTreg cell function. The data of marker genes analysis were shown in Fig2. It indicated that the different proportion of iTreg cells could show the different expression profile of these genes. Obviously, the *FoxP3* gene expression increased in a great level. Based on our previous

experiments, iTreg cells induction could be TGF-b1 dependent. After different amount of TGF-b1 induction, the genes expression profile also showed the coincidence of the data in Fig.2 (Fig.3). Using the same iTreg populations, MLR assay have been investigated for 5 days. The T cell suppression percentage would be dependent on the iTreg cells proportion (Fig.4A and B). It indicated that the gene expression levels can represent the biological function of iTreg cells. It's the better way to identify the iTreg cells. Further, we have used PBMNC for Treg cell induction, the marker genes expression analysis also showed in Fig.5. After comparing with IL-2 cultured T cells, the gene expressions revealed the difference in between iTreg cells and un-induced T cells.
Summary/Conclusions: Our study showed that MLR assay should spend 3 to 5 days for identification of the functional iTreg cells, however, the marker genes analysis took only one day for that. Besides, MLR assay is a more complicated method than qPCR analysis. Using simple analysis for human iTreg cells functional identification could save the time for clinical application and might prevent GVHD occurrence effectively.

E1568

OXIDANT-ANTIOXIDANT SYSTEM IN PATIENTS WITH MULTIPLE MYELOMA
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Background: Multiple myeloma (MM) is one of the most widespread malignant B-cell lymphoproliferative disorders and is characterized by a clonal proliferation of atypical plasma cells in bone marrow or, less frequently, in extramedullary locations synthesizing monoclonal immunoglobulins. Currently, autologous hematopoietic stem cell transplantation (auto-HSCT) is recognized as the standard method of treatment for young patients (≤65 years old) with MM. Moreover, the best auto-HSCT results are observed in patients who have received new medication (thalidomide, bortezomib, and lenalidomide) during induction therapy and who have achieved at least a very good partial response, which leads to a significant increase in overall survival. However, studies reflecting the impact of this kind of treatment on the dynamics of oxidant-antioxidant indicators are virtually non-existent. At the same time, the possibility of treating developing diseases by prescribing medication makes the problem highly relevant.
Aims: The aim of the study was to investigate the state of OS-AOS in patients with MM during auto-HSCT.
Methods: We studied 20 patients (11 men and 9 women, mean age 49 years) who followed auto-HSCT after high-dose melphalan. The control group consisted of 50 age- and sex-matched healthy persons. The plasma levels of malonic dialdehyde and cerulloplasmin as well as activities of superoxide dismutase and catalase were measured by standard biochemical techniques. In erythrocytes, the level of non-protein thiol groups was studied. The state of OS-AOS was investigated in each patient four times: before and after conditioning with melphalan, at the moment of maximal leukocyte decrease and after complete reconstitution from cytopenia.
Results: We have found the features of impaired balance in OS-AOS in MM patients before as well as in course of auto-HSCT. The level of malonic dialdehyde in MM patients was not significantly different from that in the control group. At the same time, cerulloplasmin plasma level as well as catalase activity were significantly increased in patient group ($p<0.05$), whereas the level of non-protein thiol groups was decreased in MM ($p<0.05$). The results of our study have shown, that an imbalance of OS-AOS is frequently seen in MM patients and, possibly, could influence the course of auto-HSCT.
Summary/Conclusions: The results of the study indicate a high frequency of occurrence of disturbance of the condition of OS-AOS in patients with MM. The imbalance in the functioning of this system is not entirely eliminated in the process of treating the patients with MM using auto-HSCT. The question of the necessity and methods of the possible correction of OS-AOS in patients with MM, particularly during auto-HSCT, requires further study.

E1569

SURFACE RECEPTOR EXPRESSION PROFILE DEFINES ALLOREACTIVE DONOR CD8+ T-CELLS AFTER MURINE ALLOGENIC HEMATOPOIETIC CELL TRANSPLANTATION
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Background: Acute graft-versus-host disease (aGvHD) is a severe and often life-threatening inflammatory complication of allogeneic hematopoietic cell transplantation (allo-HCT). aGVHD is mediated by alloreactive donor T cells attacking the gastrointestinal tract, liver, and skin of the host. Efficient strategies to improve aGvHD-related morbidity and mortality will rely on more precise methods than preemptive immunosuppression to consistently predict aGvHD and abrogate disease manifestation without exposing patients to an unwarranted risk for infectious complications. Recent insights into the multistep-pathophysiology of aGvHD provide a good basis for the development of new tests to identify individual patients at risk before the onset of aGvHD.
Aims: As pathologic T cell responses rely on spatiotemporally defined programs of T cell activation, acquisition of effector functions, and homing to GvHD target tissues it appeared attractive to assess receptor expression profiles of peripheral blood T cells as potential predictive markers.
Methods: Therefore, we characterized the surface receptor expression profile of peripheral blood donor lymphocytes early after allo-HCT in two independent murine models across minor histocompatibility antigens (miHAg) with multicolor flow cytometry. C57Bl/6 (H-2b, Thy1.1+) or B10.D2 (H-2d, Thy1.1+) T cells plus bone marrow cells were transplanted in conditioned (8Gy) miHAg mismatched BALB/B (H-2b, Thy1.2+) and syngeneic C57Bl/6 (9Gy) or BALB/c (H-2d, Thy1.1+) recipients. To identify suitable predictive markers, we compared the expression pattern of allo-HCT recipients to syngeneic HCT recipients and untreated wild type controls.
Results: Comparing a panel of T cell surface receptors, we found the homing markers α4β7 integrin, and P- and E-selectin ligand highly up-regulated on allogeneic peripheral blood donor $CD8^+$ T cells at peak time points of cell migration. The combination of these homing markers with the activation markers CD25 and CD69 at later time points and low expression levels of L-selectin allowed to define alloreactive donor T cells.
Summary/Conclusions: Based on this data we propose that alloreactive CD8+ T cells can be identified in miHAg allo-HCT recipients upon their homing receptor expression pattern as soon as six to ten days before the onset of aGVHD.

Thalassemias

E1570

SOLUBLE FORM OF TRANSFERRIN RECEPTOR IS ASSOCIATED WITH AGE AT DIAGNOSIS AND RISK OF THERAPEUTICAL INTERVENTION AND IRON OVERLOAD IN PATIENTS WITH NON-TRANSFUSION-DEPENDENT THALASSAEMIA

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Background: The soluble transferrin receptor (sTfR), that fully reflects the marrow erythropoietic activity, was found to have not only a striking diagnostic accuracy in predicting the risk of extramedullary haematopoiesis (EMH), but also in scoring disease severity in non-transfusion-dependent thalassemias (NTDT).
Aims: We retrospectively evaluated the relationship between sTfR and some fundamental events in the life and in the management of patients with NTDT.
Methods: We considered 111 NTDT patients with four genetic entities of NTDT: homozygous or compound heterozygous state for β-thalassemia, triplicated α genotype associated with β heterozygosity, deletional HbH, and combination of a β defect plus a β chain variant. sTfR was measured with a *commercially available kit*. A group of patients was enrolled in the Myocardial Iron Overload in Thalassaemia (MIOT) network and underwent hepatic iron overload assessment by the T2* Magnetic resonance Imaging (MRI) technique.
Results: The group with homozygous or compound heterozygous for β-thalassemia had the higher sTfR levels. sTfR values were negatively related to age at diagnosis (R=−0.462, P<0.0001), and to age at first transfusion (R=−0.703, P<0.0001). At ROC curve a sTfR>5.3mg/L discriminated the patients with a previous history of occasional transfusions. sTfR values were significantly higher in splenectomized patients. sTfR values were negatively related to age at splenectomy (R=−0.328, *P=0.044)* and in unsplenectomized patients a significant positive correlation was found between sTfR values and spleen diameter (R=0.572; *P<0.0001)*. sTfR values were negatively related to age at starting chelation therapy (R=-0.564, P=0.044). Patients never chelated showed significantly lower sTfR values than patients under chelation therapy (see Figure). sTfr values were significantly correlated with serum ferritin levels (R=0.321, *P<0.0001).* but no with LIC values.

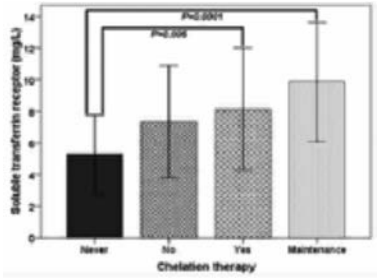

Figure 1.

Summary/Conclusions: The heterogeneity of patients with NTDT is an emerging cause of complex management and treatment of the disease. Our data indicate that the measurement of sTfR level, a common laboratory test, could contribute to correctly stratify the disease history and the chelation strategy in NTDT.

E1571

LOW SERUM FERRITIN LEVELS DO NOT PROTECT FROM CARDIAC AND HEPATIC IRON IN PATIENTS WITH THALASSEMIA MAJOR

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Background: The estimation of serum ferritin levels is the most commonly employed test to evaluate iron overload in Beta Thalassaemia Major (TM).
Aims: The aim of this mulicenter study was to assess the distribution of serum ferritin levels in a cohort of well treated TM patients and the possible protective role of really low levels *versus* iron accumulation in the heart and in the liver.
Methods: We considered 1548 TM patients regularly transfused and chelated consecutively enrolled in the MIOT (Myocardial Iron Overload in Thalassemia) Network. Myocardial and hepatic iron burdens were quantified by the T2* technique. For the heart a multislice approach was adopted in order to calculate segmental and global T2* values. Hepatic T2* values were converted into liver iron concentration (LIC) values.
Results: Mean serum ferritin levels <500ng/ml were found in 342 (22.1%) patients. Three groups were identified on the basis of mean serum ferritin levels. Both transaminases were significantly lower in patients with serum ferritin <500 ng/ml and between 500 and 1000 ng/ml *versus* patients with serum ferritin ≥1000 ng/ml. Among patients with serum ferritin <500 ng/ml, 9.1% showed significant cardiac iron (global heart T2*<20 ms) and 21.6% showed hepatic iron (LIC ≥3mg/g dw). Cardiac and hepatic iron levels were significantly lower in patients with serum ferritin <500 ng/ml than in the other two groups and in patients with ferritin between 500 and 1000ng/ml *versus* patients with serum ferritin ≥1000 ng/ml (see Figure). Compared to patients with serum ferritin levels <500 ng/ml, the other two groups showed a significant higher risk of cardiac iron overload (odds ratio-OR=2.03, P=0.002 for patients with ferritin 500-1000 ng/ml and OR=5.96, P<0.0001 for patients with ferritin ≥1000ng/ml) and of hepatic iron overload (OR=3.44, P<0.0001 for patients with ferritin 500-1000ng/ml and OR=25.43, P<0.0001 for patients with ferritin ≥1000ng/ml).

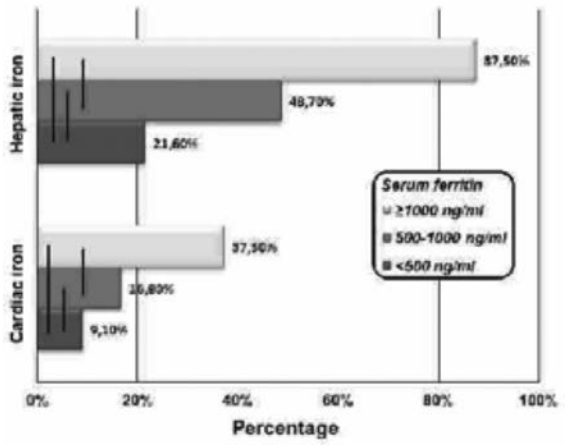

Figure 1.

Summary/Conclusions: Low serum ferritin values, even in the normal range, do not per se exclude cardiac and hepatic iron overload, although decreasing the risk. Before to consider a reduction of the chelator dose in patients whose serum ferritin levels have reached the target, a MRI scan should be performed in order to measure iron levels in the different organs.

E1572

ISCHEMIA MODIFIED ALBUMIN AS A MARKER OF OXIDATIVE STRESS IN CHILDREN AND ADOLESCENTS WITH B-THALASSEMIA: RELATION TO LIPID PEROXIDATION, IRON OVERLOAD AND VASCULAR DYSFUNCTION

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Background: Patients with β-thalassemia major (β-TM) are under significant iron driven oxidative stress. Ischemia modified albumin (IMA) is an altered type of serum albumin that forms under conditions of oxidative stress and an independent predictor of major adverse cardiovascular events
Aims: To measure the levels of IMA in 45 children and adolescents with β-TM compared with 30 healthy controls and assess its relation to lipid peroxidation, vascular complications and subclinical atherosclerosis
Methods: β-TM patients without symptoms of heart disease were studied focusing on transfusion history, chelation therapy, serum ferritin, malondialdehyde (MDA) and IMA levels. Echocardiography was performed and carotid intima media thickness (CIMT) was assessed.
Results: IMA and MDA levels were significantly higher in β-TM patients compared with controls (p<0.001). IMA was higher among patients with heart disease and pulmonary hypertension (PH) risk than those without. Serum IMA and MDA levels were elevated among patients with serum ferritin ≥2500 µg/L compared with patients below this cutoff. TM patients compliant to chelation had a significantly lower IMA levels than non-compliant ones. Receiver operating characteristic (ROC) curve analysis revealed that a cutoff value of IMA at 75 U/mL could differentiate β-TM patients with PH risk with 90% sensitivity,

91.4% specificity and positive predictive value of 75% and negative predictive value 97%; area under the curve 0.883 (95% confidence interval 0.752-0.959). In addition, the cutoff value of IMA at 17.5 U/mL could differentiate β-TM patients with heart disease with 80.5% sensitivity, 88.9% specificity and positive predictive value of 96.7% and negative predictive value 73.3%; area under the curve 0.887 (95% confidence interval 0.758-0.962). Significant positive correlations were found between IMA levels and disease duration (r=0.311, p=0.045), white blood cell count (r=0.322, p=0.031), serum alanine aminotransferase (r=0.388, p=0.01) and aspartate aminotransferase (r=0.382, p=0.037). IMA and MDA levels were positively correlated (r=0.503, p=0.001) and there was a significant positive correlation between these two markers and mean serum ferritin (IMA; r=0.645, p<0.001 and MDA; r=0.567, p<0.001) among TM patients. IMA levels were positively correlated to TRV (r=0.621, p=0.008), while negatively correlated to ejection fraction (r=-0.412, p=0.014) and fractional shortening. Both IMA and MDA were positively correlated to CIMT (r=0.607, p<0.001 and r=0.590, p<0.001, respectively).

Summary/Conclusions: Our results highlight the role of oxidative stress in the pathophysiology of vascular complications in thalassemia. IMA could be useful for screening of β-TM patients at risk of cardiopulmonary complications and atherosclerosis because its alteration occurs in early subclinical disease.

E1573

SERUM N-TERMINAL PRO-BRAIN NATRIURETIC PEPTIDE LEVEL AND ECHOCARDIOGRAPHIC TISSUE DOPPLER ABNORMALITIES IN PATIENTS WITH BETA THALASSEMIA MAJOR

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Background: Heart disease remains the major cause of morbidity and mortality in thalassemia patients. Multiple pathologies have been implicated in the development of cardiac dysfunction in these patients including: cardiac iron overload leading to right ventricular diastolic then left ventricular systolic dysfunction, chronic anemia and tissue hypoxia. Because congestive heart failure is the main cause of death in these patients, early recognition of cardiac dysfunction may be useful in modifying therapy in a timely manner. Tissue Doppler imaging (TDI) echocardiography and serum brain natriuretic peptide (BNP) level may be promising tools for such a purpose.

Aims: This study aimed to assess serum NT-proBNP level and echocardiographic tissue doppler abnormalities among a cohort of Egyptian beta thalassemia major patients and to detect possible assocoations between them as well as other disease variables including iron overload.

Methods: Thirty beta thalassemia major patients with a mean age of 12.93±2.07 years regularly followed up at Pediatric Hematology Clinic, Cairo University and thirty aged matched healthy control subjects were included. Conventional, M-Mode and TDI echogardiography were performed to all patients and control subjects in addition to cardiac magnetic resonance (CMR)for studied patients. Serum NT-proBNP level was measured using enzyme linked immunosorbant assay (ELISA).

Results: Tissue doppler imaging revealed a significant difference of ratio of the early (e') to late (a') right ventricular filling velocities (Rv e'/a' ratio) between cardiac and non-cardiac iron overloaded patients reflecting early diastolic dysfunction in cardiac iron overloaded patients. Myocardial performance index of left ventricle (LV_TEI index) by TDI showed significant difference in cardiac iron overloaded patients compared to non cardiac iron overloaded patient (mean 0.45± .02 *versus* 0.39± .04 with p value=0 .003) indicating decrease in ventricular relaxation due to iron overload and restrictive cardiomyopathy. SerumBNP level was significantly higher among patients compared to controls (mean 99.18± 72.43pg/ml *versus* 18.93± 9.65pg/ml respectively with p-valu< 0.001) and among cardiac iron overloaded patients compared to non cardiac iron overloaded (mean 212.31±57.18pg/ml *versus* 64.75±26.69pg/ml respectively with p value <0.001). We found positive correlation between level of BNP and frequency of the blood transfusion/year, RVe'/a' and LV TEI_TD index with (p value 0.006, <0.001) and 0 .030 respectively) denoting early diastolic impairment in asymptomatic thalassemia patients.

Summary/Conclusions: Asymptomatic thalassemia major patients under chelation therapy may have diastolic and or systolic dysfunctions that could not be detected by conventional echocardiography but could be highlighted by TDI. CMR, TDI and serum BNP level measurement are promising tools for accurate assessment of cardiac functions and iron overload in thalassemia patients.

E1574

PRENATAL DIAGNOSIS OF HEMOGLOBINOPATHIES IN NORTHERN GREECE. 15 YEARS REPORT

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Background: Hemoglobinopathies constitute the most frequent monogenic disorders worldwide and thalassemias are the most frequent genetic disorders in Greece. Beta-thalassemia carrier frequency is approximately 8%, while 1.5% of the population are carriers of the Hb S mutation. The rate of β-thal carriers could be as high as 15-20% in some areas. The risk of giving birth to an affected child depends on the incidence of the thalassemic gene and this may vary from 1/24 to 1/150 in married couples. The National Program for prevention of Thalassemia was established in 1973. Through population screening and prenatal diagnosis programs Greeks and immigrants are screened and counseled.

Aims: We report our findings on prenatal diagnosis of thalassemias and hemoglobinopathies in Northern Greece over a 15 year period (2001-2015).

Methods: During the 15 year period, a total of 33.837 subjects were screened individually or as couples at our Thalassemia Prevention Unit. There were 3.659 couples screened for hemoglobinopathies. In 371 couples both partners carried an abmormal Hb gene and counseling was offered and 329 pregnancies were found at risk of giving birth to an affected child. The genes interactions were in 245 pregnancies at risk for thalassemia major offsprings and 84 for sickle cell disease ones. Prenatal diagnosis was mainly carried out by CVS at 11-12 weeks of gestation (n=298), in few cases by amniotic fluid sampling (n= 21) collected at 16-18 weeks. Few late comers were tested by fetal blood sampling at 20 week of gestation(n=5). The remaining 42 pregnancies involved couples who were double heterozygotes for mutations that did not cause severe clinical disease and were exempted from prenatal diagnosis. The gene interactions were as follows β-thal / α thal, β-thal in combination with Hb E-Saskatoon or D-Punjab, HbE /HbE, Hb E-Saskatoon /with carrier of HbS, and Hb O/ Hb O, β-thal or α thal in combination with D Punjab, Hb Brugg/β-thal, silent β-thal/ silent β-thal. 91% of the couples were of Greek origin, and 9% were immigrants from Albania, Somalia, Nigeria, Fyrom, Ruanta and Thailand. We had an average of 15-32 prenatal diagnosis per year.

Results: The results of DNA analyses of the samples were as follows: 76 fetuses (23%) were found to be homozygote or double heterozygote for clinical significant mutations. These couples were informed of the danger of having an affected child but the termination or continuation of the pregnancy was left to the couples to decide. Nevertheless all, except three couples, preferred to terminate the pregnancies so we had one case of thalassemia major offspring and two cases of silent β-thal/ O Arab offsprings born. Selective abortion of the affected fetus was performed in the cases of the twin pregnancies (n=6). There have been no cases of misdiagnosed pregnancies and only one obstetric complication (rupture of membrane that lead to miscarriage) was reported.

Summary/Conclusions: It is universally accepted that thalassemia prevention programs are successful in countries with a high frequency of Hb mutations, and prenatal diagnosis is mandatory in all at risk couples. The National Thalassemia Prevention Program has effectively decreased the incidence of thalassemia major and sickle cell syndromes in our country and in our region.

E1575

THE IMPACT OF LIVER STEATOSIS ON THE ABILITY OF SERUM FERRITIN LEVELS TO PREDICT LIVER IRON CONCENTRATION AMONG NON-TRANSFUSION-DEPENDENT THALASSAEMIA PATIENTS: A CROSS-SECTIONAL EVALUATION

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Background: Fatty liver is a common abnormality encountered in western countries among patients undergoing imaging of the abdomen and is associated to systemic inflammation and to increased ferritin levels, frequently unrelated to iron overload.

Aims: We analyzed the impact of the presence of fatty liver in the parameters of iron overload among our patients with Non Transfusion dependent Thalassaemia (NTDT).

Methods: 111 patients with NTDT were cross-sectionally evaluated; the diagnosis of liver steatosis was ultrasound-based (US). In all patients ferritin levels and serum alanine aminotransferase (ALT) to serum aspartate aminotransferase (AST) ratio were assessed. Liver iron concentration (LIC) measurements were available for 64 patients (54%) who underwent a magnetic resonance Imaging (MRI) scan within the Myocardial Iron Overload in Thalassaemia (MIOT) network.

Results: Liver steatosis was frequently (35.5%) encountered among our patients with NTDT and was significantly more prevalent in males with respect to females (49.0% *vs* 24.6%, p=0.008). Patients with liver steatosis had significantly higher levels of ALT, AST, ALT/AST ratio and ferritins than those without, but LIC values were comparable (Table 1). At ROC curve analysis, a ALT/AST ratio >0.89 predicted the presence of liver steatosis with a sensitivity=0.872 and a specificity =0.901 (P<0.0001). Overall, ferritin levels positively correlated with LIC values (R=0.558, P<0.0001) but in patients without steatosis there

was a strong relationship between ferritin and LIC values (R=0.656, P<0.0001) while in patients with steatosis the correlation was moderate (R=0.426, P=0.05).

Table 1.

	No steatosis (N=71)	Steatosis (N=39)	P-value
ALT (U/l)	14.80±5.29	31.74±16.42	<0.0001
AST (U/l)	21.29±7.73	27.97±11.27	0.001
ALT/AST ratio	0.72±0.16	1.12±0.35	<0.0001
Ferritin (ng/ml)	374.10±400.74	614.67±788.22	0.002
LIC (mg/g dw)	4.21±4.45 (N=41)	4.78±6.74 (N=23)	0.905

Summary/Conclusions: Our data show that liver steatosis affected also patients with NTDT and should be suspected in presence of a ALT/AST ratio >0.89. Recently, serum ferritin thresholds to predict clinically relevant liver iron concentrations for guiding chelation therapy when MRI is unavailable in patients with (NTDT) have been provided. Our data show that the presence of liver steatosis may lead to overestimate the magnitude of iron burden and may be responsible for anticipating or exceeding chelation treatment in patients with NTDT in absence of a LIC evaluation.

E1576

CIRCULATING CELL-FREE DNA (CFDNA) AND INEFFECTIVE ERYTHROPOIESIS IN BETA-THALASSEMIA INTERMEDIA

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Background: Low concentrations of circulating cell-free DNA (cfDNA) are found in the plasma of healthy individuals and increase in a number of conditions in relation to clinical severity, including cancer, chronic inflammation, autoimmune diseases and trauma. The mechanisms of release of cfDNA in the bloodstream are not well understood: DNA could originate from cells undergoing apoptosis/necrosis in tissues or from cells released in the blood and subsequently lysed. Also the tissue origin of cfDNA is mainly unclear. It has been suggested that cfDNA, at least after bone-marrow transplantation, could be mostly of hematopoietic origin. This finding prompted us to explore whether cfDNA is increased in patients with ineffective erythropoiesis (IE), a condition characterized by the over-proliferation and lysis/removal of erythroid precursors. This situation is common in thalassemias, mainly in non transfusion-dependent patients (NTDT).
Aims: The present study was designed **i**) to evaluate the behaviour of cfDNA in IE caused by beta-thalassemia, and **ii**) to assess whether cfDNA could be useful to quantify IE.
Methods: We studied 49 beta-thalassemia intermedia (TI) patients (mean age 41 years, range 16-65), 23 of whom were splenectomized. No evidences of tumor, trauma or autoimmune diseases have been observed in any patient at time of the study. Eighteen healthy subjects were also included as control group. The study was approved by the local ethical committee. DNA was extracted by QIAgen silica-based micro-spin columns from 200 mL of K_2EDTA plasma and its concentration determined fluorometrically using the fluorescent dye PicoGreen. Biochemical and hematologic parameters were determined in all patients as a part of laboratory routine. Reticulocytes and peripheral erythroblasts (EBL) were counted by automated procedures. Soluble transferrin receptor (sTfR) and growth differentiation factor 15 (GDF15) were also measured by immunometric ELISA assays.
Results: In the 49 patients studied, plasma cfDNA concentrations ranged from 6.3 to 93.1 ng/mL and are significantly higher than in controls (median 21.8 *vs* 10.4, p<.0001). Comparing non splenectomised (non-SPX) with splenectomised (SPX) patients, we observed a significant increase of cfDNA in the SPX group (median 29.4 *vs* 19.3 ng/mL, p=0.0085). In the whole TI group, cfDNA concentration was significantly correlated with EBL (p<,0001), LDH (r=0.52, p=.0001) and AST (r=0.56, p<.0001). Correlations of cfDNA were also observed with sTfR (r=0.45, p=.0014) and GDF15 (r=0.56, p<.0001). Notably, correlations with EBL (r=0.75, p<.0001), AST (r=0.58, p=.0036) and unconjugated bilirubin (r=.54, p=.0083) were observed only within the SPX group and not in non-SPX.
Summary/Conclusions: In this study we found that plasma cfDNA rises in TI patients compared to controls. Its concentration appears to correlate with both the amount of IE based on high number of EBL and the lysis of circulating erythroid precursors (both increased after splenectomy). We obtained preliminary evidences that circulating cfDNA concentration may be a suitable indicator of erythropoietic activity in TI patients. Results need to be extended on larger samples of patients' population to investigate the possible use of plasma cfDNA as a feasible and reliable biomarker to describe/monitor the severity of IE and TI complications.

E1577

LEFT VENTRICULAR HYPERTRABECULATION BY CARDIAC MAGNETIC RESONANCE IN THALASSEMIA INTERMEDIA PATIENTS: FREQUENCY AND PROGNOSTIC ROLE

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Background: Differentiation of left ventricle non-compaction (LVNC) from hypertrabeculated LV due to a negative heart remodeling in thalassemia intermedia (TI) can depends on the selected CMR criterion. The recently proposed Piga's criterion (NC/C ratio threshold of >2.5, Am J Haem 2012) seems to have a low specificity to identify the true LVNC in TI. Anyway, the Piga's criterion could easily detect a negative heart remodeling in TI patients.
Aims: The aim of our study was to prospectively assess whether the Piga's criterion had a prognostic role for adverse cardiovascular outcomes in TI patients.
Methods: We studied prospectively 168 TI patients (81 males, mean age 38.32 ±11.61 years) consecutively enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) Network. Standard cine steady-state free precession sequences were acquired and used for the calculation of biventricular function parameters (short-axis) and for the calculation of the thickness of the non-compacted and the compacted myocardium (three diastolic long-axis views) in all 16 segments. The maximal NC/C ratio was considered. Late gadolinium enhancement (LGE) images were acquired to detect myocardial fibrosis.

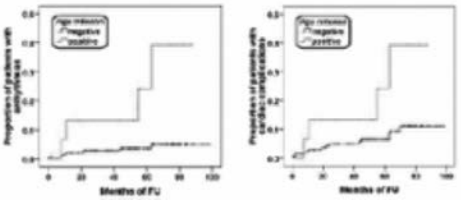

Figure 1.

Results: Eight patients were excluded because a cardiac complication was present at the first CMR. The baseline mean age of the considered 161 TI patients was 38.32±11.61 years and 81 patients were males. The study population was divided into two groups: patients with Piga's positive criterion (n=15, 9.31%) and with Piga's negative criterion (n=146, 90.68%). No significant differences were found between the two groups in terms of demographic features and CMR parameters. The mean follow-up time was 57.50±21.87 months. Sixteen new cardiac events were recorded: 1 heart failure, 10 supraventricular arrhythmias and 5 pulmonary hypertension. Due to numerical reasons, it was possible to perform a Cox regression analysis only for arrhythmias and cardiac complications globally considered. Patients with Piga's positive criterion had a significant higher risk of developing arrhythmias (hazard ratio-HR=7.19, 95% CI=2.02-25.51; P=0.002) and cardiac complications (HR=3.66, 95% CI=1.18-11.36; P=0.025). The figure shows the Kaplan-Meier survival curves. The Piga's positive criterion remained a significant prognosticator also in a multivariate models including previous and resolved events (14 cardiac complications, of which 7 arrhythmias) (HR for arrhythmias=23.67; HR for cardiac complications =7.09).
Summary/Conclusions: Based on our data a NC/C ratio >2.5 provides prognostic information for patients with TI about the risk of developing cardiac complications.

E1578

NITRIC OXIDE DYSREGULATION IN BETA-THALASSEMIA MAJOR: RELATION TO PULMONARY HYPERTENSION

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Background: Pulmonary hypertension (PH) is emerging as one of the most devastating complications of beta-thalasssemia major. Chronic hemolysis and iron overload constitute a major source of strong oxidative stress. Free heme radicals and red cell membrane elements resulting from hemolysis have a negative effect on the intrinsic nitric oxide (NO) production and arginine bioavail-

ability. Deficiency of both biochemical mediators promotes vasoconstriction of the pulmonary vasculature resulting in further endothelial dysfunction, with subsequent intensified reduction of nitric oxide. The role of nitric oxide dysregulation is well-studied in non-transfusion dependent thalassemias and in sickle cell disease, but yet not very well-characterized in beta thalassemia major.
Aims: The aim of our work is to study the relation between intrinsic nitric oxide level and the evolution of pulmonary hypertension in beta thalassemia major.
Methods: This is a case-control study, including all patients with beta thalassemia major above 12 years of age, undergoing follow up in pediatric hematology unit and in medical research institute, university of Alexandria, Egypt throughout a period of 6 months fom 1st of July till 31st of december 2016. All patients were screened for pulmonary hypertension by echocardiography, and those who have high tricuspid regurgitanr jet velocity (TRJV >2.5m/sec.) underwent cardiac catheterization.
Results: The present study included 52 thalassemic patients, 28 males and 24 females. Their age ranged between 11 and 26 years. The patients were subdivided into two groups (17 patients with pulmonary hypertension (PH), proven by cardiac cathetrerization and 35 patients without pulmonary hypertension). Nitric oxide level (measured by ELISA) was significantly lower in patients as a whole compared to controls [median of 19 micromol/L *versus* 30 micromol/L] (p=0.02). Similarly, nitric oxide was significantly lower in PH group compared to non-PH patients (p=0.001). In addition, there was a statistically significant negative correlation between serum NO level and serum ferritin level in all patients (r=-0.444, p=0.001).
Summary/Conclusions: In conclusion, NO reduction might contribute significantly to the development of pulmonary hypertension in patients with beta thalassemia major. This effect could be related to the degree of hemolysis, iron overload and the duration of disease. Further studies on the adverse pathophysiologic effects of nitric oxide deficiency in beta thalassemia major *e.g.* its relation to coagulopathy and platelet aggregation are recommended.

E1579

Abstract withdrawn.

E1580

SPECKLE-TRACKING ECHOCARDIOGRAPHY FOR DIAGNOSIS OF EARLY MYOCARDIAL DISEASE IN EGYPTIAN BETA THALASSEMIA MAJOR PATIENTS

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Background: The new parameters of cardiac function, derived from two-dimensional speckle-tracking echocardiography could be useful for an early diagnosis of cardiac involvement in transfusion dependent β-TM patients.
Aims: In this cross sectional study, our goal was to detect early myocardial disease in transfusion dependent β-TM patients using Echocardiography (Speckle Tracking) and to assess its specificity and sensitivity in comparison with cardiac MRI T2*.
Methods: This cross sectional study included 30 transfusion dependant β-thalassemia patients aged between 11-20 years recruited from the Pediatric Hematology Unit, Children Hospital, Ain Shams University. All included patients were subjected to detailed medical history{including transfusion, chelation, hepatitis C virus history with calculation of mean serum ferritin in last 2years) Radiological investigation included Echocardiography (Tissue Doppler and Speckle Tracking),MRI T2* were done.Cardiac affection by speckled was defined as decreased longitudinal strain less than 11 percentage or affection of any segment less than 11 percentage.
Results: Cardiac affection by speckled echocardiography was found in 10 patients(33.3%), 8 of them (80%) had normal ejection fraction and normal shortening fraction ,while 9 had iron overload by Cardiac MRI T2*. Patients with mean serum ferritin>2500 ng/mL in the last 2 years prior evaluation showed a significantly lower longitudinal strain (GLPSLAX) (P=0.043) which was further proved by a significantly negative correlation with the mean serum ferritin (P=.002). No significant differences were found between both splenectomized and non splenectomized patients as regard speckle tracking echocardiographic measures. The ROC curve analysis revealed that GLPS A4C a cutoff value of ≤21% was able to detect B- thalassemia patients having myocardial disease by cardiac MRI T2* with a sensitivity of 87.50% and specificity of 63.64% .Patients with cardiac iron overload by MRI T2* had significantly lower GLPSLAX &GLPSA4C and higher Ao Diam than those without cardiac iron overload (P=0.016, P=0.008, P=0.047 respectively). No significant difference between beta thalassemia patients with cardiac affection and those without cardiac affection as regard the duration of the disease, type and compliance of chelation therapy.
Summary/Conclusions: Although, Magnetic Resonance Imaging T2* technique is still considered the reference standard in myocardial iron overload, its routine use is limited by its high costs, poor availability. We demonstrated in this study an abnormal global longitudinal strain despite preserved LV systolic functions among BTM patients; thus speckle tracking echo techniques might be considered as an alternative effective method to detect early myocardial disease before evident systolic dysfunction.

E1581

EFFICACY, SAFETY AND GENETIC BASIS OF VARIABILITY OF RESPONSE TO HYDROXYUREA THERAPY IN BETA THALASSEMIA: A SYSTEMATIC REVIEW

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Background: Pharmacological agents such as hydroxyurea promote fetal hemoglobin production via a reactivation of γ-genes. In β-thalassemia there is an imbalance in globin chains which could be ameliorated by the newly synthesized γ-chains which neutralize the excess α-chains and therefore improves symptoms.
Aims: Systematic review of literature to evaluate the efficacy, safety and the genetic basis of variability of response to hydroxyurea therapy in beta-thalassemia patients
Methods: Research sources used were: MEDLINE (PubMed), EMBASE (Ovid) and Cochrane from June 1993 till June 2016. Eligible articles were reviewed and data including patients 'characteristics, duration of treatment, outcome, toxicity and impact of genetic mutation on response to hydroxyurea therapy was extracted. Major responders were those who became transfusion independent after hydroxyurea treatment, partial responders had significant decline in transfusion requirements, poor responders did not respond to hydroxyurea therapy. Statistical analysis software package 16 was used for data analysis.

Table 1.

Type of Beta Thalassemia	Major Response	Partial Response	Poor Response
b-thalassemia major	563 (52%)	359 (33%)	160 (15%)
b-thalassemia intermedia	620 (84%)	80 (11%)	38 (5%)

Results: Thirty eligible studies comprising of a total of 1822 patients with beta thalassemia were identified. Of these (n=9, 30%) evaluated the effect of hydroxyurea therapy on beta thalassemia major patients, (n=11, 36%) evaluated beta thalassemia intermedia patients while (n=10, 34%) included both beta thalassemia major and thalassemia intermedia patients. Mean age of patients was 13.5 years. Mean duration of hydroxyurea therapy was 3.4 years. The mean dose of hydroxyurea was 10mg/kg per day (8-15mg/kg). Table I showing number and percentage of patients having major, partial and poor response to hydroxyurea therapy. Only (n=12, 36%) studies evaluated the role of underlying genetic mutation on hydroxyurea response, out of these (n=6, 50%) studies found no significant correlation while (n=6, 50%) showed a positive correlation between common genetic mutations and hydroxyurea response. Hydroxyurea was found to be well tolerated, only (n= 09, 01%) had transient myelosuppression.
Summary/Conclusions: Hydroxyurea is an effective and well-tolerated agent in the management of β-thalassemia (both intermedia and major). It reduces blood transfusion requirements either partially or completely in majority of patients. No significant correlation between response to therapy and underlying genetic mutation was found. More studies are required to fully establish the association of genetic mutation to drug response.

E1582

EVALUATION OF CONTINUOUS BLOOD GLUCOSE MONITORING METHOD FOR DETECTION OF ALTERATIONS IN GLUCOSE HOMEOSTASIS IN BETA-THALASSEMIA PATIENTS

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Background: Glucose metabolism disturbances, among other endocrinopathies, are a common feature of β-thalassemia major (β-TM). Pancreatic iron overload and diabetes mellitus (DM) are common in β-TM patients. However, the relationship between iron stores and glucose disturbances is not well defined. Continuous glucose monitoring system (CGMS) enables more diagnostic accuracy and a better achievement of an optimal glycemic control.
Aims: To assess the pattern of glucose homeostasis in patients with β-TM and detect early impairment in glucose metabolism and prediabetic state in β-thalassemia patients comparing oral glucose tolerance test (OGTT) and CGM system.
Methods: This cross sectional study was conducted on 200 patients β-TM patients. Patients were studied focusing on transfusion history, transfusion index, iron chelation therapy and compliance to chelation. Complete blood picture, markers of hemolysis, serum ferritin and random blood glucose (RBG) were measured. Patients with RBG ≥140mg/dL were subjected to OGTT, insertion of CGMS for 3 days, measurement of fasting C peptide, and serum insulin with calculation of HOMA-IR and assessment of HbA1c.
Results: Screening with RBG revealed that 20 patients (10%) had RBG ≥140mg/dL. Using OGTT, 7 (3.5%) patients were in the diabetic range, 7 (3.5%) had normal OGTT while 6 (3%) had impaired glucose tolerance. The CGMS showed that 7 (3.5%) patients had IGTT (6.5%) and 13 patients had diabetes

mellitus. The percentage of diabetic patients diagnosed by CGMS was significantly higher than that with OGTT (p= 0.012). According to CGMS readings, 10 of the 13 patients with diabetes had abnormal HbA1c readings of diabetic range (6.5-9.9%) while 5 of the 7 patients with impaired glucose tolerance had HbA1c readings in the prediabetic range (5.5-6.1%). Serum ferritin were significantly higher among patients with RBG ≥140mg/dL (p= 0.001). It was noted that 85% of patients with RBG≥ 140mg/dL were noncompliant and 75% of patients on desferrioxamine therapy had RBG≥140mg/dL. There was a significant positive correlation between HbA1C% and FBG among the studied thalassemia patients with elevated RBG≥ 140mg/dL, while HbA1c% was negatively correlated with fasting C-peptide. Serum ferritin was positively correlated with RBG. As regards CGMS data, HbA1C was positively correlated to maximum blood glucose, average blood glucose, SDS blood glucose and area under the curve≥140mg/dL. The only significant independent factor for elevated RBG ≥140mg/dL was serum ferritin.

Summary/Conclusions: The use of CGMS in the diagnosis of early glycemic abnormalities (prediabetes) among patients with β-TM appears to be promising and superior to other known diagnostic modalities namely OGTT and HbA1c.

E1583

LEFT VENTRICULAR REGIONAL FUNCTION IN CHILDREN WITH BETA THALASSEMIA WITH NO CARDIAC MANIFESTATIONS (FOUR-DIMENSIONAL ECHOCARDIOGRAPHIC STUDY)

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Background: Early detection of myocardial dysfunction is essential for the management of patients with thalassemia. Four -dimensional echocardiography imaging technique that analyzes the motion of tissues in the heart may be useful for detecting subclinical cardiovascular disease.
Aims: To evaluate the 4-dimensional echocardiographic strain in children with beta thalassemia major and correlate it with other echocardiographic parameters.
Methods: This is a cross sectional cohort Study included 200 children, 1-18 years-old. They were divided into: One hundred children with β-Thalassemia major with no clinical cardiac manifestations and 100 healthy children as a control group. They were subjected to the following investigations: Complete blood count, serum ferritin and Four-dimensional echocardiographic strains (Longitudinal, Circumferential, Radial and Area strains).
Results: There was no significant difference between the two groups as regard mitral annulus systolic velocity (S wave), E/A ratio and iso-volumic acceleration but there was significant difference as regard to ejection fraction, left ventricle mass, sphericity index and myocardial performance index. The mean values of Left ventricular Strains (Longitudinal, Circumferential, Radial and Area strains) were significantly lower in patients with thalassemia (-14.86±12.131, -8.01±3.829, 33.13±10.613, -19.45±6.866) than controls (-19.13±1.502, -16.32±1.34, 37.28±4.209, -22.94±3.064) than controls respectively with a positive correlation with 2-Dimensional strain.
Summary/Conclusions: Strain parameters of the left ventricle obtained by four-dimensional echocardiography can be a novel and promising technique for early detection of left ventricular dysfunction in children with thalassemia.

E1584

THE IMPORTANCE OF SERUM GDF-15 LEVELS TO ASSESS IRON OVERLOAD IN PATIENTS WITH THALASSEMIA MAJOR

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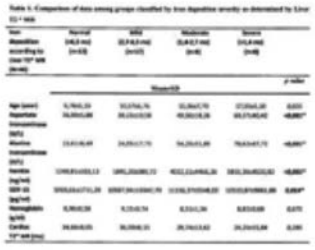
Background: There is growing interest in noninvasive assessment of iron accumulation in patients with thalassemia major. Magnetic resonance imaging (MRI) have become widely available in recent times.
Aims: We aimed to evaluate the importance of serum GDF-15 levels for monitoring the iron overload in patients with beta thalassemia major.
Methods: Forty-six patients aged between 1 and 25 years were included in the study. Serum levels of GDF-15, ferritin, troponin, AST and ALT were studied. T2*MRI was performed for all patients. The relationship between GDF-15 hormone levels and T2 * MR, ferritin levels, sex, annual transfusion volume, splenectomy was evaluated.
Results: Of 46 patients, 20 were male (%43,5) and 26 were female (%56,5), with a median age of 12,4 years. Mean serum ferritin level was 2752, 15±3105,78 ng/ml. Mean GDF-15 level was 9672,87±7910,36pg/ml. Mean duration of T2*MRI was 32,50±11,33 ms for hearth and 4,87±3,78 ms for liver. 12 patients were underwent splenectomy. Serum GDF 15 levels were significantly higher in thalassemia major patients than in normal levels. According to T2*MRI levels, serum GDF-15 levels were significantly higher in patients with hepatic iron overload. There was a negative correlation between hepatic T2*MR values and serum GDF-15 levels. However, there was not significant correlation between cardiac T2*MR and serum GDF-15 levels. Splenectomy had no effect on GDF-15 levels (Table 1).

Table 1.



Summary/Conclusions: We demonstrated that serum GDF-15 levels were increased in thalassemia major patients. GDF-15 levels is correlated with hepatic iron overload but not cardiac iron overload. It may be due to lower number of thalassemic patients with abnormal cardiac T2* MRI. GDF-15 may be a valuable parameter to assess iron overload in thalassemia major, but further studies are needed.

E1585

ASSOCIATION OF SP1 POLYMORPHISM IN THE COLLAGEN TYPE I ALPHA -1 (COLIA1) GENE WITH OSTEOPROSIS IN CHILDREN WITH BETA-THALASSEMIA

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Background: Osteoporosis is a progressive bone disease that is characterised by a decrease in bone mass and density that leads to an increased risk of fracture. Early detection of mutation at the Sp1-binding site on the *COLIAI* gene is mandatory in order to initiate preventive therapy before the occurrence of fractures in children with beta-thalassemia.
Aims: To study the relationship between SP1 polymorphism in the collagen type 1 alpha 1 gene and the development of osteoporosis in patients with Beta thalassemia.
Methods: A prospective case control study was carried out in the Outpatient Clinic of Hematology Unit of Pediatric Department and Clinical Pathology Department at Zagazig University Hospitals on forty thalassemic patients (21 females &19 males) aged 6-18 years during their regular follow-up visits (22 patients with thalassemia major and 18 with thalassemia intermedia)and forty age- and sex-matched healthy children as a control group. All patients and control were subjected to full medical history, thorough clinical examination and laboratory investigations in the form of complete blood count, Hb electrophoresis, Calcium level Serum ,alkalin phosphatase, Bone Density by DXA, Serum osteocalcine level and *COLIAI* gene polymorphism by using polymerase chain reaction-restriction fragment length polymorphism technique (PCR-RFLP).
Results: There was highly significant difference between thalassemia patients and control group as regards serum levels of calcium, osteocalcin and alkaline phosphatase and DEXA results but no significant difference between thalassemia major and thalassemia intermedia patients. As regard COLIA1 genotype there was high percentage of heterozygous Ss (G/T) and homozygous ss (T/T) genotype in beta thalassemia major 55.63%, 13.67% than thalassemia intermedia 50.6%, 0%, respectivily. There was significant relation between COLIA1genotypes and Calcium level (p=0.02). But there was no significant relation between COLIA1genotypes and osteocalcin, alkaline phosphatase levels and DEXA among studied groups.
Summary/Conclusions: SP1 polymorphism in collagen gene could be of clinical value in identifying the thalassemic patients at risk of developing osteoporosis.

E1586

UNUSUAL MOLECULAR MECHANISMS IN THE ORIGIN OF ALPHA-THALASSEMIA

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Background: Hemoglobin (Hb) is a protein responsible for oxygen transportation from lungs to the entire body. It is composed by four globular subunits - the globins - each with a central core containing a heme molecule. Globins are encoded by the α- and β-globin gene clusters located at 16p13.3 and 11p15.5, respectively. The pattern of globin gene expression during development is precisely controlled by the interaction of *cis*-regulatory genomic regions (located in close proximity to and far from genes) with *trans*-activating/silencing factors within permissive chromatin domains. Distally upstream of the α-globin genes there are four multispecies conserved sequences (MCS-R1 to R4) which are critical for the expression of the downstream globin genes. Deletions removing the α-globin genes and/or their distal MCSs give rise to α-thalassemia, one of the most common genetic recessive disorders worldwide, due to a reduced rate of α-globin chain synthesis. The severity of the pathology is variable ranging from a very mild microcytic hypochromic anemia to a moderately severe anemia associated with the formation of β_4 tetramers resulting in HbH disease or an even higher reduction or complete absence of α-chains resulting in hemoglobin Bart's hydrops fetalis, a condition generally incompatible with life.
Aims: The main objectives of this work were to characterize the molecular lesions underlying ten Portuguese cases of unusual α-thalassemia/HbH disease and to understand their origin and functional consequences.
Methods: After exclusion the most frequent molecular lesions associated with α-thalassemia, Multiplex Ligation-dependent Probe Amplification (MLPA) using the SALSA MLPA P140B HBA kit (MCR-Holland) was used to search for DNA deletions in the subtelomeric region of chromosome 16p. Additionally, specifically designed synthetic MLPA probes, as well as gap-PCR and Sanger sequencing were performed for more accurate deletion breakpoint mapping.
Results: We have found five distinct deletions and one indel, all in heterozygosity. The deletions range from approximately 3.3 to 323 kb and two of them are novel. The three larger deletions remove the entire α-globin cluster whereas the others remove totally or partially the distal regulatory elements keeping the α-globin genes structurally intact. The indel comprises the deletion of the MCS-R2 regulatory element and the insertion of a singular 39 bp DNA fragment possibly originating from a complex rearrangement involving chromosome 3. Finally, no α-globin gene cluster deletion or point mutation were found in a patient who revealed to be a very unusual case of acquired alpha-thalassemia associated with a myelodysplastic syndrome.
Summary/Conclusions: Our study widens the spectrum of molecular lesions and unusual molecular mechanisms by which α-thalassemia/HbH may occur and emphasizes the importance of diagnosing large α^0-deletions to provide patients with appropriate genetic counseling.

E1587

Abstract withdrawn.

E1588

VALUE OF HBA2 IN THE DIAGNOSIS OF BETA-THALASSEMIA MINOR "ATTENTION TO THE GRAY ZONE"

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Background: The homozygosity for the alternative splicing mutation *HBB*: IVSI-6 (C>T) is the most frequent genotype of beta thalassemia intermediate in our population and was even termed "beta thalassemia intermediate type Portuguese" (Tamagnini et al, 1983). The IVSI-6 (C> T) carriers (heterozygous) are characterized by mild hypochromia and microcytosis, with a moderately increased in HbA2, that may be even less than 3.5%. The correct identification of these carriers is important, especially when facing a couple who intends to have children.
Aims: To evaluate the percentage of individuals with hypochromia and microcytosis and Hb A2 between 3.2% and 3.4%, who are beta thalassemia carriers, alerting for the need to adapt the cut-offs of HbA2 values to the genetic background of different populations.
Methods: Parameterized search of all the consecutive individuals evaluated in our laboratory from January 2007 to January 2016. The inclusion criteria were the simultaneous presence of hypochromia and microcytosis (adjusted to the age) and HbA2 values between 3.2% and 3.4% inclusive. The exclusion criteria were the presence and/or clinical information of sideropenia or sideropenic anemia, hemoglobin variants or alpha thalassemia. Sequencing of the entire HBB gene was preformed by Sanger Sequencing.
Results: Respecting the inclusion and exclusion criteria we have identified 43 individuals with hypochromic and microcytic anemia, HbA2 ≥3.2% and ≤3.4%, in which the *HBB* gene mutations were screened. Among the 43 subjects, nineteen presented HbA2=3.2% (19/43), eleven HbA2=3.3% (11/43) and thirteen had HbA2=3.4% (13/43). The IVSI-6 (T>C) mutation was identified in 2 subjects with HbA2=3.2% (10%), 5 with HbA2=3.3% (45%) and 7 with HbA2=3.4% (54%). No other *HBB* gene mutations were detected. The remaining individuals were classified as probable alpha thalassemia and suggested continuation of the study, if warranted.
Summary/Conclusions: We have identified 14/43 (32%) individuals as beta thalassemia carriers who, for the conventional cut-off of HbA2 ≥3.5%, would not have been diagnosed. Based on this data, we propose that individuals with hypochromia and microcytosis, with normal or slightly elevated RDW, without sideropenia, with HbA2 between 3.2-3.4%, should be screened for mutations in the *HBB* gene, in order to rule out beta thalassemia carriers due to Beta+ mutations. As *HBB* IVSI-6 (T>C) mutation is one of the most frequent beta thalassemia mutations in Portugal, and in Mediterranean basin, it is necessary to keep in mind that the classic rule of HbA2> 3.5% for the diagnosis of beta thalassemia minor may underdiagnose this pathology and lead to an incorrect genetic counseling.

E1589

DIAGNOSIS OF HEMOGLOBINOPATHIES BY CAPILLARY ZONE ELECTROPHORESIS: EXPERIENCE WITH 925 CASES

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Background: Hemoglobin capillary zone electrophoresis is a relatively newer technique as compared to HPLC for detection of abnormal hemoglobins. We share our first hand experience of using Capillary 2 Flex piercing instrument for diagnosis of hemoglobinopathies as a primary diagnostic modality
Aims: The main aim was to evaluate a new technology for diagnosis of hemoglobinopathy.
Methods: The capillary 2 Flex piercing instrument with Phoresis software for hemoglobin electrophoresis at alkaline pH was evaluated at our centre over a period of 1 year. A total of 925 sample runs were included in the analysis. The equipment was assessed on the following parameters: ease of operation, pre-analytical factors, identification, quantification and precision of hemoglobin variants including the rare variants. Further, we evaluated if capillary zone electrophoresis can be useful as a single method for diagnosis of hemoglobinopathies.
Results: The automation provided by capillary zone electrophoresis eased the problem of errors during sample preparation. The option for low sample volume mode is a great help in samples from children. The instrument could readily identify all common hemoglobins and the diagnosis was straightforward in 829 (89.7%) cases. In the rest 96 (10.3%) cases, the sample was required to be rerun because it lacked Hb A or Hb A2. This posed inconvenience because the electrophoretic zones get displaced and have to be derived after mixing it with normal sample. The machine is not specifically standardized for cord blood samples hence we are not performing tests on neonatal cord blood sample.
The instrument could separately identify Hb E from Hb A2 which is a big scorer over HPLC, however, we found mild high Hb A2 both in heterozygous and homozygous Hb E cases (heterozygous Hb E, n-28 mean Hb A2- 3.9% and homozygous Hb E, n- 7 and mean Hb A2- 4.2%) leaving the doubt whether some adducts are still left. Identification of small peaks of Hb H could be difficult and requires other modalities to confirm. Two cases where Hb H was strongly suspected clinically and HB H inclusion test was positive showed small peaks of HB H (1.2% and 0.9%) on HPLC. Hemoglobins falling into the same zone (eg Hb D- Punjab and Hb Q India) needed identification with second modality. Whenever encountered with problem of identifying certain abnormal peak, we resorted to HPLC for confirmation. Spectrum of hemoglobin variants encountered (n-298 cases, rest 627 showed normal results) in the study is listed in table below.

Table 1.

	Type of Hemoglobin	No of cases (n- 298)
1.	Beta Thalassemia	194
2.	Hemoglobin S	30
3.	Hemoglobin E	35
4.	Hemoglobin D	9
5.	Hemoglobin J Meerut	4
6.	Hemoglobin H	4
7.	Hemoglobin Q India	2
8.	Haemoglobin Lepore	3
9.	Hereditary persistence of fetal hemoglobin	1
10.	Compound heterozygotes (eg Hb E-beta thalassemia or Hb S- beta thalassemia)	16

Summary/Conclusions: Capillary zone electrophoresis is an alternative method which brings automation. However, since the diagnosis of Hemoglobin variants mandates confirmation by a second method, HPLC cannot be replaced completely. Based upon the availability, workload and cost effectiveness, any of these two methods can be used as primary modality.

Thrombosis and vascular biology

E1590

RELEVANT ROLE OF VON WILLEBRAND FACTOR-ADAMTS13 AXIS IN HEPATIC ISCHEMIA- REPERFUSION INJURY

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Background: Hepatic ischemia-reperfusion (I/R) injury is a liver damage occurring during liver surgeries such as hepatic resection or transplantation, and denotes the major basis for graft dysfunction after transplantation. Although detailed mechanisms of hepatic I/R injury remain to be clarified, an excessive inflammatory response is thought to play a role in this regard.
Aims: Since recent studies suggest that von Willebrand factor (VWF) plays a pivotal role in a cross-talk between inflammation and thrombosis, we assumed that VWF may be involved in the pathophysiology of hepatic I/R injury. To test this hypothesis, we have used a mouse experimental model of hepatic I/R injury.
Methods: Mice were anesthetized with sodium pentobarbital and a midline laparotomy was then performed on a heating pad. Blood supply for left lateral and median lobes of liver (approximately 70% of the liver mass) was interrupted by cross-clamping the hepatic artery and portal vein with a microvascular atraumatic clip for 90 min. Then a clip was taken off to provoke the reperfusion of hepatic blood flow, which was monitored on the surface of left lateral lobe by Laser Doppler flowmetry (ALF21, Advance Co, Tokyo, Japan). The hepatic blood flow was measured again 24 h after reperfusion and mice were then sacrificed for blood collection and histological analysis of liver tissue. We compared 16 wild-type (WT) and 12 VWF-gene deleted (knock-out; KO) mice (from The Jackson Laboratory, Bar Harbor, ME), all of which were 8-12 weeks of age, healthy and fertile. Excess blood loss was not observed in all mice (WT or KO) during whole surgical process.
Results: As compared to WT mice, restoration of hepatic blood flow was significantly greater in VWF-KO mice at 24 h after reperfusion (WT; 61± 17% *vs* KO; 87±17%, expressed as the percentage of pre-ischemic value). Consistent with the hepatic blood flow, the time-course analysis of serum alanine aminotransferase (ALT) at several time points after reperfusion revealed the lesser liver damages of KO mice (WT; 6898±3270 and 1313±621 IU/L *vs* KO; 3043±1320 and 478±330 IU/L, at 3 h and 24 h after reperfusion, respectively). In addition, histological analysis confirmed that neutrophil infiltration in the liver tissue of KO mice was significantly reduced as compared to WT mice at 24 h after reperfusion. These impaired hepatic blood flow and ALT values as well as intensified neutrophil infiltration in WT mice were significantly improved to an extent comparable to those of KO mice by the bolus injection of recombinant human ADAMTS13 (3 μg/mouse equivalent to 2800 U/kg, n=12) just prior to the I/R operation.
Summary/Conclusions: Our results altogether indicate that VWF-dependent inflammatory responses with neutrophil recruitment at ischemic sites are involved in pathophysiology of hepatic I/R injury, and functional regulation of VWF by ADAMTS 13 may serve as a promising therapeutic option for hepatic I/R injury.

E1591

THE IMPORTANCE OF THE FULL BLOOD COUNT, JAK II AND ADAMTS 13 TESTING IN STROKE EVALUATION: A REVIEW OF 619 CONSECUTIVE YOUNG STROKE AND TIA PATIENTS

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Background: Young ischaemic stroke patients undergo extensive investigations yet around 40% remain of undetermined cause. Complex and costly thrombophilia testing is routinely sent despite limited evidence linking to arterial disease. A full blood count may be ignored but is potentially more helpful in suggesting myeloproliferative disease or thrombotic thrombocytopenic purpura (TTP) as causative.
Aims: We retrospectively reviewed full blood counts, specifically haematocrit and platelet count, and whether these were documented and further investigated if outside of the normal laboratory range. We examined whether less common primary haematological disorders known to cause stroke were considered: JAK II mutation analysis for myeloproliferative diseases such as polycythaemia vera (PV) and essential thrombocythaemia (ET), and ADAMTS13 analysis for TTP.
Methods: We retrospectively reviewed consecutive clinical and laboratory records for all stroke and TIA patients <60 years presenting to a regional hyperacute stroke unit and daily TIA clinic from January 1st 2015- August 7th 2016. All those with thrombocytosis (defined as platelet count >400x10^9/L) and/ or raised haematocrit (defined as Hct >0.45) were reviewed to see if a cause could be determined, and if not, whether JAK II analysis was considered and tested. We similarly examined patients presenting with thrombocytopenia (defined as platelet count <150x10^9/L), and if no cause determined, whether ADAMTS13 testing was contemplated.
Results: 610 patients <60 years were included: 379 ischaemic stroke (62.1%), 193 TIA (31.6%) and 38 haemorrhagic stroke (6.2%). 161 (26.4%) had abnormalities in haematocrit or platelet count: 116 (19%) had a raised haematocrit, 19 (3.1%) thrombocytosis, and 26 (4.2%) thrombocytopenia. Of these, 7 patients demonstrated abnormalities of both cell lines. Of these initial 161 abnormal results, 119 (73.9%) were repeated but 42 (26.1%) were not. JAK II testing was deemed warranted in 17 (2.8%): a persistently raised or progressively raised haematocrit or platelet count respectively, with normal liver and renal function and no other explicable cause. JAK II mutational analysis was performed in 3 patients (0.5%). One was proven positive for the V617 F mutation, hence diagnosed with polycythaemia vera. Of the 2 negative JAK II results, one patient was subsequently diagnosed with chronic myeloid leukaemia. Fourteen patients had no further testing or monitoring. 26/610 (4.3%) patients had thrombocytopenia. ADAMTS13 testing was not warranted in 17 of these (subsequent resolution of platelet count n=7, HIV n=2, liver derangement n=7, known ITP with no MAHA n=1). ADAMTS13 testing was indicated in 9 of these patients (34.6% of thrombocytopenic patients), defined as a persistent thrombocytopenia with no clear cause, normal liver and renal function and negative HIV status. Seven of these patients did not have ADAMTS13 considered, according to the clinical documentation, nor sent. Of the 2 tested for ADAMTS13, one result was normal, helping to resolve the clinical diagnosis of ITP. In the other patient, ADAMTS13 was <5%, confirming TTP and facilitating life-saving plasma exchange to take place.
Summary/Conclusions: In stroke patients <60 years, one quarter had abnormalities in haematocrit or platelets. Myeloproliferative disease or TTP was present in 3 patients of 5 specifically investigated in the cohort. From a haematological perspective, at least 21 further patients merited further investigation. However, this number may be higher since a quarter of those patients with initial discrepancies of haematocrit and/ or platelet count did not have repeated testing. Although primary haematological disorders are rare as a cause of stroke, a basic full blood count result should not be ignored in considering the aetiology of arterial thrombosis in a younger cohort.

E1592

PERIPHERALLY INSERTED CENTRAL CATHETER (PICC) RELATED THROMBOSIS IN 230 PATIENTS WITH HEMATOLOGICAL MALIGNANCIES. A 6 YEARS SINGLE EXPERIENCE CENTER

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Background: The use of peripherally inserted central catheters (PICCs) is widely extended in patients with hematological malignancies, not only to be treated with chemotherapy, blood cell transfusions, but also parenteral nutrition support or frequent analytical extractions. However, catheter-related thrombosis is one of its main complications. There are a few studies that evaluate this complication. We reported the experience of the PICC-related thrombosis (PRT) in our center.
Aims: To analyze the incidence of PRT, describe the clinical characteristics and management of these patients and identify the risk factors of PRT.
Methods: We performed a retrospective chart review of 230 adult patients diagnosed with hematological malignancies, in whom, experimented nurses tunneled PICCs with different technique: blinding Seldinger from 2010 to 2014 and guided by ultrasonography (US) from 2015 to 2016. PRT diagnose was confirmed by Doppler US. Statistical analysis was performed using the SPSS program (v.20).
Results: The median age was 58 years (14-86) and 55.7% of the patients enrolled in the study were male. The most frequent hematological malignancies were: Non-Hodgkin's lymphoma (NHL=105; 45,7%) myeloid malignancies (acute myeloid leukemia and myelodysplastic syndromes (AML/MDS=60; 26,1%), acute lymphoblastic leukemia (ALL=22; 9,6%), multiple myeloma (MM=19; 8,3%) and Hodgkin lymphoma (HL=17; 7,4%). In 188 patients (82%), PICC was tunneled when the active disease was presented. Only 51 patients (22%) received thromboprophylaxis based on low molecular weight heparin (LMWH=27), aspirin (ASA=21) or vitamin K antagonist (VKA=3). PICCs were tunneled guided by US in 127 patients (55,2%), and the main location of tip catheter was in cava-right atrium region (66%). The overall incidence of PRT was 7% (n=16). The main diagnoses related to PTR were ALL (6), NHL (5), and HL (3). All except one had active disease when PICC was tunneled (15/16=94%). Fourteen patients (88%) were treated by chemotherapy based in L-asparaginase (L-ASA), immunomodulatory drugs or other treatment combined with corticosteroids. The median onset of PRT was 26 days, (range: 0-230) and 8 of them (50%) in the first 30 days after insertion. In 11 cases (69%) D-Dimer was elevated. All PICC were removed within 72 hours of PRT and treated with LMWH to a median of 4 months (range: 1-11). During follow-up, no patient had progression of thrombosis, or pulmonary thromboembolism. Finally, in the univariate analysis ALL, HL and L-ASA had significant impact on

PRT (LLA:OR 15,75; CI 2,91-85,12; p= 0,001; HL:OR 9; CI 1,38-58,78; p=0,022; L-ASA:OR 7,82; CI 2,45-24,95; p=0,001). However, in the multivariate analysis, HL was the only risk factor of PRT (OR 8,38; CI 1,05-66,5; *p*=0,044).
Summary/Conclusions: Patients with HL may be predisposed to developing PRT compared with those with other types of hematological malignancies. Identifying the mechanism underlying this relationship will require further study.

E1593

A STUDY OF VENOUS THROMBOEMBOLISM SUSCEPTIBILITY LOCI FACTOR XI, ABO AND FIBRINOGEN IN A PORTUGUESE POPULATION SAMPLE
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Background: Venous thromboembolism (VTE) is a multifactorial disease caused by environmental/acquired risk factors and complex gene-gene and gene-environment interactions. VTE results from the development of a thrombus, usually in the deep veins of the leg (deep vein thrombosis, DVT) that can subsequently embolise to the lung (pulmonary embolism, PE). Classical inherited risk factors for VTE in European-ancestry populations include protein C and S deficiencies, factor V Leiden and prothrombin gene mutation (FII G20210A). Several other common and low-frequency susceptibility variants, mainly single nucleotide polymorphisms (SNPs) in loci *ABO,FXI, FII, FV, FGG, GP6, KNG1, PROCR, SLC44A2, STXBP5,TSPAN15* and *VWF,* have been also found robustly associated with VTE. However, in the Portuguese population, the genetic background for VTE for most of these genetic susceptibility variants remains to be evaluated.
Aims: To investigate the association of five SNPs in the loci ABO (rs2519093 and rs8176719), FXI (rs2036914 and rs2289252) and FGG (rs2066865) with VTE in a sample of Portuguese patients.
Methods: A retrospective (2012-2015) case-control study with 119 cases of unprovoked VTE and 148 healthy controls of Portuguese origin was conducted, to evaluate allele frequencies of the five VTE risk alleles in the Portuguese population and to assess the association between these alleles and the risk for VTE. FXI (rs2036914 and rs2289252) and FGG (rs2066865) SNPs were genotyped by real-time PCR with TaqMan probes. *ABO* rs2519093 and rs8176719 SNPs were genotyped by restriction fragment length polymorphism (RFLP). PLINK software was used to determine the allelic frequencies, concordance with Hardy Weinberg equilibrium and association between risk alleles and VTE through logistic regression, in the additive model, estimating OR with 95% confidence intervals (95% CI) and *p*-values. The association between the cumulative number of risk alleles and the risk of VTE was assess through Pearson χ^2 using the *Simple Interactive Statistical Analysis software* (SISA).
Results: The estimated risk allele frequencies in the overall study population sample were: 0.212 for *FGG* rs2066865 (T), 0.62 and 0.50 for *FXI* rs2036914 (C) and rs2289252 (T), respectively, and 0.295 and 0.417 for *ABO* rs2519093 (T) and rs8176719 (C), respectively. The genotype distributions were in agreement with the Hardy-Weinberg equilibrium (P>0.05) for all SNPs. The logistic regression under an additive model showed that *FGG* rs2066865 was associated with VTE (nominal *p*=0.029; OR=1.57, CI 95% 1.05-2.37) as well as *ABO* rs8176719 (nominal *p*=0.0064; OR=1.65, CI 95% 1.15-2.36). Both SNPs remain significantly associated when adjusting for age and sex (P=0.019 and P=0.0065, respectively). ABO rs2519093 did not reach significant association with VTE in our population sample (P=0.184) as well as *F11* rs2036914 and rs2289252 SNPs (P=0.76 and P=0.16, respectively). In addition, there was an increased risk of VTE associated with the increment in the total number of risk alleles: 0 *vs* 1 risk allele: X^2=5.8, *p*=0.015, OR=2.31; and 0 *vs* 2 or more risk alleles: X^2=12.2, *p*=0.00048, OR=3.36).
Summary/Conclusions: Our data suggest that the alleles *FGG* rs2066865 T and *ABO* rs8176719 C may contribute to the VTE susceptibility in the Portuguese population. The absence of significant associations for the remaining loci could be the result of a limited statistical power, consequence of a modest effect size of polymorphisms or lower sample sizes, or because of differences in genetic backgrounds between populations.

E1594

PEDIATRIC VENOUS THROMBOEMBOLISM: INCIDENCE, RISK FACTORS AND MANAGEMENT OF HOSPITALIZED PATIENTS IN A TERTIARY CARE TEACHING HOSPITAL
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Background: Venous thromboembolism (VTE) is considered a rare event in childhood. In spite of this, the incidence of VTE is on the rise in hospitalized patients. Medical progress in the treatment of critically ill patients has increased the use of central venous catheters (CVC) and interventional procedures, especially in children with cardiac defects and malignant disease. Therefore VTE is increasingly recognized as a major secondary complication of advanced tertiary care in infants and children.
Aims: To study the incidence, demographics, risk factors, diagnostic tests, therapy, and complications of pediatric acute VTE in our tertiary care hospital.
Methods: A retrospective single-center study of patients<18 years of age who were discharged from January 2014 to December 2016 by using diagnostic codes for acute VTE from our hospital database. We studied demographic characteristics, clinical presentation, diagnostic tests, risk factors, treatment strategies and outcome.
Results: We report an incidence rate of 10.7 cases per 10,000 patient-years (70 acute VTE events / 21,892 discharge cases over a 3-year period). Patients were predominantly male (57%). Mean patient age was 3.3 years, with the greatest proportion of cases, 54.3%, in the infant cohort (from 1-12 months), while children above 1 year comprised 37% and neonates (<1 month) formed 8.6% of our sample. Patients were mostly born at full term (71.4%), although 45.7% of the neonatal and infant cases were premature. Catheter-related (CVC-VTE) comprised 55.7% of VTE cases. On the other hand, non-catheter-related (NCR) diagnoses were most frequently intracranial in 35.5% of cases, in inferior extremity deep vein thrombosis (DVT) in 29% and intracardiac in 19.3%. Only 3 cases of NCR-pulmonary embolism (PE) and 2 cases of NCR-upper extremity DVT were reported. Doppler ultrasound was the most common diagnostic test used (75.7%), followed by MRI, CT and CT angiography in equal proportions. Critically ill patients encompassed most of the cases (88%). Mean duration of hospitalization was 89 days (range 2-156) and time from admittance to VTE diagnosis was 25.6 days. A large proportion had congenital heart defects (32.9%) requiring interventional procedures. Half of the patients (51.4%) had surgery around the time of VTE diagnosis. Malignancy was identified in 5 cases (2 of which were CVC-VTE). Transient triggers, such as infection (12 cases) and use of asparaginase (2 cases) were also reported. Most patients were not tested for thrombophilia (n=44, 62.9%) since they were classified as provoked VTE and from those who were tested 10% were diagnosed with a thrombophilia. All except for one patient initiated anticoagulant therapy: 78.6% (n=52) were initially treated with low molecular weight heparin (LMWH) and while most continued treatment with LMWH, 8.6% (n=6) received vitamin K antagonists and 8.6% received direct oral anticoagulants. LMWH dosing was adjusted using anti-Xa assays (AXA) in 85.7% of cases, documenting a median of 5 AXA per patient, out of which 3 were within therapeutic range. Mean duration of treatment was 5.8 months. Recurrence rate was 17%, half of which were in patients with CVC-VTE. On the other hand, bleeding rate was 15.7% most of which were mild (10%) or provoked bleeds (4.3%). Mortality was 10%, although cause of death was not directly related to VTE in any of the cases.
Summary/Conclusions: Pediatric VTE is a substantial complication arising from tertiary care hospitalization where critically ill infants are at greater risk. Potential risk factors of VTE include use of CVCs, patients with complex congenital heart defects, surgical procedures, infection and malignancy. Further studies on VTE prophylaxis and identification of VTE predictors in a critical care setting are required.

E1595

CELL-BASED EVALUATION OF CHANGES IN COAGULATION ACTIVITY INDUCED BY ANTINEOPLASTIC DRUGS FOR THE TREATMENT OF ACUTE MYELOID LEUKEMIA
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Background: Idarubicin (IDR), cytarabine (AraC), and tamibarotene (Am80) are effective for treatment of acute myeloid leukemia (AML). In acute leukemia, the incidence of venous thromboembolism or disseminated intravascular coagulation is associated with induction chemotherapy.
Aims: How some drugs for the treatment of AML affect the procoagulant activity is unclear. Thereby, in this study, we investigated the procoagulant effects of IDR in comparison with AraC and Am80.
Methods: Procoagulant effects of IDR, AraC, and Am80 were investigated in a vascular endothelial cell line EAhy926 and AML cell lines HL60 (AML M2), NB4 (AML M3, APL), and U937 (AML M5), focusing on tissue factor (TF), phosphatidylserine (PS), and thrombomodulin (TM). Normal human plasma-based recalcification time assay, flow ctytometric anayses, and RT-PCR are applied for the evaluation.
Results: IDR induced procoagulant activity on the surface of vascular endothelial and AML cell lines. Expression of TF antigen, TM antigen, and PS were induced by IDR on the surface of each cell line, whereas expression of TF and TM mRNAs were unchanged. Increased TF and PS expression may overcome increased TM expression and the overall effect may be procoagulant. Conversely, Am80 decreased TF exposure and procoagulant activity, and increased TM exposure on NB4 cells. In NB4 cells, we observed downregulation of TF mRNA and upregulation of TM mRNA by Am80. But Am80 did not sufficiently exhibit anticoagulant activity on NB4 cells when applied simultaneously with IDR.
Summary/Conclusions: These data suggest IDR may induce procoagulant activity in vessels by apoptosis through PS exposure and/or TF expression on vascular endothelial and AML cell lines. Am80 may suppress blood coagulation through downregulation of TF expression and induction of TM expression. Our methods could be useful to investigate changes in procoagulant activity induced by antineoplastic drugs.

E1596

DESCRIPTION OF THROMBOTIC EVENTS AND/OR PREGNANCY LOSSES IN A COHORT OF HOMOZYGOUS CARRIERS FOR THE C46T POLYMORPHISM OF THE F12 GENE

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Background: The intrinsic pathway of coagulation is initiated by a serine protease named factor XII (FXII) in a reaction involving the contact system and triggers fibrin formation through activation of factor XI. In vitro, FXII triggers activation of the classic complement pathway and initiates the fibrinolytic system via plasma kallikrein mediated urokinasa activation, whereas *in vivo* its role remains uncertain. A C—>T polymorphism at nucleotide 46 in the 5'-untranslated region of the F12 gene (F12 C46T) is associated with lower levels of FXII. Its frequency varies widely across populations and ethnic groups, ranging from 0.18 in the Spanish population to 0.67 among Japanese. Homozygosity for the C46T polymorphism of the F12 gene has proved to be an independent risk factor for thrombosis and unexplained recurrent spontaneous abortion. However, the precise role of this polymorphism as a thrombotic risk factor is controversial, and the evidence for an association between F12 C46T, venous thromboembolism (VTE) and myocardial infarction is weak.
Aims: To describe the occurrence of thrombotic events and/or pregnancy losses and the existence of other risk factors for thrombosis in a cohort of homozygous individuals for F12 C46T.
Methods: We retrospectively analyzed all the homozygous F12 C46T cases diagnosed in our laboratory from January 2015 to January 2017. Allelic discrimination PCR with TaqManmgB probes was performed to detect homozygous individuals for F12 C46T mutation. The following variables were collected: age, gender, race, cardiovascular risk factors (CVRF) (hypertension, diabetes mellitus, dyslipidemia, smoking and overweight), history of cancer, VTE (type, recurrence), arterial thrombosis, familiar history of thrombosis, number of pregnancy losses and other inherited/acquired thrombophilia.
Results: 122 cases were evaluated: 45 (36.88%) male and 77 (63.12%) female. Mean age: 46.2 years (1-86). Race: 65.57% caucasian, 13.1% american, 2.4% black, 1.6% asiatic, 4.1% other. Decreased factor XII plasma levels were found in 81.42% of them, with mean factor XII levels 53.73% (27.5-107.5). Overall, 34.48% of the subjects had at least one thrombotic event. Type of thrombosis: 64.4% VTE and 35.6% arterial thrombosis. One (26.7%) or more than one (46.7%) additional thrombotic risk factors were found in patients with any thrombotic event. Presence of one or more CVRF was found in 66.7%. Familiar history of thrombosis was found in 16%, whereas 13% had a recent or active malignant neoplasm. Among women, 28.57% and 12.98% had one and more than one pregnancy loss respectively. Additional thrombotic risk factors were found in 60% of women with recurrent losses. One (43%) or more than one (13%) additional thrombotic risk factors were found in women with any pregnancy loss. Presence of one or more CVRF were found in 30% of them. Familiar history of thrombosis was found in 34.7%, whereas none of them had a recent or active malignant neoplasm.
Summary/Conclusions: Most patients with a thrombotic episode had one or more additional risk factors. Nevertheless, up to 26.7% presented no other risk factor than homozygous F12 C46T, suggesting a relevant role in the pathogenesis of thrombosis. According to our results, the risk of abortion could be increased by the presence of homozygosity for F12 C46T, since it was the only thrombotic risk factor in 40% of women with recurrent pregnancy losses. Further studies are needed to clarify the real contribution of F12 C46T to thrombosis and pregnancy losses on prospectively selected patients.

E1597

ANALYSIS OF CHARECTERSISTICS OF HOSPITAL ASSOCIATED THROMBOSES

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Background: Hospital associated thrombosis (HAT) is now commonly monitored but expected targets of HATs remains poorly reported.
Aims: We analysed HATs in our hospital group over a 40 month period to assess any trends or paterns of HAT incedence and charecteristics over time.
Methods: We reviewed all VTE episodes in 2013-2017 across our hospital group, identifying those patients with hospital-associated thrombosis (HAT), defined as patients having had a hospital inpatient episode, including day case surgery and admissions of 4-24 hours, in the 90 days prior to their VTE episodeRoot cause analysis was undertaken on these cases, recording information of the index episode including risk assessments performed and the corresponding prescribing and administration of thromboprophylaxis (TP) consisting of LMWH or GCS.
Results: Results: a total of 2222 VTEs were identified (1051 PE's and 1178 DVTs) of which 581 (26%) HATs were identified (312 PE's, 269 DVT's). This represents an excess of PEs over the expected rate based on total VTE distribution between PE and DVT (p=0.0002 Fishers exact test). The majority of patients had a medical (non-surgical) index admission with 58.5% admitted as acute medical admissions and 41.5% surgical admission (trauma and orthopaedics 18.4%, general, vascular and GI surgery 12.2%, urology 4% and Obstetrics and gynaecology 4%). Not all surgical patients underwent operations. In 526 HAT cases, root cause analysis (RCA) revealed that 101 (19.2%) were deemed preventable and 367 (69.8%) were not thought to be preventable. The remaining 57 cases had the index admission outside of our trusts, largely having orthopaedic procedures which were not included in further analysis.
Of 394 HAT cases with sufficient data, 80 (20.3%) had a preventable cause, 27 receiving insufficient TP, 9 receiving delayed TP, 26 having no TP given though indicated and 18 not having a VTE risk assessment. Some cases of insufficient TP were deemed due to underdosing standard patient >90kg. Off those HAT cases deemed unpreventable, 37 patients had contraindications to TP, 166 had TP failure i.e. full TP given and in 102 TP was not indicated. 9 patients were on full anticoagulations at time of index admission.
Summary/Conclusion: HAT rates remain stable and the majority are though unavoidable by cureent techniques. Key errors in avoidable cases are failure to perform a timely VTE risk assessment and action with appropriate thromboprophylaxis. Full integration of electronic patient records with electronic prescribing modules may reduce further these errors.

E1598

THROMBOSIS DURING INFANCY AND NEWBORN PERIOD: AN UNRESOLVED ISSUE

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Background: Reported incidence of thrombosis is higher among newborn infants that can be explained by age related deficiency of anticoagulants, overproduction of procoagulants and deficiency/ dysfunction of fibrinolysis in addition to exposure to multiple risk factors and wide use of catheters which may eventually lead to the transient prothrombotic phenotype in this age group.
Aims: Our aim is to evaluate clinical and laboratory data, risk factors, outcomes of infants (>1-≤12months) and newborns(≤1month) with thrombosis in our center.
Methods: Our database revealed 752 children having various types of thrombosis between January 2003 to December 2015 and 77 out of 752 were diagnosed as thrombosis under one year of age. We retrospectively evaluate their clinical and laboratory results by searching their paper based & electronic files. Thrombotic risk factors included inherited and acquired hypercoagulable states, catheter, infection, trauma, surgical operations were also recorded.
Results: There were 51 male and 26 female with a median age of 4 months (0-12 months) in this group. Among 77 thrombotic events 22 (28%) were observed during neonatal period (≤1month) with a male predominancy (n=15, 68%) and from those 22 events 2 were arterial thrombosis (purpura fulminans(1), cerebral(1)) whereas 4 intracardiac, 5 sinovenous and 11 venous thrombosis (deep veins(4), renal veins(3), portal veins(3) cerebral vein(1)) were noted. In 2(9%) thrombosis was diagnosed on the first day of life and 11 out of 22 patient had underlying risk conditions such as prematurity(3), perinatal hypoxia(2), necrotizing enterocolitis(1), congenital cardiac disorders(3),congenital nephrotic syndrome(1) and adrenal insufficiency(1).Moreover 6 out of these 22 thrombotic event catheter insertion was the associated risk factor and 4/22 had infection. Factor V Leiden mutation was found to be homozygous in 1/18 and normal in 17/18. Heterozygous prothrombin 20210A mutation were detected in 1 out of 18 and homozygous MTHFR C677T mutation was found in 3/13 patient. Half of them 12(%54) were initially treated with LMWH and TPA were used as a thrombolytic agent in 5 case without any complication. During the follow up period 1patient had an amputation, 5 patient decased; one because of sepsis and the rest 4 had primary disesase and thrombosis .The site of location in 55 thrombotic events during the infancy period involved deep venous thrombosis (22), cerebral sinovenous thrombosis (10),cardiac(8), portal(3), renal(1) veins and cerebral arterial (7),femoral arterial(3),abdominal aortic thrombosis(1).In this group 42(76%) out of 55 had an underlying disorder and most common associated risk factor for this age group was inserted catheter related thrombosis, infection and surgical operations.Initial treatment choice was LMWH in 25(45%) and during the median follow up time of 2 years 10 had chronic thrombosis, 21 resolved, 10 had parsiel thrombosis, 4 deceased and 10 loss to follow up.
Summary/Conclusions: During the first month of life thrombotic complications is 40 times higher than at any other pediatric age. As previously reported venous thrombosis which mainly affect the limbs, the right atrium and renal veins are more frequently seen than arterial thrombosis in newborn infants with a male predominancy is compatible with our findings. In the absense of randomized clinical trials the choice of anticoagulation and the duration of treatment for this age group is still controversy beside the complex mechanism and a high mortality&morbidity rate. Although clinical and laboratory data of neonates were compatible with infants, treatment choices differ between these two groups and it sems that thrombolytic treatment was tend to be used more commonly in the neonates without any complication.

E1599

THE QUALITY COMPOSITION OF SOLUBLE FIBRIN MONOMER COMPLEX FRACTION FOR ACUTE AND POST ACUTE ISCHEMIC STROKE PATIENTS

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Background: Soluble fibrin monomer complexes (SFMC) are the early marker of thrombophilia that represent the complexes of monomeric fibrin with fibrinogen or their products of degradation (FDP). SFMC levels are not directly affected by therapy with thrombolytic agents. Detection of SFMC formed due to the activation of blood clotting by thrombin reveals a pathological process in the early, preclinical stages.
Aims: We explored the quality difference between the SFMC fraction obtained from acute ischemic stroke patients and one year post acute phase of stroke in the absolutely the same patients.
Methods: SFMC fraction was obtained from each tested groups: 35 healthy donors as well as 66 patients with atherothrombotic ischemic stroke (AIS) and 56 patients with cardioembolic ischemic stroke (CIS) during the acute phase of disease; 56 patients with AIS and 56 patients with CIS one year past acute phase. SFMC were collected from blood plasma of each tested subtypes of ischemic stroke by incubation with 0,78% o-phenanthroline per 5 min. For Size-exclusion chromatography SFMC in volume 1 ml was applied on Healthcare Life Sciences "HiLoad 16/60 Superdex 200 pg" column.
Results: Results suggest presence of proteins with Mr from 45 up to 330 kDa in SFMC fraction. The content of SFMC was similar for all stroke fractions with some exception. The difference between results of separation of stroke factions and fractions obtained from healthy donors was obvious. Mostly the proteins content of the SFMC fraction is similar for stroke and healthy fractions. But amount of the proteins as mean peaks high is different (Figure 1). In fact, the first three peaks whitch correspond to the 330, 280 and 250 kDa of chromatogram of SFMC are common for all tested fractions and were verified only in their height. Accordingly, the most widely represented variations peaks for AIS, even a year after stroke soluble fibrin monomer complex content was higher comparing to the healthy donors index. Healthy donors also had some of these complexes, but in trace amounts. For acute CIS situation was similar as for AIS, but past one year it got closer to healthy donors.

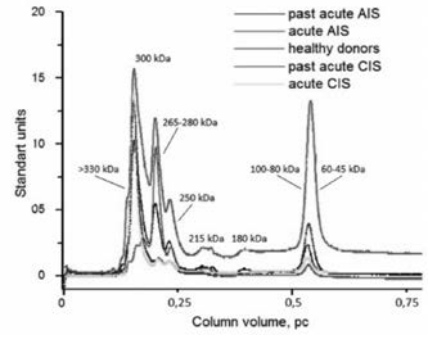

Figure 1.

Summary/Conclusions: It was shown that development of ischemic stroke accompanied by the formation of SFMC in the bloodstream that could take part in disease complication.

E1600

EVALUATION OF A RAPID NANOPARTICLE-BASED LATERAL FLOW IMMUNOASSAY (STIC EXPERT HIT) FOR THE DIAGNOSIS OF HEPARIN-INDUCED THROMBOCYTOPENIA IN A CARDIOTHORACIC HOSPITAL

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Background: Heparin Induced Thrombocytopenia (HIT) is a severe complication of heparin anticoagulation treatment that could be life threatening. HIT diagnosis is therefore of crucial importance in clinical practice especially for the cardiothoracic patients that are often exposed to heparin before surgery (e.g. during a PTCI). Laboratory testing for the presence of IgA, IgM and IgG or IgG only antibodies against PF4/Heparin (namely HIT antibodies) along with the 4Ts scoring system (Thrombocytopenia, Time of platelet count fall, Thrombosis, Other cause of thrombocytopenia) is used to evaluate the probability of HIT syndrome. At the Onassis Cardiac Surgery Center the methods for routine laboratory testing for HIT comprise Enzyme-linked Immunoassay testing for IgG, IgA, IgM HIT antibodies and Heparin-Induced Platelet Aggregation assay for the presence of platelet activating antibodies.
Aims: We evaluated a rapid nanoparticle-based lateral flow immunoassay (Stic Expert HIT) for assessing the presence of IgG antibodies to PF4/Heparin in patients plasma or serum in cases of emergency diagnosis of HIT needed for patients requiring urgent cardiothoracic surgery over a six-month period.
Methods: Stic Expert HIT, a rapid-nanoparticle based lateral flow immunoassay was performed on plasma from 35 patients from July 2016until January 2017 and the reading was done independently by two different technicians or biologists. The diagnosis of HIT was confirmed when both H/PF4 ELISA and the 4Ts score were positive.
Results: In 22 cases the Stic Expert was negative for the presence of IgG H/PF4 antibodies in the patients' plasma and HIT syndrome was easily excluded in combination with the "4Ts". In the rest 13 cases the rapid test provided doubtful results that were considered as positive and then H/PF4 ELISA was performed. Following the ELISA test, 10 out of the remaining 13 patients were found negative for the presence of IgG H/PF4 antibodies, whereas 3 patients were found positive with a relative low O.D. value (0.400). The last 3 patients that were positive for the presence of IgG H/PF4 antibodies by ELISA were found not to have HIT syndrome in combination with the '4Ts" scoring system.
Summary/Conclusions: In conclusion the Stic Expert HIT was useful for the quick exclusion of HIT (along with the 4Ts scoring system) when emergency HIT diagnosis is needed in 34% of the cases (12/35) in our Hospital. However in the rest 13/25 (66%) of the cases, laboratory testing for HIT was much more complicated and time consuming since ELISA or other assays (i.e.HIPA test) had to be performed. Nevertheless all 13 patients were found not to suffer from the HIT syndrome with the "4Ts' scoring system.

E1601

AUDIT OF 'DOOR TO NEEDLE' TIME IN ADMINISTRATION OF PROTHROMBIN COMPLEX CONCENTRATE TO PATIENTS REQUIRING URGENT REVERSAL OF ANTICOAGULATION

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Background: Anticoagulants are used to treat or prevent thrombotic events but their most worrying side effect is major haemorrhage. The British Committee for Standards in Haematology (BCSH) recommend reversal of major/life threatening bleeding in both VKAs and DOACs with Prothrombin Complex Concentrate (PCC).
Aims: We aim to reduce delays in the administration of PCC in our trust and introduce the term 'Door To Needle' time (DTN) in the context of anticoagulant reversal.
Methods: We analysed the DTN in bleeding anticoagulated patients defined as time of recognition of haemorrhage to PCC administration. In Heart of England NHS Foundation Trust between May and July 2016, 29 patients were included; 19 patients were taking Warfarin and 10 taking DOACs. All patients received PCC (Beriplex®).
Results: Sixty-nine percent of patients were male and 31% female. The majority (69%) of patients were treated for stroke prevention in AF and 24% had a history of VTE. The two commonest major haemorrhage types were cerebrovascular (including intracranial and subdural haemorrhage) in 36% and gastrointestinal bleeding in 39%. The remaining indications (25%) were pre-urgent procedure/surgery, and soft tissue haematoma. The average time for recognition of haemorrhage was 3 hours 20 minutes (range 4 minutes to 21 hours 27 minutes), and the DTN was 4 hours 50 minutes (range 33 minutes to 13 hours 24 minutes), which means an estimated average of 6 hours 27 minutes (range 2 hours 49 minutes to 13 hours 59 minutes) between hospital admission and receiving PCC. Six of the total number of patients died within 30 days of hospital admission, 4 taking on Warfarin and 2 taking on DOACs.
Summary/Conclusions: This audit demonstrates the continuing delays between recognition of major/life-threatening bleeding events and receiving PCC since previous audits despite raising staff awareness. We plan to introduce the term 'DTN' in the context of anticoagulant reversal, store PCC in the emergency department pharmacy cupboards (as a PoM) as opposed to blood bank, and introduce a reporting system 'Serious Hazards of Warfarin (SHOW)' which may further reduce delays, morbidity and mortality.

E1602

THE IMPORTANCE OF PLATELET MEMBRANE FLUIDITY AND OXIDATIVE STRESS IN THROMBOTIC COMPLICATIONS ACQUIRED BY CHRONIC MYELOPROLIFERATIVE NEOPLASMS PATIENTS

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Background: Patients with chronic myeloproliferative neoplasms (MPNs) and chronic myeloid leukemia (CML) have a variety of structural and functional abnormalities of platelets. Many of them have thrombotic or hemorrhage complications. Platelet function is influenced by changes in membrane fluidity (MF) which has an important role in the expression of platelet receptors, modulating the activity of protein membrane.
Aims: The importance of reactive oxidative species (ROS) in alteration function of platelet membrane and expression of platelet receptors in patients with MPNs and CML.
Methods: We present a retrospective study on 36 cases MPN (20 JAK2-positive MPN) and 24 CML admitted in Colentina Clinical Hospital Bucharest. The determination of platelet membrane fluidity was performed by fluorescence anisotropy measurements using as marker 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene p-toluenesulfonate (TMA-DPH). We analyzed the fluorescence anisotropy of platelet membrane and correlate the result of with a

different kind of treatment. Production of ROS was examined using fluorescence method with DCFDA and Fluorolog spectrophotometer Platelet receptor expression was analyzed by flowcytometric method studying adhesion marker (CD 42 and CD 42b) and aggregation marker (CD61, CD41).
Results: Patients with MPN and JAK2 mutation present have a high level of fluorescence anisotropy than the JAK-negative group. Median value for JAK2 positive MPN group 147.2 95% CI for median value (138.7-150.6)) *vs* JAK2 negative MPN group 130.8 (124.6-138.3) p=0.001. There are no differences between CML and MPN group. Our results confirm that fluorescence anisotropy is influenced by medication taken. MPN patients who have taken Hydroxyurea alone had a high-level fluorescence anisotropy than the patient who have taken association Hydroxyurea and Anagrelide; median value and 95% CI for median value 151 (137.1-158.6) *vs* 136 (126-137.5) p=0.03. A patient who have treatment with tyrosin kinase inhibitor (TKI) - Sprycel or Glivec, had a low level of fluorescence anisotropy, median value and 95% CI for Hydroxyurea group 151 (137.1-158.6) *vs* TKI group value 138 (124.4-147.8) p=0.04. No differences of fluorescence anisotropy was observed between the group of MPN patients who received JAK inhibitor (Jakavi) or Hydroxyurea The CD42b expression is low in patients *versus* controls (median: 17.87% *vs* 94.16%, P<0.01), there is no difference in the CD42a value range (P=0.51). The CD61/CD41 expression (GP IIb-IIIa) presents also lower values in patients (median: CD 61= 84.13%; CD 41=71.13%) *versus* controls (median: CD 61= 98%; CD 41=93,17%), statistical significance obtained only for CD61 expression.Production of ROS is higher for patients with MPNs and CML patients compared with healthy controls. CML patients in accelerate or blastic phase have higher level of ROS production compared with patients in chronic phase (1.23 *vs* 1.09, p=0.03). Our results of anisotropy measurements did not reveal any influence of ROS in MF modifications (0.15 *vs* 0.13, without statistical significance) or with platelet receptor expression.
Summary/Conclusions: The presence of JAK 2 mutation in MPN patient is associated with a low fluidity of platelet membrane. Association of Anagrelide or TKI inhibitor is associated with lower level of fluorescence anisotropy. The fluidity of platelet membrane could be an important parameter which influenced the expression of platelet receptor. We have to observe in the future if this group with high level of fluorescence anisotropy had a high risk of thrombosis. All these results will be verified in a higher patients lot and need to be checked any correlation between modification of fluidity membrane production ROS and expression of microparticles platelet derivated.

E1603

USE OF ROTATIONAL THROMBOELASTOGRAPHY TO PREDICT CENTRAL VENOUS CATHETER RELATED VENOUS THROMBOSIS IN CHILDREN: PRELIMINARY RESULTS

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Background: Central venous catheters (CVCs) have been widely used in hospitalized pediatric patients, however, CVCs increase venous thrombosis frequency. In pediatric age group exact risk factors for CVC related venous thrombosis haven't been shown yet. Rotational thromboelastography (ROTEM®) measures clot formation and stability and evaluate cogulopathy.
Aims: We aimed to predict CVC related venous thrombosis via ROTEM prospectively in pediatric age group patients.
Methods: Study included patients who required CVC insertion due to any reason and who were not on any anticoagulation treatment during the week before the CVC insertion. On the day of CVC insertion clotting time (CT), clot formation time (CFT), maximum clot firmness (MCF), and alpha angle (AA) were measured for intrinsic (INTEM), extrinsic (EXTEM), and fibrinogen (FIBTEM) pathways via ROTEM. At one week of insertion and at removal of CVC, Doppler ultrasound imaging was performed to the vein that catheter was removed.
Results: A total 14 patients were included in the study. Median age was 3.9 years (0.3-17.8 years). Ten (71%) of the patients had juguler, four (29%) patients had femoral CVC. Median duration until removal of CVC was 15.5 days (7-56). Thrombosis was detected in one patient (7%) at first week of CVC insertion (Patient 10). When the the ROTEM parameters were examined, this patient had lowest CT and highest AA in EXTEM, and the highest AA in INTEM, indicating more pro-coagulant status (Table 1). Also patient 14 had similar AA as patient 10 in EXTEM and INTEM but was not found to develop thrombosis by the 18th day of insertion. However, CVC of that patient wasn't removed yet

Table 1.

Patient n°	Thrombosis	EXTEM CT (38-79)*	EXTEM CFT (34-159)	EXTEM MCF (50-72)	EXTEM AA (63-83)	INTEM CT (100-240)	INTEM CFT (50-110)	INTEM MCF (50-72)	INTEM AA (70-83)	FIBTEM CT (38-62)	FIBTEM CFT *	FIBTEM MCF (9-25)	FIBTEM AAF
1	No	64	50	71	82	135	70	82	86	64	26	26	82
2	No	59	73	78	84	143	64	69	77	73	112	24	76
3	No	73	154	53	56	158	112	57	69	65	-	6	-
4	No	63	132	69	80	159	57	61	82	67	167	28	81
5	No	60	51	71	82	115	51	72	80	51	350	24	81
6	No	74	69	66	77	199	77	65	75	85	-	11	-
7	No	69	923	23	45	88	215	35	79	59	-	9	-
8	No	57	84	68	81	148	78	76	84	69	266	39	83
11	No	77.00	129.00	33.00	66.00	108	51.00	69.00	81.00	66.00	-	5.00	-
12	No	58	69	71	82	134.00	68	71	80	51	-	18	80
13	No	54	147	58	63	160	89	61	73	62	-	7	77
14	No	43	18	86	87	69	29	81	84	50	41	35	88

*CT; clotting time, CFT; clot formation time, MCF; maximum clot firmness, AA; alpha angle, * Normal range, & INTEM couldn't be measured for patient 1 due to technical reasons, β In some patients CFT and/or AA weren't in measurable level*

Summary/Conclusions: In this study we reported our preliminary results. We detected thrombosis only in one patient and according to this limited samples size, we may suggest that CT and AA in EXTEM, and AA in INTEM prior to insertion of CVC may be predictive for catheter related thrombosis development. Such patients with pro-coagulant findings at ROTEM prior to CVC insertion may need prophylactic anti-coagulation. The results in a larger sample size will be more definitive to make a conclusion.

E1604

THE POTENCIAL ROLE OF ANTINEOPLASTIC DRUGS IN THE PREDICTION OF THROMBOTIC RISK IN ONCOLOGIC PATIENTS IN ADDITION TO THE KHORANA SCORE

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Background: Venous thromboembolism (VTE) is common in patients with cancer. Several risk factors (related with patient, tumour and treatment) have been already identified. Thromboprophylaxis (TP) with low molecular weight heparin (LMWH) is associated with a reduction of symptomatic VTE but without clear benefit in survival as the number of major bleedings is increased. To guide primary TP in newly diagnosed cancer outpatients starting chemotherapy (QT), a risk assessment model (based in baseline clinical and laboratory variables) was developed (the Khorana score). Many patients with intermediate risk (without tromboprophylaxis indication according to Khorana-based clinical guidelines) develop VTE episodes. Factors as tissue factor-bearing micro particles and D-Dimmer levels in addition to lenalidomide, platin and gencitabine-based therapies are associated with VTE high risk. Its efficacy as a predictive tool is a matter of debate.
Aims: This retrospective, observational study is aimed to assess the Khorana score efficacy in predicting the VTE risk and analyze some treatment related factors as predictive complementary tools.
Methods: We analyzed the demographic characteristic, the Khorana score and the antineoplastic treatment of oncologic patients diagnosed of pulmonary embolism (PE) from December 2010 until December 2016 at the Complejo Hospitalario de Navarra. At baseline, the Khorana score classified patients as 'low risk' (0 points) intermediate risk' (1-2 points) or 'high risk' (≥3 points) for VTE.
Results: 102 oncologic patients were diagnosed of PE. Patient baseline characteristics are showed in table 1. In 27.5% (n=28) PE diagnosis preceded to cancer diagnosis, in 26.5% (n=27) PE occurred at least 1 month beyond the end of antineoplastic treatment and in 46.1% (n=47) PE was diagnosed during the treatment (chemotherapy +/- radiotherapy). In this last group the median time from the treatment beginning and EP diagnosis was 3 months (0-46). The stratification according to the Khorana score (at baseline) was: 'low risk' 21.3%, intermediate risk 61.7%, and high risk 17%. In the intermediate risk group (n=29) the drug-based therapy was: 44.8% platin (n=13), 6.9% gemcitabine (n=2), 2.5% lenalidomide (n=1) and 48.3% non-related-thromboembolic treatment (n=14). Most of cases (97.1%) were managed with LMWH (enoxaparin 1mg/kg/twice a day). Only 2 patients were treated with non-fractionated heparin and 1, enrolled in a clinical trial, was treated with direct oral anticoagulants.

Table 1.

Table1. Baseline patient characteristic

	n=102 (%)
Sex, male (%)	69 (67.6%)
Age, median (range)	66 (28-93)
Cancer localization	
Lung	27 (26.5%)
Colorectal	19 (18.6%)
Genitourinary	18 (17.7%)
Breast	9 (8.8%)
Pancreas	4 (3.9%)
Gastric	4 (3.9%)
Gynecological	6 (5.9%)
CNS	4 (3.9%)
Hematologic	4 (3.9%)
Esophagus	1 (1%)
Others	6 (5.9%)
Metastasis	54 (52.9%)
Incidental	40 (39.2%)
Ambulatory	80 (78.4%)

Summary/Conclusions: Nearly 2/3 of Khorana intermediate risk patients developed a PE while on antineoplastic treatment and inside this group over 50% were treated with well-recognized high thrombotic-risk drugs. The inclusion of antineoplastic drugs in a predictive thromboembolic model in oncologic patients could improve the benefit-risk of the use of LMWH prophylaxis in some patients without a high risk Khorana score but however at high risk of thrombosis. More prospective studies are needed to analyse the benefit of antithrombotic prophylaxis in oncologic patients receiving outpatient chemotherapy treatment.

Transfusion medicine

E1605

CLINICAL OUTCOMES AND UTILIZATION OF BLOOD BANK RESOURCES OF PATIENTS WITH THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP), HEMOLYTIC UREMIC SYNDROME (HUS), AND OTHER MICROANGIOPATHY- A 14 YEARS' EXPERIENCE

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Background: TTP, HUS and other thrombotic microangiopathy are rare, complex clinical syndromes which are characterized by thrombocytopenia, microangiopathic haemolytic anemia, (MAHA) and systemic thrombosis. The introduction of plasma exchange (PEX) had dramatically reduced the mortality of these patients, and has become standard of treatment. Although the clinical outcome of these conditions is heterogenous, with multiple clinical complications and prolonged hospital stay, there is no previously published data to provide measurement of blood bank and hospital resource utilization associated with its clinical management.
Aims: We performed a retrospective cohort study of 42 consecutively treated patients with MAHA and analyzed their clinical and laboratory characteristics, treatment outcomes and plasma product utilization.
Methods: Medical records of these patients treated from 2002-2017 were reviewed. We used the standardized criteria based on the consensus on standardization of terminology in TTP to define clinical response. (Scully et al J Thromb Haemost 2017).
Results: Results: In our series, the causes and number (%) of MAHA were TTP-HUS (18, 42.9%), autoimmune disorder-associated MAHA (13, 31% *i.e.* 9 SLE and 4 Sjögren's syndrome), cancer-related MAHA (4, 9.5%), drug-induced (3, 7.1%), post-transplant and infection-related microangiopathy (4, 9.5%). The average number of PEX sessions required to achieve overall clinical response in TTP, autoimmune-associated MAHA, HUS and drug-induced microangiopathy was 18.2±17.9, 11.5±7.6, 13.0±8.7 and 7.3±6.7, respectively. The mean follow up time was 40.8 months. 5 patients (11.6%) died during the course of treatment in index hospitalization, 12 (27.9%) were refractory to PEX and 24 patients (55.8%) responded to PEX, and 1 patient was lost to follow up. 1 patient relapsed 8 months after achieving clinical remission and was successfully treated with Vincristine. Another patient developed exacerbation and was palliated eventually. For the refractory cases, 7 patients were given Rituximab, 5 achieved clinical response while those who were given Vincristine (n=3) and Cyclophosphamide (n=2), achieved clinical response with a median of 15 days from the time second line agents were used. The 1 year overall survival of those who received second line treatment compared to patients who responded to only PEX and standard of care was 59% and 80% (p=0.51), respectively. The overall 1 year survival of the entire cohort is 74% which is comparable to the Oklahoma registry. The mean length of hospital stay was 30 days (median 27 days, SD±20.2). Complications encountered during PEX included bleeding (9.5%), seizures (11.9%), cardiovascular complications like myocardial infarction and stroke (16.7%), and nosocomial infection (16.7%). 30% of the cohort required temporary dialysis support for acute kidney injury while 38% of them ended up required lifelong dialysis. With regards to the cost of plasma units, consumables and PEX procedure, the calculated mean cost was Singapore Dollar (SGD) 25252.95 (€16991.17) (SD±20859.41 (€14035.02)) per patient.
Summary/Conclusions: The clinical outcome in terms of survival in our cohort is in keeping with that of other registry and cohort (Hovinga et al Blood 2010). Our data which demonstrate the health care resource utilization show that management of these patients is expensive. While small in terms of incidence, it poses an economic burden disproportionate to its overall size.

E1606

HEPATITIS E VIRUS: INVESTIGATION IN NORTH ITALIAN BLOOD DONORS

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Background: Hepatitis E virus (HEV) is a major cause of acute hepatitis worldwide, and a possible threat to transfusion safety. Recent data from Europe showed a HEV IgG prevalences of 6.8% in German blood donors, 27% in Dutch blood donors, and 52% in an hyperendemic area in the South of France.
Aims: The aim of this study was to determine the prevalence of anti-HEV reactivity and HEV viremia in Italian blood donors, in order to estimate the risk of transfusion transmission.
Methods: Nearly 10,000 samples were collected from anonymized, unpaid donors at the "Lecco processing and validation blood center" (Lombardy, Italy) from June to July 2016. Samples were tested individually (individual-donation nucleic acid test [ID-NAT]) for HEV RNA using the Procleix HEV assay (95% limit of detection 7.9 IU/mL). Initial TMA-reactive samples were retested and considered positive if the retest result was reactive. For the serology study, a subset of 2000 donations was tested for HEV IgG using DiaPro HEV ELISA kit (Diagnostic BioprobesSrl, Milano, Italy). HEV IgG and IgM were analyzed in ID-NAT positive samples at the time of donation and in the follow up, collected one year after the index donation.
Results: The prevalence of IgG anti-HEV in north Italian blood donors was 7.4%. Nine out of 9,726 donor samples gave reactive values by the ID-NAT assay for HEV RNA. Among them, only one sample was confirmed to be reactive in additional TMA tests. None of the 9 HEV RNA initially reactive samples had circulating IgM or IgG antibodies against HEV. In the follow up, only the repetitive reactive donor shewed a IgM and IgG seroconversion, indicating primary HEV infection. Therefore, we estimated that the risk of receiving a potentially infectious blood unit is of 1:10.000 (upper bound of the 95% confidence interval, 1:1700).
Summary/Conclusions: Anti-HEV reactivity, indicating a previous infection, was found in 7.4% of subjects admitted to blood donation in our area. We also identified a viremic blood donation, indicating that the risk of transmitting the infection through blood transfusion, although small, is not negligible. The clinical impact of HEV infection among blood recipients remains to be assessed. These data need to be considered when deciding a national policy for preventing HEV transmission.

E1607

SHORT-TERM ADMINISTRATION OF RECOMBINANT HUMAN ERYTHROPOIETIN DECREASES B CELL IN HUMAN PERIPHERAL BLOOD

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Background: Erythropoietin (EPO) is hematopoietic factors participating in red blood cell production, and accelerates proliferation and inhibits apoptosis of erythroblasts. It is reported that EPO has pleiotropic effects including anti-apoptotic action for some cells, antioxidant action, vascularization action, and promoting cell division in addition to stimulation of erythropoiesis as well, whereas there are conflicting results of small cohorts as to its effect on blood immune cells.
Aims: We analyzed peripheral white blood cell subsets in patients who received one bolus administration of recombinant human erythropoietin (rHuEPO) to examine the effect of EPO on human immune system.
Methods: One hundred nineteen autologous blood donors (male/female 62/57) in Gunma University Hospital were enrolled in this study after written informed consent. All the patients had no infections or inflammation. Forty nine patients were treated with rHuEPO (Epoetin alpha or Epoetin beta (24,000 IU, respectively)) once after blood donation because of low hemoglobin concentration and 70 were not treated. Peripheral blood samples were obtained at the time of the first phlebotomy and after 1week from the same patient.We measured the number of WBC, lymphocytes, myeloid dendritic cells (mDC), plasmacytoid dendritic cells (pDC), CD4+ T cells, CD8+ T cells, Natural killer (NK) cells, B cells, monocytes, and neutrophils of peripheral blood before and after rHuEPO administration by flow cytometry. Also we compared treatment group with non treatment group. Paired and unpaired Student's t-test were used to compare absolute counts and percentages of each cell, P values<0.05 were considered significant. This study was approved by the research ethics committee of our hospital.
Results: In treatment group, absolute number and percentage of lymphocyte in WBC decreased significantly after rHuEPO administration from 1985.0±520.8/μl to 1798.7±439.0 /μl, in absolute number (p=0.019), and from 33.2±8.57% to 30.0±7.32% in percentage (p=0.023). The numbers of whole WBC, mDC, pDC, monocyte and neutrophil did not change significantly. In respect of lymphocyte subsets, absolute number of CD8+ T cell, NK cell and B cell significantly decreased from 358.9±257.0 /μl to 311.5±210.9/μl (p=0.04), from 290.6±157.6 /μl to 257.4±141.8 /μl (p<0.01) and from 289.3±192.4 /μl to 239.9±158.2 /μl (p<0.01), respectively. Regarding B cell subsets, absolute number of naïve B cell and IgD^{-} $CD27^{-}$ B cell significantly decreased from 171.3±93.5 /μl to 153.0±84.2 /μl (p<0.01), and from 16.5±13.6 /μl to 12.9±12.7 /μl (p=0.045), respectively. Moreover, other B cell subsets, such as transitional B cells, memory B cells and marginal zone B cells, also showed a trend of decrease. However, percentages of naïve B cell and $IgD^{-}CD27^{-}$ B cell in total B cell did not change. These suggested that whole B cell decreased, not a specific subset of B cell. In non treatment group, there was no significant change in any type of immune cell.
Summary/Conclusions: These findings suggested that just one administration of rHuEPO influenced human immune system, especially via reduction of B cell in peripheral blood, with unknown mechanism so far.

E1608

BLOODLESS TREATMENT OF HEMATOLOGIC MALIGNANCIES AND APLASTIC ANEMIA - JEHOVAH'S WITNESSES (JW)

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Background: At most centers, the majority of patients who request bloodless medicine are members of the Jehova's Witness (JW) faith. But, there are no standard, established guidelines to manage pancytopenia in these patients, nor are there many studies to inform optimal treatment approaches. The most troublesome patients who request bloodless medicines are patients with hematologic malignancy. The treatments of these patients are considerable challenges. They have not only problems of severe pancytopenia, but also require intensive chemotherapy. Since 2000, our hospital has been a bloodless center. This study was retrospectively analyzed for hematologic malignancies and aplastic anemia with bloodless treatments in Soonchunhyang university hospital.
Aims: This study was retrospectively analyzed for hematologic malignancies and aplastic anemia with bloodless treatments in Soonchunhyang university hospital.
Methods: A retrospective review of medical records was performed of 44 patients with hematologic malignancies and aplastic anemia who request bloodless medicine from January 2006 to December 2015 at Soonchunhyang university hospital.
Results: Of 44 patients, 48% were men (n=21) and 52% were women (n=23). The median age of the study population at the time of diagnosis was 62 years (range 16-87). Thirteen patients (29.5%) were acute leukemia, 15 (34.1%) patients with non-Hodgkin's lymphoma (NHL), 2 (4.5%) patients with aplastic anemia (AA), 6 (13.6%) patients with chronic myeloid leukemia (CML), 4 (9%) patients with myelodysplastic syndrome (MDS) and 4 (9%) patients with multiple myeloma (MM). Thirty one patients were treated with chemotherapies and 13 patients were treated with supportive care only. Among 44 patients 27 patients were died. Most common cause of attribution to death was anemia (92.5%). And Chief complaint at death was dyspnea (88%). Median survival of acute leukemia was 1 month (95% CI, 0.41-1.59).

Table 1.

Acute leukemia (15 patients)

Gender	Age	Diagnosis	Regimen	Chemotherapies	Response	Survival	death
F	87	Acute leukemia		no		0.0	death
M	84	Acute leukemia		no		1.2	death
F	81	Acute leukemia		no		0.7	death
M	16	ALL		no		3.9	death
M	17	ALL	R-CHOP	yes	refractory	4.4	death
F	81	AML		no		1.0	death
M	81	AML		no		1.4	death
M	40	AML	low dose Ara-C	yes	refractory	1.0	death
M	74	AML	hydroxyurea	yes	refractory	0.7	death
48	F	AML	Ara-C/Idarubicin	yes	refractory	0.6	death
70	F	AML	mFLAI → CR1 (induction#1,consol #2)	yes	CR	16.5	death
51	F	APL	ATRA	yes	refractory	0.3	death
47	M	APL	ATRA → CR1 Relapse ATO → CR2	yes	CR	34.7	alive

Summary/Conclusions: In bloodless treatment, CML, MM and lymphoma had a relatively good prognosis. However, AML and MDS were showed a poor prognosis. Therefore, further studies are needed to improve survival for bloodless patients with hematologic malignancies.

E1609

PREOPERATIVE ANEMIA: A SINGLE INSTITUTION EXPERIENCE IN SPAIN

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Background: Preoperative anemia is considered as a strong predictor of postoperative red cell transfusions, and has also been linked to increased morbidity and mortality in surgical patients, but it is frequently overlooked.
Aims: The objective of this study is measure of real impact of preoperative hematological assesment and optimization of anemic patients in terms of decreasing blood cells transfusions.
Methods: We analyzed 85 patients undergoing elective surgery in subgroups of high or low risk of bleeding. All the patients were referred from pre-anesthesia consultation for performing a 4-week hematological protocol in order to optimize the hemoglobin level to a near normal value. We identified the underlying cause of anemia and offered the treatment according to the etiology. The primary outcomes were the response to therapy defined as reaching the Hb level >13 gr/dL or increasing of >2 gr/dL from basal level, and the rate of blood transfusion.
Results: Mean age was 70.4 years, with a male-female ratio of 1:2, and the patients were divided into 2 groups according to the bleeding risk: high risk 74% (hip and knee replacement, cystectomy, colectomy, maxilofacial surgery), and low risk 26% (mastectomy, gynecology or spine surgery), with a median hemoglobin of 10,9% and 10,1%, respectively. A diagnostic workup was performed in order to provide appropiate treatment: iron deficiency anemia (83,9%), anemia of chronic disease (10,3%), folate or vitamin B12 deficiency (5,8%). The patients with iron deficiency anemia received oral (62%) or intravenous iron (38%), but third of patients had to change from oral to intravenous iron by intolerance or poor response. The response to treatment was reached by 44.7% of patients, in an average time of 26,4 days. The rate of blood transfusion was 18% in good responders (0,5 packed red blood cells per patient) and 63% in poor responders (1,6 packed red blood cells per patient).
Summary/Conclusions: Treatment strategies over preoperative period, and the effort to reach a near to normal hemoglobin level, could minimize the amount of red blood cell transfusion the patients will be exposed in the postoperative period. Our data provide evidence about the effectiveness of a prompt evaluation and correction of preoperative anemia in a maximum time of 4 weeks.

E1610

RED BLOOD CELLS (RBC) AND PLATELET (PLT) TRANSFUSIONS IN TRANSPLANTED AND NOT-TRANSPLANTED PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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Background: Patients with hematological malignancies require often and prolonged hospitalisations during the course of their treatment, in part due to increased and frequent transfusion demands.
Aims: The objective of the study was to assess the factors affecting transfusion needs in a Hematology Department (bone marrow transplant unit- BMTU, post-transplant unit-PTU, hematology clinic).
Methods: The patients that were hospitalized between 1/1/2015 and 31/12/2015 were analyzed. Data regarding the underlying disease, the disease status, type of transplant, duration of marrow aplasia and donor-patient blood group mismatch were obtained from the medical records. The analysis was restricted to the transfusion of packed RBCs and units. Differences between groups were assessed using non-parametric statistics (Kruskall-Wallis and Mann-Whitney U-test).
Results: There were 523 admissions of 256 different patients. Complete data for analysis could be obtained for 487 admissions of 237 patients (92.6% of patients, 93.1% of admissions), corresponding to 10,673 days of hospitalization. Total number of blood products transfused was 2284 packed RBC units, 13883 PLT units (apheresis platelets counted as 5 units). Values are reported as median (range), unless otherwise specified. In the BMTU, the type of transplant was correlated with transfusion needs: number of RBC units transfused per admission was 2 (1-5) for autologous transplanted (AUTO) patients, 4 (1-28) for allo-transplanted (ALLO) (no difference between sibling and matched unrelated donors), and 7 (1-14) for haplo-identical transplantations (HAPLO), p=0.001. Platelet units requirements were respectively 15 (5-45) for AUTO, 20 (5-205) for ALLO and 50 (30-130) for HAPLO, p<0.001. The length of stay was 18 (13-23) days in AUTO, 22 (16-44) in ALLO, 30 (29-40) days in HAPLO transplantation, p<0.001, while the duration of aplasia in days was 9 (4-19) in AUTO, 13 (5-32) in ALLO and 25 (20-38) in HAPLO, p<0.001. The longer duration of aplasia and hospitalization was correlated with greater transfusion needs. In the PTU there was no statistically significant difference in transfused RBC or PLT units with regard to transplant type. Disease status (response *versus* active disease) was only correlated with RBC units transfused in PTU [2 (1-29) *vs* 6 (1-56) units respectively, p=0.006]. Donor – patient blood group mismatch was correlated with increased transfusion demands in BMTU for RBCs [4 (1-28) *vs* 2 (1-5), p<0.001] and PLTs [25 *vs* 15, p<0.001]. In hematology clinic, the underlying disease was correlated with transfusion needs in RBC and PLTs, as shown in table 1. Patients with AML had the higher needs in RBCs and PLTs, whereas patients with lymphoma had the lowest needs in RBC transfusions. Disease status was not correlated with transfusion needs. The duration of aplasia was correlated with the number of RBC units (Pearson's r=0.66, p<0.001, r^2=0.435) and of PLTs transfused (Pearson's r=0.78, p<0.001, r^2=0.61).

Table 1. Units transfused in hematology clinic

	ALL	AML	Lymphoma	MPN	Myeloma	MDS/AA	
RBCs	3 (0-7)	5 (0-30)	2 (0-45)	5 (0-10)	3 (0-8)	3 (0-15)	p<0.001
PLTs	10(0-70)	30(0-250)	5 (0-240)	20(0-55)	10(0-60)	0(0-180)	p<0.001

Summary/Conclusions: The main determinants of transfusion requirements are the duration of aplasia, the type of transplant and the disease, with myeloid malignancies requiring more transfusions. The establishment of haplo-identical transplantations has increased the transfusion needs due to longer period of aplasia.

PUBLICATION ONLY

Acute lymphoblastic leukemia - Biology

PB1611

BOTANICAL ALKYL HYDROQUINONE HQ17(3) EXERTS CYTOTOXICITY TO T(9;22) PHILADELPHIA CHROMOSOME SUP-B15 ALL CELLS THROUGH INDUCING ENDOPLASMIC RETICULUM STRESS, AUTOPHAGY, AND AIF TRANSLOCATION

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Background: Patients suffering from Acute lymphoblastic leukemias (ALLs) harboring t(9;22) genetic abnormality are classified very high risk (VHR) ALLs displaying poor clinical outcome irrespective of intensive chemotherapies and tyrosine kinase inhibitor treatment. Development of new adjuvant therapeutics will provide great value. HQ17(3)[10'(Z),13'(E),15'(E)-heptadecatrienyl hydroquinone] isolated from sap of the lacquer tree showed potent cytotoxic effect within 24 hours at micromolar concentration on several ALL cell lines, including TKI (Imatinib, IM)-refractory Ph$^+$ B-ALL cell line SUP-B15 cells, but spare normal PB leukocytes, and were non-toxic in experimental rats after 28-day HQ17(3) injection. Thus HQ17(3) presents as a potential anti-leukemic agent and serves a model for design anti-leukemic regimen. We previously showed HQ17(3)-induced rapid cell demise, characterized by oxidative stress, loss of membrane integrity, mitochondrial membrane potential disturbance and nuclear DNA fragmentation. Neither pan-caspase inhibitor nor Nec-1 (RIP-1 inhihitor) protected SUP-B15 cells from HQ17(3)-induced cell death. The cell death program elicited by HQ17(3) is caspase-independent, and is different from the RIP1-mediated controlled necroptosis.
Aims: To investigate the characters of, and the molecular pathways involved in the HQ17(3)-induced non-classical death on VHR-ALL SUP-B15 cells and help developing effective therapeutic strategies for the VHR-ALLs.
Methods: Cell growth inhibition in response to HQ17(3) w/wo inhibitors was analyzed by ACP assay. Cells were stained by Annexin V/PI and analyzed by flow cytometry for cell death. Lysosomal protease inhibitors (AEBSF (serine protease inh.), pepstatin/CA074-Me (cathepsin D/B inh.)) or autophagy inhibitors (Bafilomycin A1) were used in combination with HQ17(3) in some experiments. Acridine orange stain and confocal microscopy are used to visualize the changes of acidic vesicles. Autophagic flow in response to HQ17(3) was revealed by aggregation of ectopically expressed EGFP-LC3. Western blot analysis were used to analyze p- eIF2a, ER chaperone Grp78, spliced XBP-1 (markers for ER stress). Lenti-viral delivery of shRNAs was used to repress the expression of Beclin-1. Nuclear accumulation of apoptosis inducing factor (AIF) was revealed by fluorescence microscopy.
Results: Enlarged acidic vesicles accumulated soon after HQ17(3) treatment, and deminished when cell death ensued. HQ17(3)-induced cell death could not be attributed to cathepsins released from lysosomal membrane permeabilization (LMP) as cathepsin inhibitors did not attenuate the cell death. HQ17(3) enhanced autophagy as revealed by aggregation of ectopically expressed EGFP-LC3. Inhibition of autophagy by Bafilomycin A1 or knockdown the essential autophagy-related Beclin1 by shRNA could partially attenuate HQ17(3)-induced cell death. Further, HQ17(3) treatment gave rise to early ER stress as revealed by enhancement of eIF2a phosphorylation and up-regulation of ER chaperone Grp78. HQ17(3) induced nuclear translocation of AIF, in compatible with mitochondria disturbance and caspase-independent cell death thereafter.
Summary/Conclusions: In Ph$^+$-ALL SUP-B15 cells, HQ17(3) acts in multifacet: a) lead to oxidative stress and perturb mitochondria membrane integrity, b) induce ER stress and calcium mobilization to mitochondria, cleave and release AIF to mediate nuclear chromatin cleavage, c) HQ17(3)-induced autophagy may be implicated cell death. This study shows agents that are capable of eliciting an intricate effector network in therapy-induced cytotoxicity will have potential as adjuvants controlling the VHR-Ph$^+$-ALL cells refractory to conventional high dose chemotherapies and TKI regime.

PB1612

TARGETED MUTATIONAL PROFILING OF CHILDHOOD AND ADULT ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

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Background: Acute lymphoblastic leukemia (ALL) is the most common cancer in children, representing about 80% of acute leukemias, whereas it is less common in adults (20%). Identification of cytogenetic aberrations and a small number of molecular abnormalities are still the most important risk and therapy stratification methods in clinical practice today.
Aims: The aim of the present study was to assess mutational profile of both childhood (cALL) and adult acute lymphoblast leukemia (aALL) patients, by applying targeted next generation sequencing (NGS) on MiSeq System.
Methods: We analyzed DNA samples from 34 de novo ALL patients (17 cALL and 17 aALL) using TruSeq Amplicon – Cancer Panel (TSACP) that targets mutational hotspots in 48 cancer related genes (212 amplicons). The bioinformatics analyses was conducted using processing pipeline composed of both freely available open source bioinformatics tools as well as tools developed in-house. The average coverage of high-quality sequences was 2609 × per amplicon. Ten genes were discarded due to insufficient coverage, therefore we analyzed a total of 183 amplicons from 38 genes. Variants were identified in relation to the GRCh37 reference genome by applying a Bayesian approach and compared to public genetic variation databases and in-house databases.
Results: We identified a total of 331 (159 cALL, 172 aALL) variants in the coding regions (median per patient: 9, range: 6-12; median per cALL: 9, range: 6-12; median per aALL: 10, range: 7-12) and 429 (211 cALL, 218 aALL) variants in the non-coding regions (median per patient: 13 range: 10-15; median per cALL: 13, range: 10-14; median per aALL: 13, range: 10-15**)**. Overall, a total of 98 variants (median per patient: 2.8, range: 1–6) were potentially protein-changing, including nonsense, frameshift, and missense (NFM) mutations. There were no significant differences in the number of NFM mutations between cALL (total 47, median per patient: 3, range: 1–5) and aALL patients (total 51, median per patient: 3, range: 1–5). Moreover, we identified 5 NFM mutations in *STK11* gene, 3 in *ABL1*, *RET, KRAS* and 2 in *HNF1A, NRAS,* and *NOTCH1.* Observed in individual patients detected mutations predominantly disrupted Ras/RTK pathway (*STK11*, *KIT*, *MET*, *NRAS*, *KRAS*, *PTEN*). Additionally, we identified 5 patients with the same mutation in *HNF1A* gene coding for transcriptional factor, disrupting both Wnt and Notch signaling pathway. Notch pathway was disrupted in two patients in which detected variants affected *NOTCH1* gene. *HNF1A* and *NOTCH1* variants were mutually exclusive, while genes involved in Ras/RTK pathway exhibit a tendency of mutation accumulation.
Summary/Conclusions: Our targeted NGS study showed low number of recurrent mutations in both cALL and aALL patients. Detected mutations affect few key signaling pathways, primarily Ras/RTK and Notch pathways. This study contributes to knowledge of ALL mutational landscape, leading to better understanding of molecular basis of ALL and better stratification and treatment of ALL patients.

PB1613

RELAPSED LOW HYPODIPLOID ACUTE LYMPHOBLASTIC LEUKAEMIA IN A LI-FRAUMENI PATIENT

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Background: A 7-year old girl presented with backache, leg pain and difficulty walking. She was referred to the local paediatric oncology service, as she was known with Li-Fraumeni Syndrome (LFS). Cancers linked to this disorder include soft tissue sarcomas, osteosarcoma, breast cancer, brain cancer, leukaemia and adrenocortical carcinoma. The patient's mother had breast cancer twice, and both her (monozygotic) twin sister and older sister had adrenal cortical carcinomas removed. The patient was born with left sided twinning defects (absent left kidney, malrotation, absent left ear and ear canal, Arnold-Chiari malformation with spinal cord syrinx and hydrocephalus). At presentation there was a suspicion of a disseminated malignancy. She underwent an MRI scan which showed extensive changes in her spinal column and hips. Bone marrow biopsy revealed Acute Lymphoblastic Leukaemia (ALL). Paucity of diagnostic material restricted the cytogenetic analyses. G-banding showed 46,XX[14], with FISH demonstrating loss of FOXO1/13q14.11 [44%], gain of MYC/8q24.21 [9%], ETV6-RUNX1 NEGATIVE, gain of RUNX1/21q22.12 [21%] and BCR-ABL1 NEGATIVE. The patient was treated according to NCRI risk criteria on the least intensive regimen of the UK ALL 2003 trial. She achieved morphological remission after a 3-drug induction, and successfully completed further treatment, including intensification/CNS directed phase, interim maintenance and 2 delayed intensification blocks. She completed 5 years of follow-up and was transferred to the Long Term Follow-Up Clinic, when she presented with hypercalcaemia. Peripheral blood and bone marrow biopsy confirmed a diagnosis of ALL. Although the phenotype resembled the profile of the first presentation of leukaemia, the genetic aberrations appeared incongruent.
Aims: Establish the origin of the second episode of ALL in a patient with known Li-Fraumeni Syndrome. As treatment and outcome for relapsed ALL in comparison with a second, primary, ALL are completely different, this information was critical to guide further management.
Methods: We set out to comprehensively characterise the second ALL, including conventional G-banding and fluorescence *in situ* hybridisation (FISH). The

acquired results were compared with those derived from the first ALL diagnosis.
Results: The G-banding analysis showed 36,XX,-2,-3,-4,del(5)(q31q35),-7,-12,-13,-14,-15,-16,-17[13]/46,XX[7]. Extensive FISH analysis confirmed the diagnosis of low hypodiploidy ALL. This result was in line with the reported association between *TP53* gene mutations in 90% of cases of low hypodiploid ALL, with these mutations often present in normal cells. [Holmfeldt, Nat Gen, 2013] At first sight, this did not recapitulate the original cytogenetic analysis and suggested the occurrence of a second episode of ALL. In order to further characterise the diagnostic genetics, FISH probes were used on archived diagnostic slides. Careful selection of probes demonstrated that the original leukaemia sample contained two co-existing clones – one low hypodiploid clone (with an identical pattern of loss and gain of chromosomes as the second ALL) and one clone resembling a doubled up/near triploid low hypodiploid clone.
Summary/Conclusions: This case report demonstrates the value of in-depth genetic analyses to guide management of patients with ALL. This patient proceeded with re-induction according to our current relapsed therapy guidelines (R3), to which she has shown a promising response. She is considered for allogeneic bone marrow transplantation using an unrelated donor.
In hindsight, the treatment regimen used for the initial ALL was incorrect. If it had been established that she had low hypodiploid ALL the first time around, she would have been allocated the most intensive regimen within the trial. Nevertheless, she maintained remission status for 5 years with low intensity treatment and ironically relapsed when most patients are told they are cured.
Since the original diagnosis of ALL in 2007, research has vastly improved our understanding of the biology and genetic landscape of ALL. This has facilitated risk stratification, improved outcome after treatment and identified novel drug targets. Genomic profiling of low hypodiploid ALL has identified oncogenic activation of Ras and phosphoinositide 3-kinase (PI3K) signalling conferring sensitivity to PI3K inhibitors, thus providing therapeutic avenues if conventional treatment were to fail.

PB1614

IMMUNOLOGICAL CHARACTERIZATION OF PH+ ALL BONE MARROW BY MULTIPLEX IMMUNOHISTOCHEMISTRY

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Background: The treatment results in Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL) have improved significantly in the era of tyrosine kinase inhibitors (TKIs). However, many patients relapse despite having received intense treatments with initially favorable responses. TKI therapy is known to modulate the immune system, and it may play a critical role in keeping the leukemia under control. However, little is known about the status of the immune system in patients with Ph+ ALL. Especially with the emerging immunotherapies in sight, it is vital to chart the immunological landmarks that could help us direct the treatment towards a more personalized course.
Aims: To characterize the immunological microenvironment in Ph+ ALL bone marrow (BM) by multiplex immunohistochemistry (IHC).
Methods: Ph+ ALL BM biopsies from the diagnosis stage were collected from Helsinki University Hospital and Tampere University Hospital (N=31). BM biopsies from non-leukemic (NL) controls (N=14) were used as a reference. Samples were hematopathologically evaluated and a tissue microarray (TMA) was constructed by selecting two BM cores with high leukemic cell infiltration per patient. The TMA sections were stained with both fluorescent and chromogenic dyes for six markers and nuclei simultaneously enabling cytometric analysis at cell-level resolution. Marker panels included T and B lymphoid cells, NK and dendritic cells, macrophages as well as myeloid derived suppressor cells. Furthermore, we analyzed immune checkpoint molecules (PD1, LAG3, OX40, TIM3, CTLA4) and their ligands (PD-L1, PD-L2, HLA-G, HLA-ABC) alongside with various activation markers (granzyme B, CD45RO, CD25, CD57, CD27). After the staining, the cells were segmented and quantified with the image analysis software CellProfiler and the cell analysis software FlowJo.
Results: The CD4+/CD8+ ratio was lower in Ph+ ALL BM *versus* NL BM (1.3 [Interquartile range (IQR) 1.0-1.9] *vs* 2.0 [IQR 1.7-2.4], p=0.0134) indicating that there are relatively more CD8+ T cells in the leukemic than in the non-leukemic marrow. The ratio of memory CD4+CD45RO+ T cells in Ph+ ALL BM *versus* NL BM was elevated (21.0% [IQR 16.7-28.5] *vs* 13.0% [IQR 8.7-15.9] of CD4+ T cells, p=0.0044). The difference in memory CD8+CD45RO+ T cells was not significant (p=0.36). Further analysis of the T cell phenotype showed increased proportion of both PD1-positive helper T cells and PD1-positive CD8+ T cells in Ph+ ALL BM *vs* NL BM (29.7% [IQR 17.5-50.1] *vs* 6.9% [IQR 5.7-8.9], of CD4+ cells, p<0.0001 and 28.8% [IQR 13.2-38.0] *vs* 14.9% [IQR 9.6-18.7], of CD8+ cells, p=0.0107). The ratio of OX40-positive helper T cells was also higher in Ph+ ALL BM (27.1% [IQR 21.6-33.25] *vs* 18.5% [IQR 14.8-21.9], of CD4+ cells, P=0.0001), but no difference was observed in the proportion of OX40-positive CD8+ T cells (P=0.49).
Summary/Conclusions: Multiplex IHC enables ample cytometric evaluation of different immune cell subtypes in their original microenvironmental context of the bone marrow. The TMA format not only allows analysis of tens of BM samples in parallel but also serves as a retrospective, easy-access archive for any follow-up studies. Ph+ ALL BM is characterized by a decrease in the CD4+/CD8+ ratio and an increase in the proportion of CD4+CD45RO+ T cells in comparison with the non-leukemic controls. The proportion of PD1-expressing T cells is also elevated. However, the heterogeneity between patients is marked. The analysis of other marker panels is presently ongoing, as well as correlation to clinical and treatment outcome parameters.

PB1615

CDKN2A/P16INK4A DELETION IS NOT A POOR PROGNOSIS PREDICTOR IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS TREATED ACCORDING TO PROTOCOL RALL-2009

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Background: CDKN2A/p16^{INK4a} deletion is a frequent cytogenetic abnormality in acute lymphoblastic leukemia (ALL), ranging from 18% to 45%. In pediatric group of patient's p16^{INK4a} deletion was associated with T-cell ALL phenotype and poor event-free survival. The prognostic impact of CDKN2A/p16^{INK4a} deletion in adult ALL patients appear controversial.
Aims: To evaluate the prognostic impact of the CDKN2A/p16^{INK4a} deletion in adult patients with acute lymphoblastic leukemia.
Methods: We present the results of the CDKN2A/p16^{INK4a} deletion in 110 adult patients with newly diagnosed Philadelphia –negative ALL, which were treated by RALL-2009 (NCT01193933) in our center since June 2009 till September 2016. Patients characteristics: the median of age was 26 years old (range 15 -54), the median white blood cell (WBC) count was 16.9×10^9 /L (range: 0.4-785×10^9 /L), the median blasts cells count in the bone marrow (BM) was 84.4% (range: 0-98). Sixty-five (59%) of the 110 patients had a B-cell phenotype, 42 (38%) had a T-cell phenotype, 3 (2.7%) patients - biphenotypical ALL. Interphase fluorescence *in situ* hybridization (FISH) was performed for detection CDKN2A deletion, TEL/AML1, MLL rearrangement, MYC (8q24.21) translocation, TP53 deletion, iAMP21.

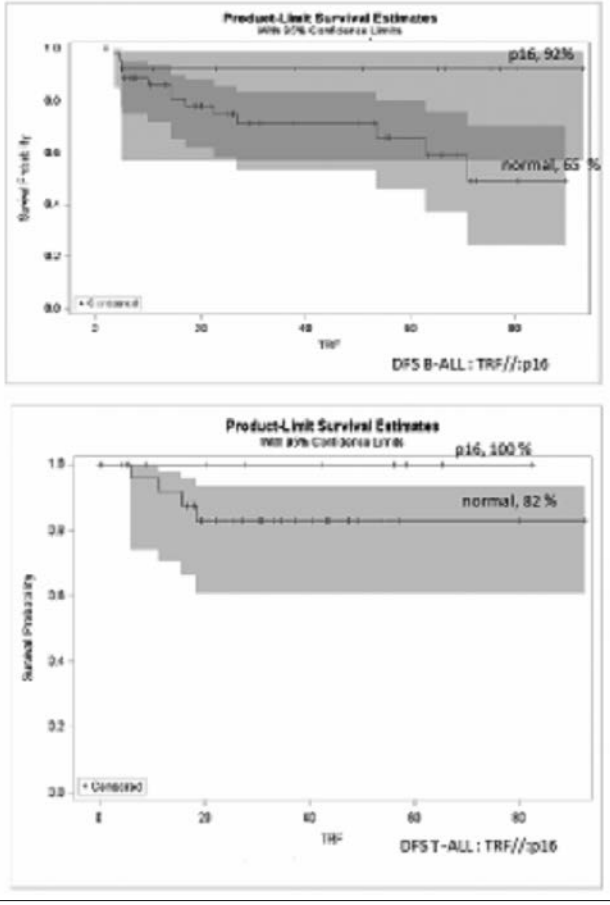

Figure 1.

Results: The prevalence of the CDKN2A deletion in all studied population was 24,5% (27 cases). The frequency of homozygous deletions was 70% (in 19 cases), heterozygous deletion was 30% (in 8 cases). CDKN2A deletion was detected in 14 (52%) patients with precursor-B phenotype, in 11 cases (41%) with T-ALL and in 2 (7%) cases with biphenotypical ALL. Our study demonstrated that CDKN2A deletion had no significant association with age, sex, WBC counts, BM blasts, risk stratification groups, complete remission (CR) and relapse rate in B-cell ALL. We didn't reveal any significant differences in OS, clinical and laboratory dates between groups of patients with homozygous and heterozygous deletion of the CDKN2A deletion. The analysis for T-ALL has detected that CDKN2A deletion was strongly associated with high WBC count (the median is 86×10 /L, p=0.006), with high lactate dehydrogenase (LDH) level (the median is 3062 E/L, p=0.0004) and no assonating with CR and replace incidence was found. We didn't revealed relationship between CDKN2A deletion and MLL, TEL/AML1 rearrangement, MYC translocation, TP53 mutation and iAMP21. CDKN2A deletion didn't have statistically significant impact on outcome of patients. The five-year overall survival (OS) for patients with and without deletion was 84% and 77% (p=0, 40); free survival (DFS) was 91% и 71% (p=0, 09), respectively. OS for patients with B-cell ALL with and without deletion was 85% and 76% (p=0, 35); DFS was 92% and 65% (p=0, 07), respectively. OS for T-cell ALL patients with and without deletion was 90% and 80% (p=0, 63); DFS was 100% and 82% (p=0, 24), respectively. (Figure 1).
Summary/Conclusions: We were unable to demonstrate prognostic value of the CDKN2A deletion in adult ALL patients and did not find significant association between deletion of the CDKN2A gene and with known cytogenetic prognostic factors. However patients with T-cell ALL and CDKN2A deletion had a more aggressive initial clinical features (high level WBC and LDH), but it didn't associate with poor outcomes including overall survival.
Deletion of CDKN2A is not adverse prognostic factor in adult ALL treated according to protocol RALL-2009.

PB1616

FREQUENCY AND CLINICAL IMPACT OF CDKN2A/B GENE LOCUS IN AN ADULT T-ALL COHORT OF PATIENTS ENROLLED IN THE SPANISH PETHEMA GROUP PROTOCOLS

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Background: Recurrent 9p21 deletions involving *CDKN2A/CDKN2B* locus are frequent in ALL. The very few data regarding their prognostic significance in adult T-ALL have shown that homozygous deletions of the *CDKN2A/CDKN2B* locus are associated with improved overall survival (OS).
Aims: We precisely characterized the copy number status (CNA) of *CDKN2A/CDKN2B* locus by discriminating deletions in A or B gene in order to elucidate its clinical impact separately.
Methods: Samples from 30 adult T-ALL cases included in high-risk protocols from the PETHEMA group were analyzed by CytoScan array (Affymetrix). Additionally, we set up a genomic qPCR to screen for *CDKN2A* and *CDKN2B* deletions in samples with few or not enough quality DNA (n=53). We corrected our CNA values for the normal cells (2N) contaminant present in the diagnosis samples. In addition, the results obtained by the array and/or qPCR were checked by FISH, when samples were available. Cumulative incidence of relapse (CIR) and OS were analyzed after censoring the patients at the time of allogeneic hematopoietic stem cell transplantation.
Results: qPCR results showed that most of the 9p21 losses corresponded to homozygous deletions in both genes (36%, 19/53), while heterozygosis deletions corresponded to 5.7% (3/53) and different CNA status between *CDKN2A* and *B* to 28% (15/53) of the samples. Globally alterations in *CDKN2A/B* locus were observed in 70% (37/53) of patients. Results obtained by the array corroborate the findings obtained by qPCR. The resolution of the array allowed us to distinguish between homozygosis in *CDKN2A* and heterozygosis on *CDKN2B*. The FISH analysis corroborated the homozygous deletion in the CDKN2A/B locus in all the cases analyzed. With that, we ask for clinical implications of CDKN2A/B CNA status in 49 cases with adequate follow-up. Median age (range) was 34 (16-68) years, 76% males, median WBC count 34 (0.6-431.0) x10^9/L. Immunophenotype: pro-T+pre-T (n=21), cortical T (n=21), mature T (n=7). CR was achieved in 92% (45/49) and MRD levels <0.1% at the end of induction were attained in 81% of patients. A trend for better OS was observed for patients with heterozygous or homozygous deletion of *CDKN2B* (61% [40%>82%]) *vs* non deleted patients (25% [0%>54%],(p=0.084), whereas no clinical impact was observed for the CNA status in the *CDKN2A* gene. No influence of *CDKN2A* or *CDKN2B* CNA status on CIR was observed. By multivariate analysis only the MRD level at the end of induction influenced on OS (p=0.028, HR=5.58 [1.21 ; 25.79]) and on CIR (p=0.07, HR= 3.67 [0.90-15.63]).
Summary/Conclusions: *CDKN2A/B* locus abnormalities, mainly homozygous deletions, were found in 70% of adult T-ALL patients. Different CNA status was found for *CDKN2A* and *CDKN2B*. Although homozygous deletion in *CDKN2B* was associated with a trend for better OS, the level of MRD was the only prognostic factor for OS in these patients.
Supported by 2014 SGR225 (GRE) from CERCA Programme/Generalitat de Catalunya, and by funds from Josep Carreras International Foundation, "la Caixa" Foundation and Celgene Spain.

PB1617

BUTEIN KILLS ACUTE LYMPHOBLASTIC LEUKEMIC CELLS *IN VITRO* AND *IN VIVO* THROUGH FOXO3A AND CASPASE-DEPENDENT APOPTOTIC PATHWAYS

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Background: Acute lymphoblastic leukemia (ALL) is a common hematological malignancy in children. Discovering and developing effective chemotherapeutic drugs are needed for ALL.
Aims: In this study, the anti-leukemic effect and the potential molecular mechanisms of butein on ALL were investigated.
Methods: We examined the rate of apoptosis of CEM-C7 (T-ALL), CEM-C1 (T-ALL), MOLT-4 (T-ALL), RS4-11 (B-ALL) cell lines and primary ALL blasts exposed to various concentrations of butein for 24 h using the flow cytometry. We tested the expression of the caspase-9, poly ADP-ribose polymerase (PARP), nuclear Forkhead Class box O3a (FOXO3a) and BCL-2 interacting mediator of cell death (BIM) using western blot assay. We established the xenograft mouse model to examine the anti-leukemic effect of butein *in vivo*.
Results: Butein was found to significantly induce the cellular apoptosis of ALL cell lines and primary ALL blasts in a dose-dependent manner. It also activated the cleavage of caspase-9 and PARP. We also found that butein promoted FOXO3a localization, enhanced the binding of FOXO3a on the BIM gene promoter and then increased the expression of BIM. Moreover, we showed that FOXO3a knockdown significantly decreased the apoptosis by butein, whereas overexpression of FOXO3a enhanced the butein-induced apoptosis. However, overexpression of FOXO3a mutation (C-terminally truncated FOXO3a DNA-binding domain) decreased the apoptosis by butein through decreasing the expression of BIM. Furthermore, treatment with butein was highly efficacious *in vivo*, with enhanced reduction of tumor burden in a xenograft model of ALL.
Summary/Conclusions: Our results therefore demonstrate the therapeutic potential of butein for ALL via FOXO3a and caspase-dependent apoptotic pathways.

PB1618

GENOMIC LANDSCAPE AT DIAGNOSIS AND RELAPSE IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA.

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Background: Childhood acute lymphoblastic leukemia (ALL) is the most common of pediatric malignancies, but intensive chemotherapy now allows to obtain complete remission in over 90% of the cases. Nevertheless, 1 out of 5 children will relapse.
Aims: In order to identify new markers prognostic of relapse, we analyzed SNP arrays of paired diagnosis and relapse samples from 8 B-ALL children.
Methods: The cohort included 3 males and 5 females, aged between 6 months and 21 years old (median age 4 years old). Bone marrow samples were analyzed by multiparameter flow cytometry, standard karyotype and FISH. SNP array (Affymetrix®) performed on cryopreserved cells at diagnosis and relapse investigated copy number alterations (CNA) and loss of heterozygosity (LOH). *TP53* mutation was studied on paired samples by Sanger sequencing.
Results: No modification was observed in the EGIL classification between diagnosis and relapse. Diagnostic cytogenetics prognosis was good for 3 children, poor for 3 (iAmpAML1, *KNMT2A* and complexe caryotype) intermediate for 2 (normal karyotype). Three patients showed additional karyotypic anomalies at relapse. SNP array showed a mean of 10 CNA and 3 LOH at diagnosis with 4 CNA and 0,6 LOH modulations at relapse. Seven of the 8 patients presented modulation in CNA and LOH during evolution with a median of 4. Some anomalies observed by cytogenetics were refined by SNP analysis, notably all chromosomal gains and losses were recovered and precisely located. More-

over, a t(4;8) translocation was found to be more complex with 7 and 8 CNA on chromosomes 4 and 8. Patients with the most CNA and LOH also had a complex karyotype. Anomalies were observed in hot spot regions in 9p (encompassing *CDKN2A/2B*, *PAX5* and *JAK2*) for 5 patients and 12p (including *ETV6*) for 3. Stable CNA were observed in the *JAK/STAT* pathway in 2 patients *(JAK2)* and LOH in the *RAS/MAPk* pathway *(NRAS)* in 1. Using the genetic classification of Moorman *et al* based on SNP array for 8 genes at diagnosis (*IKZF1, CDKN2A/2B, PAR 1, BTG1, EBF1, PAX5, ETV6* and *RB1*), SNP reclassified our patients in 3 of good prognosis and 5 of poor prognosis, with a median of 2 CNA for the 8 genes of interest. The 2 patients with cytogenetic intermediate prognosis would thus probably have been considered for a more intense therapeutic regimen, *i.e.* allogeneic stem-cell transplantation. Moreover, SNP showed that 2 patients acquired an *IKZF1* deletion, also of poor prognosis, while none of the children had *TP53* mutation at diagnosis nor relapse.
Summary/Conclusions: SNP array allowed to identify additional anomalies (compared to karyotype) in all children tested and changed the prognostic value of diagnostic anomalies. Moreover, the identification of anomalies in the *JAK/STAT* pathway could indicate a treatment by tyrosine kinase inhibitors, which would possibly have positively modified outcome. Taken together, this new technology combined with classical analyses at diagnosis might modify therapeutic options in childhood ALL, especially in the subgroup with a normal karyotype.

PB1619

SCREENING OF NUDT15 GENE VARIANTS IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: In cells, while DNA bases can be protected by double helix formation and nucleosome packaging, deoxyribonucleotide triphosphates are unprotected, thus, are vulnarable to damage. One of the enzymes which are responsible for removing damaged nucleotides is Nudix hydrolase15 (NUDT15). NUDT15 works as a negative regulator in thiopurine metabolism. Thioguanines are active metabolites of thiopurines. Mechanisms of action of thioguanines are disruption of DNA synthesis and induction of apoptosis. NUDT15 inhibits incorrect base pairing and apoptosis through catalysis of thioguanine hydrolysis. Tanaka *et al.* claimed that, besides *TPMT* variants in Japanese patients, there might be possible additional factors that may influence thiopurine toxicity. They reported that *NUDT15* variants are more specific to Asian population when compared to European people. As far as we know, this is the first study on screening of possible variants in the first exon of *NUDT15* in Turkish children with precursor B-cell acute lymphoblastic leukemia (Pre-B ALL).
Aims: In this study, our aim was screening of gene variants in first exon of NUDT15 in pediatric group of patients diagnosed with Pre-B ALL.
Methods: Our study group was composed of 83 patients aged between 1-15 diagnosed with Pre B-ALL at Lösante Hospital. DNA samples were isolated by using MagNa Pure isolation system. First exon of *NUDT15* was amplified by PCR reaction. After PCR purification, sequencing was performed.
Results: After screening of first exon of *NUDT15*, we detected two variations. First variation was intronic insertion which was defined as rs3831098 (c.158+52_158+53insGGGGCGTGCGCAGAGGGACGATCTC). The other intronic variation was defined as rs79687000 (c.158+117C>T). rs3831098 was determined in one of the 83 patients and rs79687000 was found in three out of the 83 patients (Tbale 1).

Table 1.

NUDT15 Variants in Children with Leukemia

Reports	rs Number	Nucleotide Change	Amino Acid Change	Localization	Prevalence Rate (%)
Tanaka et al. (n:92)	rs116855232	c.415C>T	Arg139Cys	Exon 3	6/92 (6.5)
Moriyama et al. (n:270)	rs186364861	c.52G>A	Val18Ile	Exon 1	
	rs554405994	c.36_37ins GGAGTC	Val18_Val 19insGly Val	Exon 1	
	rs147390019	c.416G>A	Arg139His	Exon 3	
	rs116855232	c.415C>T	Arg139Cys	Exon 3	
Yang et al. (n:657)	rs116855232	c.415C>T	Arg139Cys	Exon 3	2/657 (0.3)
Chiengthong et al. (n:82)	rs116855232	c.415C>T	Arg139Cys	Exon 3	2/82 (2.4)
Our study (n:83)	rs3831098	c.158+52_158+53insGGGGCGTGCGCAGAGGGACGATCTC	-	Intron 1	1/83 (1.2)
	rs79687000	c.158+117C>T	-	Intron 1	3/83 (3.6)

Summary/Conclusions: The changes in *NUDT15* that we found have not been previously reported in pediatric ALL patients. We do not know if these changes have an effect on pre-mRNA or "splice" regions and ALL. This issue needs further investigations in a large number of children with leukemia. We are planning the screening of other exons of *NUDT15* in order to evaluate for possible applications to clinical practice (*e.g.* cytopenia).

PB1620

COMPREHENSIVE MOLECULAR CYTOGENETIC ANALYSES OF BONE MARROW CELLS IN 64 CHILDREN WITH T-ALL REVEALED PROGNOSTICALLY RELEVANT RECURRENT FINDINGS

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Background: T-ALL represents 15% of newly diagnosed children with ALL and it is a clinically and genetically heterogeneous disease. Despite the use of intensive chemotherapy, relapse occurs in almost 25% of patients whose outcome remains dismal. Visible chromosomal aberrations are seen in approximately half of the cases, while cytogenetically cryptic aberrations are observed in almost all cases of T-ALL. However, prognostic implication of majority of them still remains unclear.
Aims: The aim of this study was to retrospectively and prospectively analyze bone marrow cells of children with T-ALL, to determine a frequency of recurrent cryptic chromosomal aberrations and to assess their impact on event free (EFS) and overall survival (OS).
Methods: Bone marrow cells of all patients were analyzed at the time of diagnosis by combination of conventional and molecular cytogenetic methods. For detection of the most frequent known chromosomal changes, *i.e.* rearrangements of *TCR* loci (*TRA*-14q11, *TRB*-7q34, *TRG*-7p14) and *TLX3* gene (5q35), deletion of *CDKN2A* (9p21) and amplification of *ABL1* (9q34), interphase FISH with locus-specific probes (Dako, Abbott Molecular) was used. Complex chromosomal rearrangements were proved by multicolor FISH and multicolor banding (24*X*Cyte/*X*Cyte Probe Kit; MetaSystems) or CGH-SNP array (SurePrint G3 Cancer CGH+SNP 4x180K, Agilent). For OS and EFS Kaplan-Maier analysis with Mantel Cox test was done.
Results: During the years 1996-2016 we examined archived material of 64 children with T-ALL (19 girls and 45 boys, median age 8.25 years). In total, chromosomal aberrations were detected in 86% of patients. The most frequent aberration was deletion of *CDKN2A* gene, which was found in 35/64 patients (19x homozygous, 16x heterozygous). Rearrangements of *TCR* loci were detected in 17/64 children (11x *TRA,* 6x *TRB)*. *TLX3* gene rearrangement was established in 15/64 patients. No aberration of *TRG* gene and amplification of *ABL1* were found. Complex chromosomal aberrations were proved in 12/64 children. In two cases, isochromosome of the long arm of chromosome 9 was found. 48 patients are living in the first/second complete remission. Relapse of the disease occurred in 17 patients, 16 children died. Best outcome (EFS and OS) was associated with *TRA* translocations ($p<0.05$). Patients with *TLX3* rearrangement had significantly shorter OS and EFS ($p<0.05$).
Summary/Conclusions: Using molecular cytogenetic methods cryptic recurrent aberrations were proved in vast majority of patients. Rearrangement of *TLX3* gene was related to poor outcome in contrast to *TRA* translocations associated with more favorable course of the disease. Our work attempts to clear up the significance of chromosomal aberrations related to childhood T-ALL in order to facilitate the patients´ stratification into cytogenetic prognostic groups and to identify patients at an increased risk of relapse similarly like it has been adopted in pB-ALL.
Supported by grants RVO-VFN64165, GACR-P302/12/G157 and NPU I nr.LO1604

PB1621

ADULT PRIMARY ACUTE LEUKEMIA SAMPLES WITH CHROMOSOMAL TRANSLOCATIONS GROW WELL IN IMMUNODEFICIENT MICE, BUT ARE DIFFICULT TO TRANSDUCE WITH LENTIVIRUSES

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Background: Acute leukemia (AL) is a severe disease of the hematopoietic system and associated with a poor outcome for patients. Patient derived xenograft (PDX) mouse models provide an attractive tool to engraft and grow primary tumor cells. In contrast to culture growth, samples can be monitored in a consisting

microenvironment. This powerful tool provides the baseline for further experiments like preclinical treatment trials or biology studies. While good engraftment rates were published for primary pediatric ALL samples, engraftment rates of adult ALL samples might be inferior, but remain largely elusive.
Aims: This study aimed to determine engraftment and growing ability of primary adult AL samples in immunodeficient mice. Genetic engineering was performed to evaluate transduction efficiencies by lentiviruses in PDX AL cells.
Methods: Primary adult ALL and AML samples were transplanted into NSG mice in the absence of total body irradiation. Both frozen as well as fresh patient material was used. Human CD45 and human CD38 were stained in blood to monitor successful engraftment. Mice were sacrificed before coming down with leukemia. Isolated cells from bone marrow and spleen were analyzed by flow cytometry. Genetic engineering was performed using lentiviral vector systems and monitored by expression of fluorochrome markers and flow cytometry.
Results: Engraftment and growth was successful in NSG mice in 12 out of 15 primary adult ALL samples. Frozen samples showed a longer median engraftment time with 114.4 days, whereas fresh samples could already be isolated with an average time of 75.29 days. Generally, the engraftment time varied form 47 days up to 166 days and was shortened for slow samples over several passages. Genetic engineering was successfully performed using lentiviral transduction to introduce expression of fluorescent colours for cell marking and monitoring in further experiments. Lentiviral transduction was performed in 8 ALL samples with BCR-ABL rearrangement and 2 MLL-AF4 ALL samples. Adult ALL PDX samples with chromosomal translocations showed very low transduction rates around 1%. Three AML samples with MLL-AF6, MLL-AF9 and MLL-AF10 translocation were analysed for this study. Interestingly and in contrast to ALL, transduction efficiency for AML rearranged samples was high with up to 60%. These values are similar to non-rearranged ALL samples having transduction rates between 30% up to 80%.
Summary/Conclusions: In summary, we observed a high engraftment rate of primary adult ALL samples in immunodeficient mice which was above what we anticipated from the literature. Adult PDX ALL samples can be transduced with lentiviruses with identical high transduction efficiency as pediatric samples, with an age independent exception of AL PDX cells with BCR-ABL or MLL translocations.

PB1622

SYNERGIC CHEMOTHERAPEUTIC EFFECT OF MENADIONE COMBINED WITH EPIGALLOCATECHINE-3-GALLATE OR DOXORUBICIN IN A HUMAN CELLULAR MODEL FOR ACUTE LYMPHOBLASTIC LEUKEMIA
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Background: Epigallocatechine-3-gallate (EGCG) and menadione (vitamin K3; MD) are known as potent apoptogens in cellular models for acute lymphoblastic leukemia (ALL) –Jurkat T cells.
Aims: The goal of this study was to explore the chemotherapeutic potential of MD combined with EGCG or DOX, and to determine whether there is a synergic interaction between these agents that could significantly enhance their antitumoral effect in a cellular model of ALL. We investigated the antiproliferative effect of EGCG and MD, applied alone or in combination EGCG:MD and MD:DOX respectively on human leukemia Jurkat lymphoblasts. Some underlying cellular mechanisms were also scrutinized.
Methods: Cell suspensions of Jurkat lymphoblasts were treated at various concentrations of EGCG, MD and DOX. Clonogenic survival was evaluated as the colony forming capacity in 96-well plates. Cell cycle and apoptosis/necrosis were determined by flow cytometry using the fluorescent indicators propidium iodide and Annexin V-FITC/7-AAD, respectively. Determination of oxidative stress and mitochondrial polarization was performed by spectrofluorimetry, using the fluorescent probes CM-H_2DCFDA and JC-1, respectively.
Results: EGCG decreased clonogenic survival (IC_{50}=117 μM and Hill coefficient *h*=3.17) and mitochondrial calcium in a dose-dependent manner (IC_{50} =97 μM, *h*=2.53). Furthermore, data show that there is no correlation between the level of mitochondrial calcium ($[Ca^{2+}]_m$) and mitochondrial membrane potential ($\Delta\psi_m$) (Pearson correlation coefficient r= −0.100) or between $[Ca2+]_m$ and reactive oxygen speacies (r=−0.437) production, thus EGCG exerted a depolarizing effect at the mitochondrial level, most likely via interference with the opening of the mitochondrial permeability transition pore. The combination EGCG:MD induced cell cycle arrest in G_2/M and S phases in a synergic manner (the measured viability was: ~91% for EGCG 50 μM, ~72%MD 25 μM, ~20% for their combination). The existence of two binding sites for EGCG is suggested, both modulated by MD, implying that MD is an allosteric modulator of the EGCG-induced depolarization. Fluorescence induced by treatment with EGCG alone, MD alone and EGCG:MD in combination was 172%, 101% and 387%, respectively, suggesting that EGCG and MD interact with the second specific target in a synergic manner. DOX induced cell cycle arrest, and clinical doses of DOX generated oxidative stress. MD augmented this effect, enhancing the antiproliferative effect of DOX most likely by increasing the affinity of DOX for nuclear DNA.
Summary/Conclusions: Our results support the notion that the combinations EGCG:MD and MD:DOX exert a strong synergic antiproliferative effect in human leukemia Jurkat cells and encourage further studies to test the clinical utility of this association in ALL therapy.

PB1623

FOCAL ERG DELETIONS AND DUX4 FUSIONS IN CELL LINES DERIVED FROM B CELL ACUTE LYMPHOBLASTIC LEUKEMIA
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Background: *DUX4* has recently been presented as new oncogenic driver in B cell acute lymphoblastic leukemia (pre B-ALL) of adolescents and young adults [1]. Translocations of *DUX4*, especially those with the *IGH* locus led to high expression of the corresponding fusion gene. *DUX4* then triggered the expression or a novel isoform of the *ETS* transcription factor *ERG* in pre B-ALL [2]. Focal deletions of exons 3-9 were a second cause for short *ERG* variants. Up to 7% of pre B-ALL showed deregulated expression of both genes, *DUX4* and *ERG* [2].
Aims: We set out to find pre B-ALL cell lines with *DUX4* translocation and *ERG* deletion as potential model systems for this novel subtype of pre B-ALL.
Methods: We screened a panel of ALL cell lines for aberrant expression of *DUX4* mRNA as potential indicator for *DUX4* translocations. Genomic PCR was performed to detect focal *ERG* deletions, qRT-PCR showed expression of alternative *ERG* exon 6, transcriptional target of *DUX4*.
Results: Genomic PCR showed that 2/66 pre B-ALL cell lines (NALM-6, SUP-B15) tested carried deletions targeting *ERG* exon 5. Results of *DUX4* qRT-PCR (Taqman probe Hs03037979_g1) were surprisingly inconsistent with Western blot analysis - which could only in part be explained by *DUX4* being a one-exon gene. NALM-6 was the only cell line expressing the DUX4 protein. Likewise, the alternative *ERG* transcript with alternative exon 6 was observed in NALM-6 only.
Summary/Conclusions: In conclusion, focal *ERG* deletions in pre B-ALL cell lines (2/66) occur at similar frequencies as in the primary tumor. Cell line NALM-6 carries the *DUX4-IHG* translocation, expresses the DUX4 protein and an *ERG* mRNA variant including the alternative exon 6. *ERG* deletions were present in cell lines NALM-6 and SUP-B15. However, cell line SUP-B15 did not express DUX4 protein and consequently also not alternative *ERG* exon 6 transcript. These results indicate that focal *ERG* deletions are not a safe indicator for aberrant expression of *DUX4*. Cell line NALM-6 is presented as model system for *DUX4/ERG* pre B-ALL.

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PB1624

NATURAL HISTORY OF SECONDARY MULTILINEAGE PROLIFERATION WITH MONOSOMY 7 FOLLOWING TREATMENT OF RELAPSED ACUTE LYMPHOBLASTIC LEUKEMIA
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Background: Approximately 90% of children with acute lymphoblastic leukemia (ALL) are cured with current treatment protocols. However, 15-20% of the patients still experience disease relapse. Moreover, a small subset of patients develop secondary therapy-related leukemia or myelodysplasia.
Aims: We present a case of a 11-year-old boy with the history of relapsed ALL followed by aberrant proliferation of several different subsets of precursor cells in bone marrow (BM), which was associated with progressive ineffective hematopoiesis.
Methods: A boy diagnosed with standard risk B-cell Precursor (BCP) ALL in 10-2009 was treated until 12-2011 with frontline chemotherapy according to ALL-IC BFM 2002 protocol. In 12-2012, one year after treatment completion he developed late combined ALL relapse (BM and testis). Unilateral orchidectomy was performed, while the biopsy of the second testis showed no leukemic infiltration. He received 2nd line chemotherapy according to IntReALL 2010 and local radiotherapy for the testicular area. Despite the borderline minimal residual

disease (MRD) at the end of induction treatment, the SCT procedure wasn't executed due to the lack of suitable matched donor. In June 2014, during maintenance treatment, the patient showed persistent pancytopenia.
Results: BM aspirate morphology showed 5% of blasts. However, detailed 8-color flow cytometry according to the EuroFlow protocols revealed no cells with BCP-ALL-specific immunophenotype, but several subsets of BCP with abnormal maturation (total 3.5%) and plasmacytoid dendritic cell precursors (2.1%). After cessation of maintenance therapy in 02-2016, continuous progression of infiltration was observed. Subsequent BM aspirates revealed increasing proliferation of five different cell populations which show rare, aberrant immunophenotypes. Three of them represented immature BCP: B1 (CD34+/CD19-/CD10+dim/CD20-/nTDT+dim/CD22+dim/CD38++/CD117+/CD123/HLA-DR+/++/SSCintermediate), B2 (CD34+/CD19+/CD10+heterogeneous/CD20-/nTDT+/++/ CD22+/CD38++/CD117-/CD123-/HLA-DR+/++/SSClow), and B3 (CD34-/CD19dim/CD10dim/CD20-). The fourth population corresponded to non-lymphoid/non-dendritic cell precursors (CD34+/CD19-/CD10-/CD20-/nTDT-/CD22-/CD38++/CD117-/CD123-/HLA-DR++/SSChigh) and the fifth population showed the features of plasmacytoid dendritic cell precursors with aberrant CD10 expression (CD34+dim/CD19-/CD10+/CD20-/nTDT-/CD22+/CD38+dim/CD117-/CD123++/HLA-DR+/SSC intermediate). Analysis of clonality via PCR assessment of *IGH* gene rearrangements revealed polyclonal pattern in all BCP subsets. Cytogenetic analysis showed an altered 45,XY,del(4)(q31?),-7,der(9) [20] karyotype, while interphase FISH showed monosomy 7 in >80% of all BM cells. Retrospective FISH analysis at 1st relapse showed normal chromosome 7 in all cells. CytoScan® 750K array (Affymetrix®) analysis in 10 sorted cell populations showed a complex karyotype highlighting monosomy of chromosome 7 and loss of chromosome 4q (del4q21.1-q25; 40Mb) associated with gain of chromosome 14 (14q32.33; 200Kb). In addition, several gains of minor chromosomal regions were detected in CD34+/CD19-/CD10- and CD34+/CD10+/CD19- cells. Due to progressive increase of all subsets of abnormal precursor cells (27,5% in total) and hepatosplenomegaly, further treatment direction was set at haploidentical stem cell transplantation.
Summary/Conclusions: We present an abnormal secondary proliferation, with increased numbers of aberrant BCP, myeloid and plasmacytoid dendritic cell precursors resulting from stem cell defect hallmarked by monosomy 7.

PB1625

IDENTIFICATION OF KEY GENES AND CONSTRUCTION OF MICRORNA–MRNA REGULATORY NETWORKS IN MULTIPLE MYELOMA BY INTEGRATED MULTIPLE GEO DATASETS USING BIOINFORMATICS ANALYSIS

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Background: T cell acute lymphoblastic leukemia (T-ALL) is a hematopoietic clonal malignancy caused by the malignant transformation of T lymphocyte driven by gene mutation. The prognosis of T-ALL is poor and early relapse is common.
Aims: We aimed at looking for specific and effective therapeutic target for T-ALL and eventually cure this form of leukemia by targeted therapy.
Methods: Bone marrow mononuclear cells (BMMC) are collected from bone marrow samples of T-ALL patients, including at initial presentation (n=46), during first CR (n=23) and at relapse (n=6). The expression level of mRNA encoding L-cellular Fas-associated death domain-like interleukin-1β converting enzyme inhibitory protein (c-FLIP) was assessed by realt time PCR. Changes in the expression level of HDAC before and after chidamide treatment were also assessed by western blot. Necroptosis and apoptosis after chidamide treatment were assessed by flow cytometry. Changes in expression level of c-$FLIP_L$ protein before and after treatment were assessed by western blot. Expression level of early apoptotic protein, key proteins of necroptosis were assessed by western blot.The effect of chidamide on NF-κB signaling pathway activity and expression of key molecules when inducing necroptosis were assessed by western blot. The regulating effect of chidamide on downstream genes of NF-κB pathway including cyclinD1, TNFα, IL-2, IL-8 were assessed by real- time PCR.
Results: The expression level of c-$FLIP_L$ mRNA is significantly higher in patients at initial presentation and relapse, compared to those at complete remission and healthy control. The expression level of c-$FLIP_L$ mRNA is associated with patient risk stratification, white blood cell count at initial presentation, serum level of lactate dehydrogenase (LDH), serum level of hydroxybutyrate dehydrogenase (HBDH), CD45, HLA-DR, SIL-TAL1 fusion gene and complex karyotype, and is not associated with age, sex, plasma fibrinogen level, and the chromosomal aberration 6q-. Patients who did not achieve CR during first chemotherapy had a higher c-$FLIP_L$ mRNA level than those who did ($p<0.05$).The expression level of histone deacetylase is higher in bone marrow mononuclear cells of T-ALL patients, Jurkat and HUT-78 cell lines. After treatment with chidamide, the expression level of histone deacetylase was significantly decreased in both cell lines.Chidamide induced necroptosis and apoptosis in Jurkat and HUT-78 cell lines. After apoptosis inhibitor was applied, chidamide mainly exert its effect of inducing cell death by inducing necroptosis. Chidamide inhibits the transduction and translation to c-$FLIP_L$ gene. When apoptosis is inhibited, chidamide upregulates the expression level of receptor-interacting protein 3 (RIP3) and the phosphorylation level of mixed lineage kinase domain-like (MLKL). After treatment with chidamide, the phosphorylation level of I-κB and p65 protein were both significantly decreased.
Summary/Conclusions: c-$FLIP_L$ mRNA expression level is abnormally high in T-ALL patients both at initial presentation and at relapse. The expression level of c-$FLIP_L$ is associated with risk stratification, white blood cell count, serum LDH level, serum HBDH level, CD45, SIL-TAL1 fusion gene, complex karyotype and disease outcome. c-$FLIP_L$ could be used as a prognostic marker in T-ALL.Chidamide suppresses histone deacetylation in Jurkat and HUT-78 cell lines.Chidamide induces necroptosis in Jurkat and HUT-78 cell lines by down regulating the transcription and translation of c-$FLIP_L$ gene.Chidamide induces necroptosis in Jurkat and HUT-78 cell lines via the classical NF-κB signaling pathway.

PB1626

CYP1A1 AND CXCL12 GENE POLYMORPHISMS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Acute lymphoblastic leukemia (ALL) is the most common type of childhood leukemia and represents one third of all pediatric malignancies. Despite the high survival rates (more than 80%), a noteworthy number of children relapse and for them the outcome remains poor. Epidemiological studies that examined possible risk factors of acute leukemias, proved that genetic factors play a crucial role in leukemogenesis. Recent genetic association studies on cancer risk, have focused on the effects of single nucleotide polymorphisms in genes that regulate inflammation and tumor suppression such as chemokines and P450 cytochrome. Chemokines induce the motility of endothelial and tumor cells. CXCL12, a chemokine expressed in various tumors, binds to chemokine receptor 4 (CXCR4) and is considered to play an important role in tumor growth and invasion. The polymorphism rs1801157 of this gene have been investigated concerning the disease pathogenesis. Moreover, CYP1A1 gene belongs to family 1, subfamily 1A1 of cytochrome P450. CYP1A1 protein is a phase I xenobiotic metabolizing enzyme that activates the conversion of environmental chemicals into carcinogens. The above gene contains two important single nucleotide polymorphism, CYP1A1*2A (rs4646903) and CYP1A1*2C (rs1048943), which are associated with an increased risk of leukemia.
Aims: The study of single nucleotide polymorphisms rs1801157 of CXCL12 and CYP1A1*2C (rs1048943) in children with B-lineage ALL .
Methods: Thirty children with B- lineage ALL (19 boys, mean age 6.8 years) were included in the present study and 70 healthy individuals (20 children and 50 adults blood donors) as control group. Genomic DNA was isolated from peripheral blood of participants and was analyzed for the existence of polymorphisms rs1801157 of CXCL12, and CYP1A1*2C (rs1048943) with Polymerase Chain Reaction (PCR). The PCR products were digested with the restriction enzyme MsPI for CXCL12 and BsrDI for CYP1A1. Descriptive statistics and logistic regression analysis were used to examine for differences between children with ALL and controls.
Results: In the CXCL12 loci, the frequencies of AA, AG, and GG genotype were 3.45%, 93.1% and 3.45% in children with ALL, 13.3%, 60.0%, 26.7% in children control group and 4.17%, 45.83% and 50.0% in adult control group respectively. In the CYP1A1 loci, the frequencies of AA, AG, and GG genotype were 13.3%, 86.7% and 0% in children with ALL, 90.0%, 10.0%, 0% in children control group and 81.6%, 16.4% and 2.0% in adult control group respectively. No statistical significant differences in CXCL12 polymorphism were revealed between children with ALL and healthy groups using logistic regression analysis. Regarding CYP1A1 loci, we detected a positive association for the AG polymorphism and ALL [OR: 37.7 (95% CI: 10.81, 131.37), $p<0.001$ and OR: 58.5 (95% CI: 9.66, 354.12), $p<0.001$ using only the children's control group].
Summary/Conclusions: A higher frequency of CYP1A1 heterozygote allele was observed among children with ALL compared to controls, whereas no differences were observed regarding CXCL12 polymorphisms. Future studies in larger populations are needed in order to specify the role of the above polymorphism in childhood ALL.

PB1627

INTRACHROMOSOMAL AMPLIFICATION OF CHROMOSOME 21 IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA; A RARE SUBTYPE

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Background: Intrachromosomal amplification of chromosome 21 (iAMP21) defines a rare subtype of pediatric acute lymphoblastic leukemia (pALL) occurring in approximately 2-3% of cases. The patients are older (median age is 9 years), usually have low white blood cell counts and show high relapse risk with standard therapy. Thus, it has been proposed to include ALL with iAMP21 as a distinct entity in the WHO classification of hematological malignancies.
Aims: To assess the frequency as well as the clinicopathological and genetic characteristics of ALL with iAMP21 in one of the three national diagnostic centers of pALL in Hungary. We sought to determine additional genetic aberrations associated with this rare entity.
Methods: Between 2008-2016, 175 samples of pALL patients were tested with FISH for *BCR-ABL1*, *ETV6-RUNX1* and *MLL* translocations. When available, bone marrow karyotyping was used to verify the abnormal results. In one case with iAMP21, multiplex ligations-dependent probe amplification (MLPA) was used to verify the cytogenetic aberrations as well as to detect associated copy number alterations.
Results: Among the 175 samples screened with FISH, three showed evidence of iAMP21 (1.7%). Case 1 was a 16-year-old male who presented with thrombocytopenia and hepatosplenomegaly. Flow cytometry (FCM) showed common ALL phenotype with the expression of CD13 and CD33. FISH showed >10 *RUNX1* signals in clusters in leukemic blasts, while karyotyping demonstrated r(21) with 7q deletion and +X. The lesions were verified by MLPA, which additionally revealed biallelic *CDKN2B* and *RB1* deletions. The patient was treated with ALL-IC BFM 2002 standard risk protocol. Following remission, isolated meningeal relapse occurred, for which he received radiotherapy. The patient died with recurrent meningeal disease without bone marrow involvement after 52 months. Case 2 was an 11-year-old girl, who presented with symptoms suggesting osteomyelitis of the tibia with unremarkable blood count. MRI showed multiple lesions in vertebrae as well as meningeal involvement of the spinal cord. Bone marrow biopsy and biopsy of the left tibia showed diffuse infiltration of lymphoblasts with only 5% leukemic cells in bone marrow aspirates. FISH detected 6-8 copies of *RUNX1* in leukemic blasts, while karyotyping yielded only normal bone marrow cells. She was commenced on ALL-IC BFM 2002 standard risk and was later switched to high risk protocol. She is in complete remission after 14 months. Case 3 was an 11-year-old boy who presented with anemia and thrombocytopenia. FCM showed ALL with common phenotype with two populations; one being strong CD19+/CD66c+ and one with dim CD19+/CD66c-. FISH showed >10 *RUNX1* signals in clusters in 95% of cells, while 52% showed *BCR-ABL1* positivity. Bone marrow karyotyping yielded metaphases of poor quality (Figure 1).

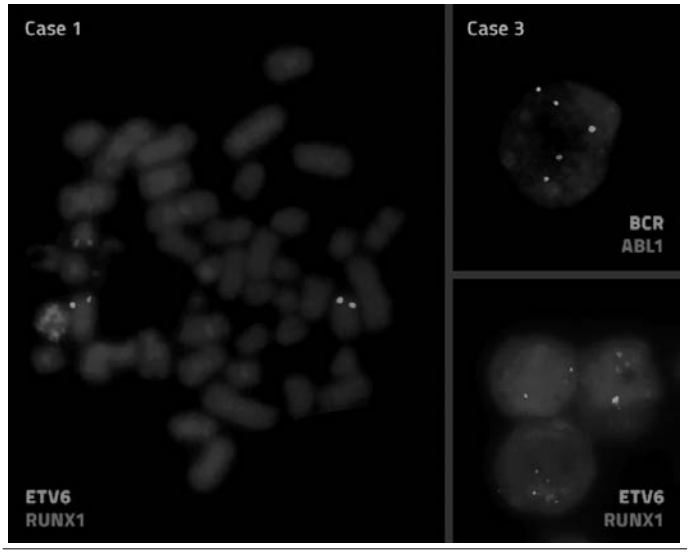

Figure 1.

Summary/Conclusions: ALL with iAMP21 is a rare subtype with distinct clinicopathological characteristics. Presenting with only mildly elevated WBC in older children is typical, relapses are frequent if standard risk chemotherapy is administered. Association with *BCR-ABL1* translocation is rare, having been reported so far in only 4 cases. Observing *BCR-ABL1* translocation in a subpopulation of leukemic cells is an intriguing phenomenon; it indicates that this translocation may occur as a secondary event even after leukemic transformation has commenced.

PB1628

HIGH RESOLUTION TECHNOLOGIES IN B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: B-cell acute lymphoblastic leukemia (B-ALL), the most common pediatric malignancy and main case of childhood cancer death, resulting from accumulation of genetic aberrations. Advances in our understanding of these aberrations is useful to improve disease classification, prognosis, therapeutic purposes, and to provide an overall understanding of the pathogenesis of the B-ALL.
Aims: Genomic characterization of childhood B-ALL.
Methods: We retrospectively examined bone marrow samples from 29 pediatric B-ALL using high resolution technology. We study copy number alteration (CNAs) and copy neutral loss of heterozygosity (CN-LOH) using Illumina CytoSNP-850K BeadChip in the Illumina HiScan platform. Analysis of more than 90 genes related with pediatric cancer was done using Next Generation Sequencing (NGS).
Results: Except for one, all patients showed copy number alterations. Losses were more common than gains. Whole and partial CN-LOH were observed in 12 cases. Only four recurrent genetic alterations were found: hyperdiploidy (44% of the cases), deletion of *CDKN2A/B* genes (22%), deletion of *PAX5* gene (16%) and deletion of *ETV6* (9%) gene. Several possible target genes were identified, including *SESN1, NME1* and *BMPR1B*, but additional studies are needed to confirm their implication in the disease. We identified high diversity of mutations 30 genes, 40% of all mutations are previously described in cancer patients. We found several mutations in Jak gene family in 5 patients that could have been the subject of therapeutic intervention with specific inhibitors of these kinases.
Summary/Conclusions: NGS and SNP arrays are powerful genetics tools capable of identifying a multitude of genetic alterations associated with B-ALL. The use of SNP arrays and NGS in clinical practice can help identify new prognostic alterations and develop individualized treatment plans for affected children.

Acute lymphoblastic leukemia - Clinical

PB1629

COMPLETE REMISSION WITH BLINATUMOMAB IN TWO PATIENTS WITH SKIN RELAPSED B-CELL ACUTE LEUKEMIA

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Background: Blinatumomab is a bispecific T cell–engager (BiTE) antibody (CD19/ CD3) indicated in relapsed/refractory B-cell Acute Lymphoid Leukemia (r/r ALL) (Topp *et al.*). Extra-medullary relapse is a rare event occurring in only 8% of the patients, of whom only 1.4% present a skin relapse which harbor a dismal prognosis (Gokbuget *et al.*).
Aims: Herein, we report the efficacy of Blinatumomab in two patients presenting with extra-medullary relapse of ALL.
Methods: The first patient (a 40-year-old man) was diagnosed a CD19+ Ph - B-ALL in 2009. He received a chemotherapy regimen according to the GRAALL protocol (Huguet *et al.*) until complete remission (CR). In 2015, he presented with a maculopapular rash of the right leg and the left flank, and two enlarged inguinal lymph nodes. Cutaneous relapse was attested by examination of skin biopsy specimen showing a blastic dermal infiltration harboring a CD10+, Tdt+ phénotype. The second patient was a 50-year-old male who presented, in 2016, a CD19+ B-ALL Ph- Ikaros- without central nervous system involvement. He obtained a first CR after GRAALL induction with negative MRD (IgH) but he relapsed 3 months later with a maculopapular rash of his chest. The skin biopsy revealed a blastic dermal infiltration. These two patients with skin relapse received salvage chemotherapy (COPRAALL 2007 regimen) *(*Domenech *et al.)*, with no efficacy (cutaneous blastic infiltrate). Both patients received one cycle of Blinatumomab from day 1 to day 28, at 28 µg per day, in an attempt to achieve CR before allogeneic stem cell transplantation, as previously described.
Results: At day 5 of Blinatumomab, an important non pruritic maculo-papular rash occurred in both patient, in the same area of the initial cutaneous involvement. Interestingly, it decreased after day 8. No new drug introduction or infection (bacterial, viral or parasitic) was documented in the days preceding or during Blinatumomab infusion. A skin biopsy performed at day 6 of Blinatumomab showed a more prominent dermal CD3+ lymphocytic infiltrate with a perivascular, but also a peri-nervous distribution (on the first patient's specimen only). Few lymphocytes marginated at the basement membrane and rare basal necrotic keratinocytes were also noted but without blast for the first, although few residual blastic cells were observed on the second's. One month later, another skin biopsy showed a CR without lymphocytic infiltrate. The medullar CR was confirmed at the molecular level (MRD negative). The first patient received allogenic stem cell transplantation (SCT) from a matched related donor one month later. He presented an acute and chronic GVHD, and is now in complete remission with a follow-up of 7 months. The second is still waiting for a SCT.
Summary/Conclusions: These observations confirm the strong efficacy of Blinatumomab in r/r B-ALL. We observed a T-cell dermal recruitment 6 days after Blinatumomab initiation clinically mimicking skin GVHD. However, we couldn't find specific histological features of GVHD, but only an "inflammatory dermatosis". Efficacy of Blinatumomab in relapsed B-ALL with cutaneous infiltration suggests promising activity in extra-medullary relapse. Further studies are required to confirm a Blinatumomab-based strategy in extra medullary relapsed B-ALL. This may provide a better understanding of how cytolytic synapses between T lymphocytes and intradermal blasts happen and the underlying homing mechanisms involved.

PB1630

A NOVEL METHOD FOR MINIMAL RESIDUAL DISEASE ANALYSIS IN PHILADELPHIA-NEGATIVE ACUTE LYMPHOBLASTIC LEUKEMIA: MODIFIED BIOMED-2 POLYMERASE CHAIN REACTION FOR IMMUNOGLOBULIN HEAVY CHAIN REARRANGEMENT

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Background: Recent studies have demonstrated the clinical importance of minimal residual disease (MRD) monitoring in adult acute lymphoblastic leukemia (ALL) as well as pediatric ALL. However, patient-specific polymerase chain reaction (PCR)-based MRD assessment, one of the most commonly recognized methods, is not widely used in clinical practice because it is expensive, time consuming, and technically difficult. Therefore, we modified the BIOMED-2 protocol, PCR for immunoglobulin heavy chain (IgH) rearrangement, to assess MRD in ALL easily and readily in our hospital.
Aims: The aim of this study was to examine the clinical utility of monitoring MRD by the modified BIOMED-2 PCR for IgH rearrangement in patients with Philadelphia-negative (Ph (-)) ALL.
Methods: We enrolled 54 patients diagnosed with Ph (-) ALL between 2006 and 2016 in our hospital. IgH rearrangement was detected in 35 patients using the standard BIOMED-2 PCR protocol. Patients who received palliative chemotherapy, never achieved remission (blasts <5%), or had no follow-up MRD data were excluded. Finally, data from 27 patients with Ph (-) ALL were analyzed. We assessed MRD with the modified BIOMED-2 PCR for IgH using bone marrow samples collected after each chemotherapy session. Patients' MRD statuses were classified as follows: Early MRD^{neg}, achievement of MRD negativity within 6 weeks after chemotherapy initiation; Late MRD^{neg}, achievement of MRD negativity more than 6 weeks after chemotherapy initiation; or MRD^{pos}, persistent MRD detection during chemotherapy. The endpoint was disease-free survival (DFS), calculated from the date of achieving remission.
Results: The median age was 38 years (16–73), and the median follow-up time was 47 months (4–106). There were 8, 14, and 5 patients with early MRD^{neg}, late MRD^{neg}, and MRD^{pos}, respectively. There were no differences in patient characteristics by bone marrow status, except for the duration to achieving remission (Table 1). There were significant differences in the 3-year DFS rates among patients with early MRD^{neg}, late MRD^{neg}, and MRD^{pos} (100% *vs* 72.9% *vs* 20%; p<0.001) (Figure 1). Patients undergoing transplantation had better prognosis than those receiving chemotherapy alone in the late MRD^{neg} group (100% *vs* 40%, p=0.028), whereas there was no difference in the early MRD^{neg} group (100% *vs* 100%, p=0.48).

Table 1. Patient characteristics by MRD status as assessed with the modified BIOMED-2 PCR for IgH protocol.

Factor	Total (n=27)	MRD^{pos} (n=5)	Late MRD^{neg} (n=14)	Early MRD^{neg} (n=8)	p-value
Sex, M/F	9/18	1/4	6/8	2/6	0.64
Age >50 years	10	1	6	3	0.87
WBC count risk*	4	1	3	0	0.37
Cytogenetic risk**	4	1	3	0	0.37
Transplantation	16	4	9	3	0.33
CR after 2 cycles***	2	2	0	0	0.03

* WBC risk: B >3 × 10^4/µL, T >10 × 10^4/µL.
** Cytogenetic risk: Hypodiploidy, complex karyotype, MLL rearrangement.
*** Achievement of remission after 2 cycles of chemotherapy.
MRD, minimal residual disease; PCR, polymerase chain reaction; IgH, immunoglobulin heavy chain; M, male; F, female; WBC, white blood cell; CR, complete remission.

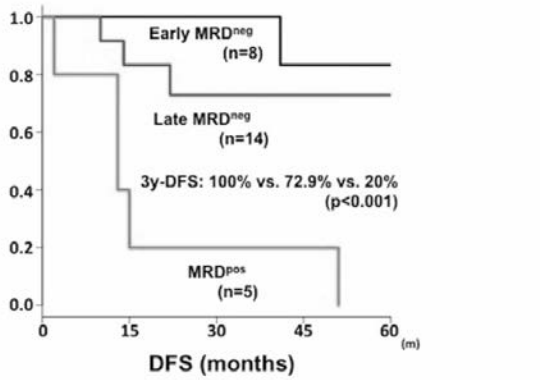

Figure 1. The status of minimal residual disease was associated with prognosis.

Summary/Conclusions: The modified BIOMED-2 PCR protocol is a highly accurate and reliable method of MRD assessment in adult ALL. It predicted treatment outcomes in adult Ph (-) ALL, and patients with late MRD^{neg} might derive a high survival benefit from allogeneic transplantation. Finally, the accuracy and reliability of the modified BIOMED-2 PCR for IgH were confirmed with a comparison to quantitative real-time PCR for BCR-ABL using samples from patients with Philadelphia-positive ALL (data not shown).

PB1631

SYSTEMATIC LITERATURE REVIEW OF PEGASPARGASE FOR THE TREATMENT OF NEWLY DIAGNOSED ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Asparaginase is a component of a multi-agent chemotherapy regimen and is now a cornerstone treatment for patients with acute lymphoblastic leukemia (ALL). Since 2006, pegaspargase (PEG-ASP) has been the gold standard asparaginase for the treatment of pediatric ALL as it offers equivalent efficacy to native *E. coli* L-asparaginase (native ASP), with less frequent dosing,

an IV administration option, and improved immunogenicity. Clinical outcomes in the adult ALL population are less well understood.

Aims: To assess the relative clinical benefit of PEG-ASP *vs* native ASP in 1st line treatment in newly diagnosed adult ALL patients in terms of event-free survival (EFS) and overall survival (OS). Safety outcomes were also examined.

Methods: A systematic literature search was conducted using a standardized search algorithm within the constraints of the National Library of Medicine database to identify available evidence for newly diagnosed patients treated with adult ALL protocols that use PEG-ASP or native ASP. Randomized, observational, and cohort studies were included, with the predefined clinical outcomes of event-free-survival (EFS) and overall survival (OS). Data was pooled with 95% confidence intervals (CIs) calculated using the logit transformation.

Results: A total of 30 studies were identified that met the pre-specified inclusion criteria, with 10 studies providing data for PEG-ASP and 23 studies for native ASP. The pooled estimate of 2-year EFS for adult ALL patients treated in 1st line with asparaginase was 48.0% (95% CI: [10.8; 85.2) for PEG-ASP and 66.0% (95% CI: [52.0; 77.0]) for native ASP.

The pooled estimate of 2-year OS for adult ALL patients was 62.8% (95% CI: [27.7; 97.9]) for PEG-ASP and 47.3% (95% CI: [8.5; 89.7]) for native ASP. Similarly, the pooled estimate of 5-year OS was 64.5% (95% CI: [61.5; 67.5%]) for PEG-ASP and 46.8% (95% CI: [33.6; 60.1]) for native ASP. In very high risk ALL patients, the pooled estimate of 5-year OS was 57.1% (95% CI: [52.4; 61.7%]) for PEG-ASP and 35.3% (95% CI: [21.7; 51.7]) for native ASP.

Findings for safety outcomes were consistent with product labeling for both asparaginases.

Summary/Conclusions: The systematic literature review highlights a positive clinical effectiveness profile for PEG-ASP in regards to EFS and OS in the treatment of newly diagnosed adult ALL patients with less frequent administration and similar safety profile as compared with native ASP.

PB1632

A COMPREHENSIVE ANALYSIS OF PATIENT- AND THERAPY-RELATED FACTORS AFFECTING THE TOXICITY OF PEGYLATED-ASPARAGINASE FOR THE TREATMENT OF ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: The application of pediatric regimens in the treatment of adult acute lymphoblastic leukemia (ALL) has led to a significant improvement in patients outcome. However, concerns about the feasibility of more intensive therapies and of the use of pegylated L-Asparaginase (PEG-ASP) in adult patients have emerged. Some patient-related risk factors as high BMI or hepatic steatosis have been already identified as risk factors, but few data are available on the synergic toxic effect from other concomitant drugs.

Aims: The aim of the present study was to evaluate the incidence of PEG-ASP related adverse events in a cohort of adult ALL patients in order to identify potential patient and therapy-related risk factors contributing to toxicity.

Methods: Since 2013, 21 adult ALL patients received PEG-ASP therapy in our institution. Median age was 44 (range 19-76); 12 patients were treated in frontline setting (7 according to a full pediatric protocol) whereas 9 patients received therapy for relapsed/refractory neoplasm. We retrospectively analyzed each single course which included PEG-ASP administration as an independent event, accounting 41 episodes. Patients' features (age, BMI, disease status) and concomitant therapies were accurately analyzed as factors potentially affecting PEG-ASP toxicity. The incidence of major thrombotic/bleeding complications and grade III/IV hepatic or pancreatic toxicity was analyzed; toxicity grading and management of PEG-ASP related complications were performed according to guidelines recently published by Stock *et al.*

Results: No grade III/IV pancreatic, thrombotic or hemorrhagic adverse events were recorded. A total of 8 episodes of grade III/IV hepatic toxicities were observed. In 3 cases, grade IV toxicity was observed. Those patients experienced unexplained severe weight gain and painful epathomegaly, a clinical picture resembling sinusoidal occlusive disease, ultrasonography showed acute liver steatosis. All 3 patients received concomitant therapy with idarubicin, vincristine and vancomycin. In univariate analysis, the incidence of grade III/IV hepatic toxicity was significantly higher when concomitant chemotherapy with at least 2 mg/sqm cumulative dose of vincristine (p 0.044, HR 4.75) or at least 16 mg/sqm cumulative dose of idarubicine (p 0.046, HR 1.45) were administered. Steroids therapy determined a borderline increase in toxicity risk (p 0.068, HR 1.688). No increase in toxicity was observed with any dosing of daunorubicin, cyclophosphamide, cytarabine, methotrexate and 6-mercaptopurine (Table 1). Among concomitant antibiotic therapies, vancomycin administration seemed to increase the incidence of grade III/IV hepato-toxicy (p 0.02, HR 1.863). No significant increase was observed with carbapenems and azoles (Table 1). Older age (>45), receiving PEG-ASP with active leukemia or a high BMI (>25) were not related with an increased incidence of grade III/IV hepatotoxicity (Table 1). Notably, none of the patients undergoing full pediatric induction (who received the highest doses of PEG-ASP), regardless of age (ranging from 21 to 55) experienced grade III/IV hepatotoxicity. A multivariate logistic regression analysis disclosed that concomitant administration of idarubicin, vincristine or vancomycin were independent predictors of grade III/IV hepatotoxicity (p 0.004, 0.027 and 0.042, respectively, Table 1).

Table 1.

Variables related to grade III/IV hepatic toxicity

	Grade III/IV hepatic toxicities [%]	HR	p [univariate]	p [multivariate]
Age >65 years	5/17 (29)	-	0.250	-
BMI >25	2/12 (17)	-	1.000	-
Higher PEG-ASP dose	8/29 (28)	-	0.250	-
Daunorubicin	4/12 (75)	-	0.690	-
Vincristine	7/29 (25)	4.75	0.044	0.027
Cyclophosphamide	5/31 (16)	-	0.698	-
Idarubicine	3/9 (50)	1.45	0.046	0.004
Cytarabine	1/15 (13)	-	0.219	-
6-Mercaptopurine	0/12 (0)	-	0.078	0.003
Methotrexate	0/7 (0)	-	0.399	-
Steroids	6/30 (27)	1.69	0.068	0.742
Vancomicine	4/7 (57)	1.86	0.020	0.042
Azoles antifungals	4/17 (24)	-	0.628	-
Carbapenemics	5/13 (39)	-	0.086	0.455
OVERALL	8/41 (20)	-	-	-

Summary/Conclusions: Our data show that the toxicity profile of PEG-ASP in adult patients is overall manageable. However, serious warnings emerge from our experience. Concomitant drugs and their timing of administration may play a crucial role in significantly contributing to PEG-ASP hepatic toxicity. In order to attempt to reduce toxicity, anthracyclines with shorter half-life, *i.e.* daunorubicin instead of idarubicin, should be used. A particular attention should be paid when administration of concomitant antibiotic therapy is required.

PB1633

COST OF CARE FOR ADULT PATIENTS WITH RELAPSED ACUTE LYMPHOBLASTIC LEUKEMIA WITH AND WITHOUT HEMATOPOIETIC STEM CELL TRANSPLANT IN GERMANY

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Background: Adult ALL is a rare but frequently fatal disease. Many patients who respond to initial therapy experience a relapse. For relapsed ALL (rALL), hematopoietic stem cell transplant (HSCT) is a potentially curative treatment option. HSCT is associated with added costs, however, which could impact overall healthcare budget.

Aims: This retrospective observational study aims to determine the cost of care and the impact of HSCT on total cost for adult rALL patients from a German payers' perspective.

Methods: A German claims database with a representative sample of approximately 7 million individuals insured within the German statutory health insurance and continuously observable over a period of 6 years was used as data source. From these data, adult patients (18 years and older) with a new diagnosis of ALL (ICD-10-GM code: C91.0*) between January 1, 2011 and December 31, 2015 and a relapse after remission to initial treatment were identified. Mean health care cost per patient per quarter, the smallest unit of time available in the database, was determined by whether or not patients had an HSCT after relapse. Costs were considered from the perspective of the German statutory health insurance and included costs for prescription medicine as well as outpatient and inpatient healthcare encounters.

Results: Of the total 116 incident adult ALL patients identified, 29 (25%) were determined to have had a relapse and 11 underwent HSCT after relapse (38%). Patients with an HSCT appear to incur higher cost than those without HSCT in each of the quarters after relapse was diagnosed (Table 1), with the highest in the first quarter after relapse, but decreasing in subsequent quarters. Inpatient cost accounted for the majority of the cost for the first three quarters for both HSCT and non-HSCT patients, but more for HSCT patients. The number of patients in the HSCT cohort remained relatively stable, while the non-HSCT cohort had only half the patients left by the third quarter post relapse.

Table 1. Costs in € per patient (with and without HSCT) by quarter after relapse

Patient group	Index quarter (relapse)			Q1			Q2			Q3		
	N	mean	% Inpatient cost	N	mean	% Inpatient cost	N	mean	% Inpatient cost	N	mean	% Inpatient cost
rALL without HSCT	18	34,616	84%	18	23,228	83%	11	10,762	61%	9	11,194	73%
rALL with HSCT	11	38,057	79%	10	54,559	88%	9	25,972	65%	9	27,055	88%
Total	29	35,921	82%	28	34,417	86%	20	17,606	64%	18	19,125	84%

Summary/Conclusions: The results of this study inform the magnitude of cost in Germany associated with adult rALL patients with or without an HSCT after relapse. The cost estimates provide a benchmark against which new treatment options for rALL can be compared. For future studies, it would be important to determine the magnitude of benefit such as long-term survival and other health consequences associated with HSCT as well.

PB1634

RETROSPECTIVE STUDY OF ADULT ALL IN MEXICO CITY: FIRST REPORT OF THE WORKING GROUP ON ACUTE LEUKEMIA

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Background: The prognosis of adult acute lymphoblastic leukemia (ALL) is dire, with a long-term survival of 40-50%. This disease entity is probably more frequent in the Latino population. Several studies have reported a worse prognosis in Hispanics with ALL as well as a greater incidence of the Ph-like genetic signature; however, the data is inconclusive in the Mexican population and there are no existing large multicenter series of ALL patients in Mexico that analyze survival.
Aims: The aim of this study was to describe the incidence, clinical and biologic characteristics as well as the survival of ALL patients in 5 referral hospitals in Mexico City.
Methods: A working group known as the *Grupo de Trabajo de Leucemia Aguda (GTLA)*, was created as a result of an initiative of the Mexican Group for the Study of Hematology *(Agrupación Mexicana para el Estudio de le Hematología*) to promote acute leukemia research in Mexico. This is the first report of the GTLA which includes 5 referral hospitals in Mexico City. A retrospective, multicenter, descriptive study of adult ALL patients treated between 2009 and 2015 was conducted.
Results: We included 559 adults in 5 centers in Mexico City. Their median age was 28 years (14-81): adolescents and young adults (AYA) 67.3%; adults 24.7% and elderly adults 8.1%. Tumor lysis syndrome was detected in 9.8% of all cases. Cytogenetic information was unavailable in 45% of cases due to lack of access or growth in metaphase. Among cases that could be analyzed, a normal karyotype was the most frequent (70.5%), followed by Ph+ (16.7%). Patients were considered high-risk in 52.1% cases. The most frequently used drug protocol was Hyper-CVAD, in 47% of cases. Complete remission (CR) was achieved in 67.1% of patients, and 18% required a second cycle for CR, while 13% were primarily refractory. A mortality rate during induction was registered as 10.6%, and there were 11.4% deaths while in CR. Among patients in CR, 59.1% relapsed. At the time of analysis, 26.7% of patients were alive, with a median OS of 12.97 months and a DFS of 16 months. Only 5.7% were able to receive an allogeneic hematopoietic progenitor cell transplant (AlloHPCT). OS at 3 years was 22.1% and by age group: AYA 25.7%, adults 17.4% and elderly adults 0% (p=0.0001). On multivariate analysis, significant risk factors for OS were the age group, ECOG, the presence of the tumor lysis syndrome and liver function test abnormalities while protective factors included early CR and an AlloHPCT.
Summary/Conclusions: Outcomes are poor in adult ALL patients treated in these referral centers in Mexico City. This may be explained by the high incidence of AYA patients and the low frequency with which they are treated with regimens containing L-asparaginase. The incidence of the Philadelphia chromosome is lower than reported, which could be due to a real difference between populations or due to aspects related to cytogenetic techniques. Based on these results, the GTLA´s objectives will be: to standardize diagnostic testing (immunophenotype and cytogenetics), improve early CR rates, standardize support care to decrease deaths during induction as well as treatment -related deaths, and increase the percentage of patients treated with AlloHPCT. Studies to determine the prevalence of the Ph-like genetic signature will be of great relevance in our population.
ClinicalTrials.gov Identifier: NCT02990104TRIAL

PB1635

IMPACT OF DISEASE STATUS ON OUTCOMES OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION WITH REFRACTORY AND RELAPSED ACUTE LYMPHOBLASTIC LEUKEMI

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Background: Refractory or relapse remains a major obstacle in improving outcomes of patients with acute lymphoblastic leukemia (ALL). And allogeneic hematopoietic stem cell transplant (allo-HSCT) was the only curative treatment option for these patients. However, whether an allo-HSCT was performed in status of advanced stage or in setting of remission after salvage chemotherapy, there is no standard of care.
Aims: To evaluate the impact of disease status on the outcomes of allo-HSCT in the treatment of patients with refractory and relapsed ALL.
Methods: 52 patients with refractory and relapsed ALL, including 19 cases in advanced stage (nonremission, NR) and 33 cases in more than or equal to second complete remission(≥CR2), received allo-HSCT after myeloablative conditioning regimen in our department.
Results: 51 patients engrafted successfully. The transplantation-related mortality (TRM) rate of NR and ≥CR2 was 10.5% *vs* 12.1% (P=0.815). The incidence of aGVHD was 52.6% *vs* 57.6% (P=0.730), including 42.1% *vs* 33.3% (P=0.527) with mild (grade I-II) and 10.5% *vs* 24.3% (P=0.399) with severe (grade III-IV) aGVHD. The incidence of cGVHD was similar also(41.6% *vs* 57.9% ,P=0.660). With a median follow-up of 12(1.8-44.5) months, the cumulative relapse rate of NR and ≥CR2 was 47% *vs* 34.3%□P=0.425) respectively. The estimated 2 year overall survival (OS) and 2 year leukemia-free survival (LFS) rate were 42.6% *vs* 45.7% (P=0.487) and 46.3% *vs* 46.2% (P=0.571) respectively. Multivariate Analysis results showed that cGVHD was independent favorable risk factor for OS and LFS of R/R ALL□For relapsed patients, OS was significantly better with first CR duration>6 month and time to transplant≤2 months.
Summary/Conclusions: Allo-HSCT is an effective salvage treatment option for patients with refractory and relapsed ALL. Our retrospective analysis showed that R/R ALL with different status prior transplant had similar outcome post transplantation.

PB1636

THE FREQUENCY AND PROGNOSTIC SIGNIFICANCE OF IKZF1 DELETIONS IN ADULT PH-POSITIVE AND PH-NEGATIVE B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS TREATED IN RUSSIAN ACUTE LYMPHOBLASTIC LEUKEMIA STUDIES.

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Background: The incidence of IKZF1 gene deletions is approximately 20% in adult patients with BCR-ABL1- negative B-cell ALL and 70–80% in BCR-ABL1-positive ALL. These mutations are associated with poor prognosis in patients with Ph-negative ALL, but not in patients with Ph-positive ALL, suggesting that IKZF1 deletions may be more prognostically valuable in patients with Ph-negative ALL.
Aims: To evaluate the frequency and prognostic impact of mutation status of IKZF1 in patients with d*e novo* BCR-ABL1-negative and BCR-ABL1-positive B-cell acute lymphoblastic leukemia.
Methods: The study included 36 adult patients (median age 27, range 17-56; m:f=15:21) with newly diagnosed BCR-ABL1- neg B-cell ALL and 15 patients (median age 34 years, range 22–68; m:f=6:9) with BCR-ABL1- pos B-cell ALL, who were enrolled in Russian acute lymphoblastic leukemia (RALL) - 2009 [ClinicalTrials.gov public site; NCT01193933] and RALL-2012 protocols since Feb 2010 till Sep 2016 and Aug 2009 till Feb 2017, respectively.
Intragenic deletions of IKZF1 were detected using breakpoint-specific fluorescent multiplex polymerase chain reaction according to the procedure described by [Aurelie Caye et al, Haematologica, 2013]. DNA for PCR was extracted from leukemia cells of frozen bone marrow samples.
Results: The IKZF1 deletions were detected in 7 (47%) of 15 patients with BCR-ABL1- pos ALL (3 cases with del 4-7 (43%), 2 – del 2-7 (28%), 1 – del 2a-8 and 1 – del 4-8 (14%)). The median follow-up time in 15 patients was 18 months (range: 4-79 month). Five patients died (33%) after relapse or progression of the disease, and 10 patients are alive. Overall survival (OS) in BCR-ABL1 - pos B-cell ALL patients with IKZF1 mutations and without was 37,5% and 57,1% (p=0,77), relapse - free survival (RFS) - 25% and 33,3% (p=0,88), respectively. In patients with BCR-ABL1- neg ALL the IKZF1 deletions were revealed in 8 (22%) of 36 patients (4 cases with del 4-7 (50%), 2 - del 2-7 (25%), 1 – del of 2-8 (12,5%) and in 1 patient all types of deletions were determined (del 4-7, del 4-8, del 2-7, del 2-8)). The median follow-up time in 36 patients was 22 months (range: 0,5-84 month). 4 patients died of the disease (11%) and 2 of infections, 30 patients are alive. OS for patients with BCR-ABL1- neg ALL with IKZF1 mutations and without was 100% and 60,2% (p=0,77), RFS - 75% and 40,2% (p=0,74), respectively. (Figure 1).
Summary/Conclusions: The frequency of IKZF1 gene deletions in patients with BCR-ABL1- pos and with BCR-ABL1- neg ALL was 47% and 22%, respectively. IKZF1 mutations seemed to be of poor prognosis for BCR-ABL1-

pos ALL and, on the contrary, more favorable for BCR-ABL1- neg ALL, though not statistically significant. Having or not IKZF1 mutations, all BCR-ABL1-pos ALL patients are candidates for allogeneic hematopoietic stem cell transplantation (allo-HSCT). Regarding BCR-ABL1-neg ALL: though the group of patients is small, we can suggest that IKZF1 mutation did not appear to influence survival due to different chemotherapy principal in RALL – 2009 – non-intensive but not-interruptive therapy with low numbers of HSCT.

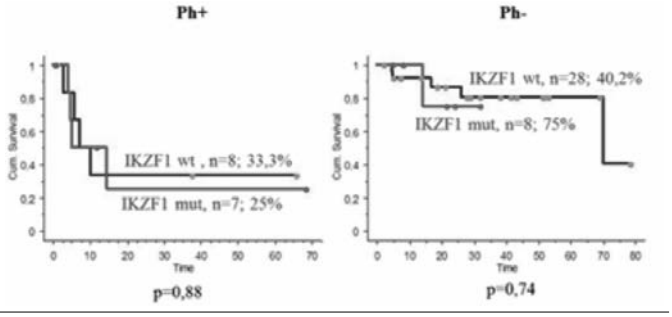

Figure 1. Relapse-free survival.

PB1637

GMALL BASED PROTOCOL, USING NATIVE E. COLI L-ASPARAGINASE, IMPROVES SURVIVAL OF ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA IN BRAZIL

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Background: Despite being the most common childhood cancer, nearly one half of ALL cases occurs in adults. Recently, it has been suggested that more intensive protocols may improve survival in adolescents and young adults (AYA).
Aims: Compare results of patients treated with BFM-based protocol to those patients treated with GMALL-based protocol, in a developing country.
Methods: This is a single center retrospective study which included all newly diagnosed adult ALL patients admitted between May/2012 and October/2016. Initially, patients aged 18-39 years (AYA group) were treated with BFM ALL 2009-based protocol and those aged 40-59 years were treated with GMALL 2003-based protocol. Since September 2013, because of high toxicity, only patients under 30 years were elegible for BFM-based treatment. Major adaptations were: (1) native *E. coli* l-asparaginase was substituted for peg-asparaginase, and (2) GMALL irradiation therapy was postponed to maintenance phase. *BCR/ABL1* positive patients received standard chemotherapy plus Imatinib. Negative MRD was defined as <0,01% by flow cytometry. Overall survival was estimated by Kaplan-Meier method. Competing risk analysis was carried out for cumulative incidence of death in CR1 or not in CR1. This study was approved by local Ethics Committee.
Results: Thirty five patients were included, 21 of them started BFM-based treatment and 14 started GMALL-based protocol. During the first three months, 7 patients migrated from BFM to GMALL-based treatment because of toxicity and were analyzed separately. Median age was 21years (18-38) for BFM-based group, 44 years (30-57) for GMALL-based, and 33 years (21-38) for de-escalated. Male predominance was observed (71%), not different between groups. T-phenotype was more frequent than expected, representing 50% of BFM-based, 50% of GMALL-based and 29% of de-escalated groups. *BCR/ABL1* was detected in 14% of BFM-based, 23% of GMALL-based and 14% of de-escalated groups (p=0,85). Seven patients (2 BFM and 5 GMALL) underwent allogeneic stem cell transplantation in first remission. Of all 35 patients, 31 achieved complete remission after first induction phase. With median follow-up of 18 months, 1-year overall survival (OS) was 60% for all patients (39% for BFM-based, 75% for GMALL-based and 86% for de-escalated groups – p=0,04; BFM-based *versus* other protocols). Cumulative incidence (CI) of death in first complete remission (CR1) at 12 months was 18%, not different between groups. CI of death at 12 months in non-CR1 (relapsed or refractory) patients was 39% for BFM-based, 7% for GMALL-based and 0% for de-escalated groups – BFM-based *versus* other HR 2,6; p 0,13. Among 31 patients who achieved CR1, MRD data was available for 26 (74%) of these at the end of first induction. OS at 18 months for CR1 patients with negative MDR after first induction was 74%, compared to 52% in MRD+ (Figure 1).
Summary/Conclusions: Our results show that GMALL-based protocol yields good overall survival in adults ALL patients in a low income country, despite major adaptations. On the other hand, overall survival of AYA patients treated with BFM-based protocol was surprisingly poor, specially because of ineffective disease control. This finding may be related to several aspects: socioeconomic impairment, inadequate supportive care for more intense therapies and ineffective cancer care network. Future prospective studies should focus on this issues.

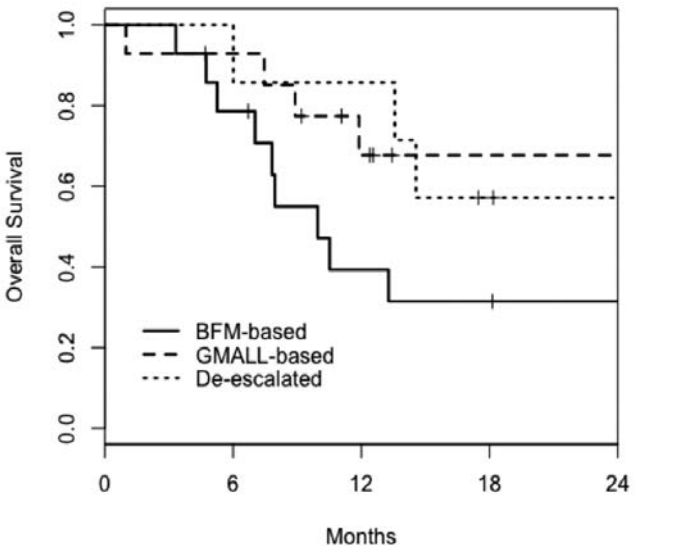

Figure 1.

PB1638

THE INVESTIGATION OF RELATIONSHIP BETWEEN COL1A1 AND FOK1 GENE POLYMORPHISMS AND DEVELOPMENT OF TREATMENT-RELATED SKELETAL COMPLICATIONS IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Cure rates for childhood acute lymphoblastic leukemia (ALL) have approached 90% with therapeutic advances over the last several decades. Many treatment related long-term complications including impaired physical growth, neurocognitive dysfunction, emotional and occupational di□culties, cardiac abnormalities, hypertension, secondary neoplasms, decreased bone mineral density (BMD) and osteonecrosis have been observed as the number of survivors increased. Bone infiltration of leukemic cells, corticosteroids exposure, poor nutrition, low vitamin D levels, poor muscle mass, genetic predispositions contribute to the development or worsening of bone pathology during therapy that may result in osteoporosis, fracture and osteonecrosis.
Aims: In this study, we aimed to investigate whether vitamin D receptor and collagen protein gene polymorphisms, which are important in bone mineral and matrix formation, have effects on bone turnover in patients with ALL.
Methods: Fifty children with ALL who were diagnosed and treated with BFM-95 protocol (25 girls, 25 boys) between 1998-2008 and 96 healty children at Dokuz Eylül University Medical School were enrolled in this study. Polymorphisms of vitamin D receptor (VDR) *Fok1* gene and the collagen *Col1A1* gene were studied from peripheral blood samples of the patients that were collected before initiation of chemotherapy protocol. After genomic DNA extraction, VDR *Fok1* gene and colloidal *Col1A1* gene polymorphisms were analyzed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). The data including age, sex, leukemia risk group, presence or absence of relapse were all noted. Bone marrow density and markers of bone metabolism including serum calcium, phosphorus, serum alkaline phosphatase, parathyroid hormone and 25-OH D vitamin levels were all screened before initiation of maintenance treatment.
Results: The distribution of *Fok1* and *Col1A1* gene polymorphisms was similar both in the patient group and healthy control group. The frequency of gene polymorhisms in the patient group were 8% ff, 46%Ff and 46%FF for the *Fok1* genotype and 62%GG, 26%GT and 12%TT for the *Col1A1* genotype. Out of 50 patients, 16 (32%) patients were found to have skeletal diseases like osteopenia (16%), osteoporosis (12%) and osteonecrosis (8%). The *Fok1* genotype and *Col1A1* genotype polymorhisms were similar in both group of patients with or without skeletal diseases. The frequency of osteopenia was signicanly higher in the male group (p=0.049) and the frequency of osteonecrosis was signicanly higher in patients older than 10 years old (p=0.001). There was no significant association between *Fok1* and *Col1A1* gene polymorphisms and leukemia subtype, risk group or relapse rate.
Summary/Conclusions: It has recently become more important to prevent treatment-related complications that we see as a consequence of high cure rates in ALL. In this context we have investigated whether there is a relationship between gene polymorhisms and treatment related skeletal diseases like

osteopenia, osteoporosis and osteonecrosis. We haven't detected a significant association between *Fok1* and *Col1A1* gene polymorphisms and frequency of skeletal complications. Studies investigating the possible underlying genetic susceptibilites to certain complications are important not only for better management of complications but also for development of new individual patient-specific treatment modalities.

PB1639

OUTCOME OF ADOLESCENTS AND YOUNG ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA TREATED WITH PEDIATRIC PROTOCOL: MONOCENTRIC STUDY

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Background: Several retrospective studies have confirmed that adolescents and young adults (AyA) with acute lymphoblastic leukemia(ALL) treated with pediatric protocols have better outcomes than similarly aged patients treated with adult protocols.
Aims: We reported results and feasibility of a pediatric-based protocol (EORTC 58951) in adolescents and young adults.
Methods: From January 2000 to December 2015, 72 patients aged 16 to 30 years with newly diagnosed ALL were treated, in the department of clinical hematology of Hedi Chaker Hospital, according to the pediatric protocol EORTC 58951. Further leukemia characteristics (Sex, White Blood cell count, Blasts phenotype, Cytogenetic results), we studied the protocol results: response to prophase), risk group stratification (average: AR1 and AR2, very high: VHR), remission rate, death rate, relapse rate and 5 years survivals (overall OS and event free EFS).
Results: Seventy two AyA ALL were treated with the pediatric protocol. The patients were 45 males and 27 females (SR=1.66). A WBC>100 G/l was noted in 32%. A T blast phenotype was noted in 53% of cases. Twenty two patients (30%) were PPR. Nine patients (13%) were treated according AR1 arm, 39 patients (54%) according AR2 arm and 24 patients (33%) according VHR arm induction. CR rate was 87% after one course and 94% after 2 courses. Induction death was noted in 3% and post-induction death was noted in 13%. Twenty four patients treated with VHR arm protocol were eligible for allogenic stem-cell transplantation (SCT), among them 15 patients had a familial donor and 10 patients were allograft (42%) and only 4 patients still in CR (2 patients died by GVH and 4 patients relapsed). Relapse was observed in 22 patients (32%), among them 12 during the first year of treatment. The median follow up was 101 months (8,4 years). The five years OS and EFS were respectively 50 and 50.4%.
Summary/Conclusions: The results of this pediatric based study show that response to therapy and prognostic in adolescent and young adults were better than those treated with adult protocols and tolerability of chemotherapy is acceptable. However OS and EFS, better than adult ALL treated by adult protocol (OS= 14%, EFS=14%: local study) was not satisfactory because the high toxic mortality rate.

PB1640

ASSESSMENT OF DEPRESSION AND SELF-CONCEPTION IN CHILDREN WITH ALL-TREATMENT

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Background: Leukemia is the most prevalent pediatric malignancy with acute lymphoblastic leukemia (ALL) being the most common accounting for 75% of leukemia cases with about 2400 newly diagnosed children each year worldwide. Treatment of ALL requires long course chemotherapy ranging up to 48 months with 20% possibility of relapse. Affected children receive in-patient treatment at the clinic for nearly six months for leukemia and related complications. The diagnosis of childhood leukemia and its stressful treatment, not only adversely impact the physical and psychological health of the children with leukemia, but also impose heavy psychological burden on their parent.
Aims: The study aimed to determine prevalance of condition of depression and self conception of children with leukemia and compare with healty children.
Methods: The study was conducted in 24 voluntary children with leukemia and 25 healthy children aged 9-16 years. Children with leukemia were evaluated at the time of diagnosis, end of induction treatment and end of consolidation treatment, just before the maintenence treatment, respectively. Psychological data including depression and low self-concept were assessed by Child Depression Scale and Piers Harris Self-Cocept Scale. The changes in psychological conditions due to long stay at the hospital were investigated. Demographic data included age, sex, school achievement, parents education, socioeconomic condition, loss of first degree relatives.
Results: The prevalance of depressive disorder in children with leukemia at the end of induction and at the end of consolidation treatment were significantly increased. Self-Concept Scales were found lower in these patients.
Summary/Conclusions: The children with ALL receive long course chemotherapy and become distanced from their family, school and milieu and as a result, these patients are vulnarable to psychological problems. They are more depressive and have lower self-conception comparing to healthy children. It is important to provide psychological support to these children in addition to their chemotherapy.

PB1641

SEVERE PSYCHIATRIC DISTURBANCES DURING THERAPY IN PEDIATRIC ALL

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Background: Psychiatric disturbances are not uncommon in patients with cancer. Their pathogenetic mechanisms are variable and comprise consequences of the therapy, underlying disease, as well as personality characteristics. These disturbances are frequently associated with the use of corticosteroids, which is an essential component of the treatment for children and adolescents with Acute Lymphoblastic Leukemia (ALL).
Aims: This study aimed to investigate the incidence of severe psychiatric disturbances in patients treated for childhood ALL.
Methods: We report the results of a retrospective analysis of the incidence of severe psychiatric disturbances, defined as behavioral and psychological changes which lead to dangerous or erratic behaviors requiring use of psychiatric medications, in patients treated for childhood ALL. All patients were treated in a single institution and followed the same chemotherapeutic protocol, according to which, corticosteroids are administered initially during the "induction" phase and then in multiple subsequent pulses.
Results: Seventy patients (mean age:4.04 years old, range:1-16) were treated for ALL during the 4 years of the observation period. During that period, 9 (12.8%) children (6 boys, 3 girls) of mean age 12.3 years old (range: 10-15) experienced psychiatric - neurological symptoms and/or mental disorders, which included major depressive disorder, withdrawal, first psychotic episode, disorientation, visual hallucinations, mood swings and behavioral outbursts. The majority of the patients (8 patients, 6 at the Intermediate Risk group and 2 at the High Risk group) experienced disturbances during the reinduction phase, while treated with dexamethasone at 10 mg/m2 for 21 days. Two patients of the High Risk group presented with behavioral effects one during the second HR2 block. Patients who had symptoms of major depression were treated with either fluoxetine, or/and risperidone, or/ and escitalopram for a period of time ranging of 5 days to 6 months. One patient experienced a psychotic episode during reinduction (Prot.II,phase 2) with aggression and violence towards others and had to be treated immediately with intramuscularly haloperidol and diazepam. All of our patients are alive and in remission, 7 off therapy for a period of 3 years, and 2 receiving maintenance therapy. Statistical analysis showed that severe psychiatric disturbances were observed more frequently in older patients and they were more common with the administration of dexamethasone than with prednisolone.
Summary/Conclusions: Severe psychiatric disturbances are not infrequent in children and adolescents receiving treatment for ALL. Awareness of this complication, appropriate parental education for identifying early signs, and prompt therapeutic interventions are essential for optimal outcome. Further studies are required for identifying patients at risk and best use of chemotherapeutic agents and of dexamethasone.

PB1642

INCIDENCE, SEVERITY AND RISK FACTORS FOR NEUROLOGIC COMPLICATIONS ASSOCIATED WITH L ASPARAGINASE TREATMENT IN PAEDIATRIC PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKAEMIA

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Background: Combined chemotherapy increased over time cure rate in patients with acute lymphoblastic leukemia (ALL). Among other things, one of the direct adverse effects of chemotherapy is affecting hemostasis inducing thrombosis or bleeding. Hemostasis can also be affected indirectly by the appearance of infections secondary immunosuppression. It has been found that L Asparaginase induces thrombotic events by reducing antithrombin III, protein C, protein S and by inducing endothelial damage. Thrombotic events may result as a direct effect of the disease, may be the effect of chemotherapy or may be related with prothrombotic status of the patient.
Aims: To evaluate incidence and severity of thrombotic or bleeding events in paediatric patients with ALL during chemotherapy.
Methods: We considered all patients hospitalized for ALL in the Pediatrics

Department of Clinical Institute Fundeni during 2010-2017 and received chemotherapy according to protocol ALL BFM 1995 and ALL BFM 2002, established after framing in the risk group.
Results: Over a period of 8 years in our department 280 patients with ALL received L-asparaginase in the induction phase. Neurological manifestation suggestive for bleeding or thrombotic events occurred in 9/280 (3,21%) patients. 2 patients were treated according protocol ALL BFM 1995 and 7 patients were treated according to protocol ALL BFM 2002. M/F ratio was 4/5. Patients had at diagnosis between 3 and 15 years (median age 9 years). All patients had thrombotic events after starting administration of L Asparaginase during induction. Most had clinical symptoms after the fourth dose of L Asparaginase. Clinical manifestations were accompanied by hypofibrinogenemia (<100 mg/dl) especially in patients who experienced bleeding. The patients who experienced thrombosis had decreased levels of antithrombin III, protein C and increased D dimer levels. The diagnosis of cerebral venous sinus thrombosis (CVST) is typically based on clinical suspicion and imaging confirmation. At 5 of these patients neuroimaging test (CT and MR imaging) documented CVST after developing neurological symptoms; one of the patients suffered major complication (extended brain injury) and died. All patients with ALL and thrombotic events received low-molecular weight heparin (LMWH) for 3 to 6 months. A follow-up CT or MRI at 3 to 6 months after diagnosis was made to assess for recanalization of the occluded cortical vein/sinuses. Survival in the patients with CVST was 84.61%. 1 patient with ALL and hemostasis alteration had intracerebral hemorrhage (ICH) with rapid progressive neurological deterioration to death. 1 patient had pulmonary embolism associated with clotting disorders and severe sepsis and he died. 2 patients had clinical manifestation (headache, confusion and seizures) and clotting disorders (decreased levels of antithrombin III, protein C, fibrinogen and increased D dimer levels), but with normal brain imaging. Survival in the cohort was 77,7%.
Summary/Conclusions: Thrombotic events have occured in all patients during induction. Clinical manifestation were depending on location, size and duration of thrombosis, from headaches, seizures or focal neurological deficits. Severe sepsis association was an additionally risk factor for thrombotic and bleeding events in patients with ALL. Screening for genetic prothrombotic defects diagnosis prior to initiating chemotherapy may represent a way to reduce thrombotic or bleeding events and appropriate management of hemostasis disorders that occur during the treatment.

PB1643

INCIDENCE AND SURVIVAL OF CHILDHOOD LEUKEMIA IN ARMENIA: A POPULATION-BASED ANALYSIS

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Background: Leukaemia is the most common cancer in children. Childhood leukemia incidence and survival varies globally, and this could be associated with environmental risk factors, genetics, and improvement in diagnosis and treatment. Armenia is considered to be a mono ethnic nation.
Aims: We aimed to quantify the incidence of and mortality from acute leukaemias among children population in Armenia and their variation with gender, age, year of diagnosis.
Methods: In this work we included children diagnosed with de novo acute leukaemia, 0–18 years of age from 2006 to 2016. The initial data for this survey have been derived from ambulance/dispensary cards, hospitalization journals, and clinical data from the Registry of Blood Diseases at the R.Yeolyan Hematology Center, Yerevan, Armenia. The data has been supplemented by the data from the Registry of Oncological Diseases of the V. Fanarjyan NCO, as well as from death certificates. The demographic data has been obtained from the National Statistics Board of Republic of Armenia. The obtained data has been statistically analyzed using EPI INFO-2002 program.
Results: A total of 277 cases of childhood acute leukemia were identified, 174 (62.8%) of whom were male. The overall incidence of leukemia was 3.4 per 100 000 children-years. The higher incidence rates were noted in 2007, 2012, 2010 (accordingly 4.0, 4.0 и 3.9), and the lower rates in 2011, 2014, 2009 (accordingly 2.4, 2.4 и 2.9). There are three registrated regions in Armenia-Lori, Vayots Dzor and Tavush with higher incidence rate (respectively 0.020, 0.012 and 0.010 per 100 000 children-year). Currently 83.8% of studied patients are alive. The 5-year survival rate was 72%, 100%, and 100% among children diagnosed at 3–7, 7–13, and 13–18 years of age, respectively. The results indicated that the children diagnosed between ages of 3 and above had the lowest risk of mortality and higher survival rates.
Summary/Conclusions: This is the first general population study to describe the incidence of and mortality from childhood acute leukaemias in Armenia during 2006-2016. It forms the basis for quality assessment of acute leukaemia treatment in Armenia and offers a unique opportunity for population-based research. Age at diagnosis remained to be a crucial determinant of the survival variability of pediatric ALL patients, after adjusting for sex, race, therapy, primary tumor sites, immunophenotype, and year of diagnosis. Further research is warranted to disentangle the effects of age-dependent biological and environmental processes on this association.

PB1644

LONG-TERM SURVIVAL OUTCOMES OF ADULT PHILADELPHIA CHROMOSOME-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS TREATED WITH IMATINIB OR DASATINIB

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Background: Acute lymphoblastic leukemia (ALL) with positive Philadelphia chromosome (Ph+) is a unique subset of ALL with poor prognosis. Recent studies have demonstrated improved survival outcomes in adult patients with Ph+ ALL with the use of tyrosine kinase inhibitors (TKIs) along with chemotherapy. However, there are very few studies that describe the comparative effectiveness of various TKIs in this patient population.
Aims: To characterize long-term survival outcomes including leukemia-free survival (LFS) and overall survival (OS) for Ph+ adult ALL patients treated with imatinib *versus* dasatinib.
Methods: Retrospective chart review was conducted at our institution. Patients >= 18 years old and diagnosed with Ph+ ALL between 2002 and 2015 were included. Analysis was done by intent to treat for patients initiated with imatinib or dasatinib at the time of Ph+ diagnosis. The primary endpoints were 2-year LFS and OS and secondary endpoints were complete molecular response (CMR; BCR-ABL1/ABL1 ratio <0.01% by PCR) and major molecular response (MMR; BCR-ABL1/ABL1 ratio <0.1% by PCR).
Results: Among 46 patients with Ph+ ALL, 74% (n=34) were in imatinib group and 17% (n=8) in dasatinib group; 9% were treated with other or no TKI (1 ponatinib and 3 with no TKI). Thirty-eight percent (n=13) of patients in imatinib group and 13% (n=1) in dasatinib group switched to a different TKI due to adverse effects or failure to achieve remission. There was a trend towards increased 2-year LFS for patients on dasatinib (HR 0.40, 95% CI: 0.14-1.14, p=0.09) and no difference in 2-year mortality (HR 1.00 95%CI: 0.46-2.17, p=0.99). Molecular response data was available for 61% (n=28) of patients; 75% of imatinib group achieved CMR or MMR (65% CMR) compared to 76% of dasatinib group (63% CMR) (p=0.98) (Figure 1).

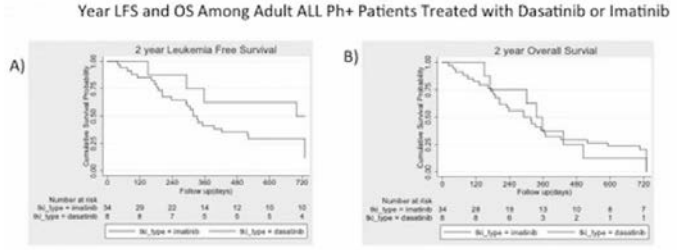

Figure 1.

Summary/Conclusions: In conclusion, dasatinib, compared to imatinib, in combination with chemotherapy, may prolong LFS in patients with Ph+ ALL and may be a suitable first-line agent. Large, randomized studies are needed to better define a detailed treatment protocol in this high-risk patient population.

PB1645

OUTCOME OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN A MIXED COHORT OF PEDIATRIC AND ADULT PATIENTS WITH KMT2A-AFF1 ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Acute lymphoblastic leukemia with poor-risk translocation t(4;11)(q21;q23)/*KMT2A-AFF1* occurs in all age groups with a clear dominance in children, especially up to 1 year. To date, allogeneic hematopoietic stem cell transplantation (allo-HSCT) is considered to be potentially curative treatment in high-risk acute leukemia patients, including the abovementioned.
Aims: To evaluate the prognostic impact of the different clinical and cytogenetic characteristics on the results of allogeneic hematopoietic stem cell transplantation in *KMT2A-AFF1* acute lymphoblastic leukemia patients.
Methods: Retrospective analysis of treatment results was performed for a mixed cohort of the patients with *KMT2A-AFF1* ALL who received allo-HSCT, including haploidentical one at our University over 2008 to 2015. Twenty-one patients (12 females and 9 males aged from 3 months to 48 years; median 18.9 years) were examined.

Results: Eight of 21 (38%) patients exhibited an isolated t(4;11) translocation. Additional chromosome abnormalities (ACA) were revealed in 11 (52%) patients, including 8 (42%) subjects with 3 and more chromosome aberrations. In univariate analysis, significance was shown for clinical stage at HSCT (1st remission *vs* other stages, 75% *vs* 0% p=<0,001 for OS; 58% *vs* 0%, p=<0,001 for EFS), complex chromosomal aberrations (<3 abnormalities *vs* ≥3 abnormalities, 58% *vs* 13%, p=0.04 for OS; 46% *vs* 0%, p=0.04 for EFS). According to multivariate analysis, the clinical stage at HSCT (HR 26.8, 95% CI 3.28-218.80; p=0.002 for OS; HR 11.18, 95% CI 2.92-42.80 p=0,0004 for EFS) was only independent prognostic factor for clinical outcomes.
Summary/Conclusions: The study has shown the stage of disease at the moment of allo-HSCT to be independent prognostic factor in a mixed cohort of *KMT2A-AFF1* ALL patients treated with HSCT. The good results of allo-HSCT can be obtained using a haploidentical transplantation from parents that removes the problem of searching the HLA-matched donors in the Registers and, therefore, greatly simplifies the treatment.

PB1646

DERMATOLOGIC COMPLICATIONS ASSOCIATED WITH TYROSINE KINASE INHIBITORS FOR THE TREATMENT OF ACUTE LEUKEMIA

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Background: Despite of targeted effects of tyrosine kinase inhibitors (TKIs), they are not absolutely selective in relation to their target. Hair pigmentation is regulated by factors including the interaction of the ligand stem cell factor (SCF) with its class III receptor tyrosine kinase, c-kit. Hair depigmentation observes during therapy TKI with action directed against class III receptor tyrosine kinase (PDGFRα, PDGFRβ, C-KIT, CSF1R, FLT3). But other TKI such as BCR/Abl TKI can also inhibit class III receptor tyrosine kinase by non-targeted actions. Skin reactions are the most common observed during the epidermal growth factor receptor-tyrosine kinase inhibitor treatment.
Aims: To describe the spectrum of skin and hair reactions in patients with acute leukemias (Ph+/Ph- acute lymphoblastic leukemia and acute myeloid leukemia) during the treatment by second-generation TKI (bosutinib), third-generation TKI (ponatinib) and multikinase inhibitor (sorafenib).
Methods: From 2016 to March 2017 6 patients (pts), age 24-53 (median 29,5), 1 male, 5 female, received second or third line therapy with target tyrosine kinase inhibitors in National Research center for Hematology. One pt (pt 1) with AML had been receiving chemotherapy (decitabine, cytarabine, idarubicin) with continuous treatment of sorafenib. Three pts with Ph+ ALL received TKIs. Two of them with T315I mutation (pts 2, 3) received ponatinib and one pt (pt 4), without molecular remission on dasatinib and nilotinib therapy, received second-generation TKI (bosutinib). One pt with B-ALL was treated by sorafenib due to refractory disease on the first-line therapy (pt 5). And one patient (pt 6) with T-cell ALL received sorafenib with nelarabine containing chemotherapy due to early relapse after allogeneic stem cell transplantation.

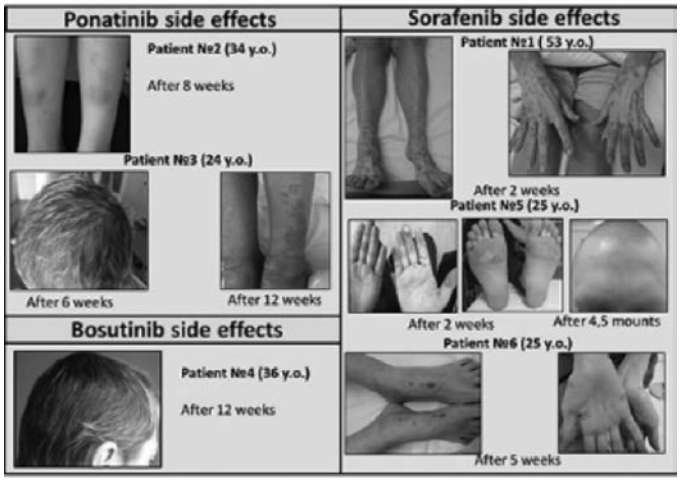

Figure 1.

Results: All of the 6 patients who had taken second-generation TKI (bosutinib), third-generation TKI (ponatinib) and multikinase inhibitor sorafenib developed dermatologic reactions (skin rash or grey hair). Generalized maculo-papular skin rash grade II evolved after two weeks of sorafenib treatment in pt1. Both patients on ponatinib therapy developed localized maculo-papular skin rash grade I in pt 2 after 8 weeks of therapy. In pt 3 after 6 weeks of ponatinib treatment gray hair observed. Skin rash with pigmentation grade I evolved in pt 3 after 12 weeks of therapy. Pt 4 had gray hair after 12 weeks second-generation TKI (bosutinib) treatment. Palmar-plantar erythrodysesthesia syndrome grade II and hair and total skin depigmentation were evolved after 2 weeks and after 4,5 months respectively observed during the sorafenib treatment in pt 5 (with psoriasis anamnesis). Pt 6 developed localized maculo-papular skin rash grade I after 5 weeks of sorafenib treatment. Despite of all patients developed dermatological side effects, temporarily discontinuation of TKI therapy was required in only three (50%) cases. In the other cases the treatment was continued. The therapy was restarted in all pts with temporarily discontinuation after skin lesions disappearing (Figure 1).
Summary/Conclusions: Dermatological adverse events in acute leukemia pts who have taken second-generation TKI (bosutinib), third-generation TKI (ponatinib) and multikinase inhibitor sorafenib they were not serious. Temporarily dose reduction or interrupting of TKI therapy led to complete regression of skin lesions. Restarting TKI at full dose did not lead to dermatological adverse reactions reappearing. Moreover, the temporary cancellation did not reduce its effectiveness.

PB1647

CYTOKINE RELEASE SYNDROME AFTER THE FIRST INTRATHECAL CHEMOTHERAPY IN A PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA WITH AN EARLY MENINGEAL RELAPSE

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Background: Central nervous system (CNS) is a frequent site of recurrence in childhood acute lymphoblastic leukemia (ALL), and Triple Intrathecal Therapy (TIT) with Methotrexate (MTX), Cytarabine (ARA-C) and hydrocortisone, at doses for age, is the mainstay of the treatment of CNS relapse. Severe neurotoxicity is well known TIT complication, usually related to repeated infusions and neurotoxic concomitant systemic drugs.
Aims: We describe a case of a massive acute leukoencephalopathy after only one TIT, in a 5 year-old child with an early isolated CNS relapse of ALL (26 months after the first diagnosis), rapidly proceeding to comatose status.
Methods: At admission for disease restaging at the end of first-line trial, the child showed physical and neurological examination completely negative, such as haematological, bioumoral and ultrasound findings. The cerebrospinal fluid (CSF) appeared turbid and liquoral pressure increased. CSF count showed 8100 cells/µl; morphology and flow citometry confirmed an early isolated CNS relapse. Due to the abnormal pleocytosis, TIT administration associated with oral dexamethasone was suddenly performed without any other concomitant chemotherapy. To prevent acute toxicities from tumor lysis syndrome, the patient received hydratation, allopurinol, acetazolamide and prophylaxis of seizures with levetiracetam. After few hours from TIT, the child developed severe headache followed by skin urticarial rash, high blood pressure and hallucinations, rapidly evolving in flaccid paralysis of lower extremities. A brain resonance (MRI) showed diffuse areas of hyperintensity of white matter, particularly cortical and subcortical areas, cerebellar region, optic chiasm and brainstem in T2-Flair sequences; spinal cord shower massive edema, especially in lombar region. The MRI pattern was interpreted as diffuse grade IV leukoencephalopathy of probable toxic nature. The child, 30 h after TIT, was transferred to intensive care unit for progressive ascending paralysis and respiratory distress that required intubation. During the following days, other three diagnostic lumbar puncture were performed that showed significant reduction of blasts cells (20, 10 and 0 cells /µL, respectively).
Results: Patient persisted in deep coma for 5 days, until he restart a spontaneous breathing. After waking up, the child showed rapid neurological ameliorations, such as reappearance of reflexes, spontaneous movements of eyes, hand and feet fingers. The subsequent MRI highlighted improvement of hyperintensity at midbrain, brainstem and bridge brain areas and spinal cord with persistence of altered signals in subcortical white matter. The visual evoked potentials were normal and the motor and sensory conduction velocity appeared slowed without axonal damage; EEG showed slow waves spread. At the moment, after three week from severe neurological complication, the child is fully awake, moving all four limbs, but requires motor and phoniatric rehabilitation. Systemic chemotherapy with high-dose MTX and IT ARA-C is restarted without any additional neurotoxicity. Dosage of CSF levels of interleukin 6 and its soluble receptor is ongoing.
Summary/Conclusions: Although leukoencephalopathy following IT MTX or ARA-C administration are described, the severity and rapidity of event's onset, associated with CSF remission after a single TIT administration, suggests us that neurotoxicity could be related to massive blast cytolysis with subsequent cytokine release syndrome causing an inflammatory leukoencephalopathy. This syndrome is a frequent complication of blinatumomab or chimeric antigen receptor T-cells administrations. The CSF IL-6 dosing could clarify the pathogenesis of the event.

PB1648

SEVERE HYPOFIBRINOGENEMIA ASSOCIATED WITH IMATINIB AND PREDNISONE THERAPY IN PHILADELPHIA CHROMOSOME–POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Hypofibrinogenemia associated to acute lymphoblastic leukemia (ALL) is rare and usually due to L-asparaginase. Consumption coagulopathy or therapy-related hepatotoxicity are other possible explanations. Severe hypofibrinogenemia, not linked to the causes listed, was rarely reported and a role of steroid therapy on fibrinogen metabolism was suggested.
Aims: Our aim was to identify the incidence of severe hypofibrinogenemia during induction phase in a cohort of consecutive ALL patients and to assess its impact on clinical decision-making.
Methods: In order to avoid confounding factor due to L-asparaginase, we revised our cohort of Philadelphia chromosome–positive (Ph^+) ALL that we treated according to pediatric-type therapy program (imatinib, intensive chemotherapy without L-asparaginase) for patients aged 18-65 years and through LAL0201-B protocol (imatinib, prednisone) for patients ≥65 years. We retrospectively analyzed coagulation tests on admission and during induction therapy of all Ph^+ALL patients diagnosed at our Institution from 2004.
Results: Twenty-one Ph^+ALL were identified; 17 patients were younger than 65 years, while the remaining 4 patients had a median age of 74 years (66-76). No alteration of plasma fibrinogen during induction was observed in younger patients. Severe hypofibrinogenemia (≤100 mg/dl) was detected in 3 out of 4 Ph^+ALL over 65 years. In these patients induction consisted of prednisone 40 mg/m^2/dfrom day 1 to 45 and imatinib at the fixed dose of 800 mg/d. On admission hemoglobin levels were ≥10 g/dl in all patients, leucocytes counts were 2x10^9/L (blasts 15%), 8x10^9/L (blasts 30%) and 18x10^9/L (blasts 61%), while platelet count was reduced in 2 cases (61x10^9/L and 65x10^9/L). Coagulation tests were normal (fibrinogen median level 380 mg/dl). Severe hypofibrinogenemia developed between 6 and 15 days after beginning treatment and lasted between 4 and 48 days. Fibrinogen nadir ranged from 47 to 100 mg/dL (median 61 mg/dL); reduced plasma fibrinogen levels at functional tests were also confirmed to immunological assays. During fibrinogen nadir, D-dimer was positive in all patients, but stable compared to the outset. Antithrombin, coagulation factors, activated partial thromboplastin and prothrombin time, common liver function tests remained in a normal range; platelet counts showed a trend to normalization. Early clearance of peripheral blood blasts was observed and when hypofibrinogenemia appeared no blast cells were detectable. At the end of induction bone-marrow evaluation demonstrated the absence of BCR-ABL transcript by qualitative RT-PCR. There were no bleeding events and only one patient received a prophylactic transfusion of fresh-frozen plasma (10 ml/kg) for fibrinogen <50 mg/dl on two occasions. Normal fibrinogen levels (≥165 mg/dl) were recovered at the end of steroid therapy.
Summary/Conclusions: We observed severe hypofibrinogenemia in Ph^+ALL patients older than 65 years treated with imatinib and high-doses steroid, while normal fibrinogen levels were detected in younger Ph^+ALL during intensive chemotherapy plus imatinib. In our experience, hypofibrinogenemia was not associated to major bleeding events, although its clinical significance should be investigated in larger series. Fibrinogen may recognize multiple metabolic pathways, also unrelated to *in vivo* coagulation and fibrinolysis; the correspondence between steroid treatment and hypofibrinogenemia seems to suggest that glucocorticoids may alter some steps in fibrinogen kinetics and could be considered as a cause of acquired hypofibrinogenemia.

PB1649

LATE EFFECTS OF CHEMORADIOTHERAPY ON THE ENDOCRINE SYSTEM IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Over the past four decades treatment of childhood acute lymphoblastic leukemia has been modified with the aim of achieving high survival rate while reducing the risk of the life threatening late-effects and promoting risk-based follow-up care of survivors.
Aims: The aim of our study is evaluation of late effects of chemotherapy and cranial radiotherapy on the endocrine system in children with acute lymphoblastic leukemia.
Methods: Fourty-eight patients, who were diagnosed and treated for ALL between 1997-2007 in Istanbul Kanuni Sultan Suleyman Education and Research Hospital Pediatric Hematology-Oncology Clinic and have disease-freee for at least 5 years after cessation of treatment, were evaluated prospectively. The study form included each patients age, gender, weight, height, target height, parental height, treatment protocol, stage of puberty, bone age, TSH, free T4, LH, FSH, estradiol or testosterone, IGF-1 and IGFBP-3 levels. Annual rate of growth was evaluated for each patient. The patients with inadequate growth rate and delayed bone age were subjected to growth hormon stimulation test with clonidine.
Results: Mean age of the patients was 14.41±2.85 (10.5-22.4) years. Thirty-one of patients had prophylactic cranial radiotherapy; five of them 18 Gy and twenty-six had 12 Gy CRT. Fifteen of the 48 patients were diagnosed with at least one endocrinological disorder. Six patients had lower height (<-2 SD), three patients had a body mass index >30kg/m². Bone age delayed in two patients. Four patients had IGF-1 value below <-2SD and two patients had inadequate levels of growth hormone. Tanner stage of the patients were appropiate for their ages except for one patient with hypergonadotropic hipogonadism and one patient with pubertas precox. Subclinical hypotiroidism was detected in two patients.
Summary/Conclusions: Significant late effects may develop over time in children treated for ALL. For this reason long-term follow-up of these children is necessary. Because of the awareness of the late effects can the treatment modifed to reducing of the late effects.

Acute myeloid leukemia - Biology

PB1650

MUTATIONAL ANALYSIS OF 231 DE NOVO AML PATIENTS BELOW 60 YEARS WITH CURATIVE THERAPY

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Background: Acute myeloid leukemia (AML) is an aggressive cancer disease of the myeloid lineage of blood cells, characterized by rapid growth of undifferentiated myeloid precursors. Analysis of the spectrum of somatic mutations in leukemic cells may help to improve the identification of individual prognostic subgroups of patients as well as to observe clonal evolution in the course of AML treatment.
Aims: The aim of the project is to identify somatic alterations in genes related to AML using next generation sequencing (NGS) in large cohort of AML patients from Czech Republic and to determine their frequency and mutual coexistence.
Methods: The analyzed group consists of 231 *de novo* consecutively diagnosed AML patients with curative therapy below 60 years from five hematological centers. The NGS libraries are prepared from peripheral blood samples from diagnosis using ClearSeq AML panel (Agilent Technologies) and sequenced on MiSeq and NextSeq machines (Illumina). As positive are determined mutations with variant allele frequency (VAF) at least 2%.
Results: At least one somatic mutation (median 2; range 0-6) was identified in 204 (88.3%) patients with *de novo* AML. In total, 526 recurrent mutations in 19 genes were identified. The most frequently mutated genes were: *FLT3* 91/231 (39.4%; from this *FLT3*-ITD 69/231 [29.9%] and *FLT3*-TKD 22/231 [9.5%]), *NPM1* 90/231 (39.0%; mutation type A 71/90 [78.9%], type B 11/90 [11.1%], other types 10/90 [10.0%]), *DNMT3A* 68/231 (29.4%; mutations in codon R882 49/68 [72.1%]), *NRAS* 51/231 (22.0%; the most frequent mutation G12D 17/51 [22.0%]; 11/51 patients [21.6%] contain more than one mutation in *NRAS* gene), *IDH2* 35/231 (15.2%) and *CEBPA* 35/231 (15.2%). The analysis also identified mutations in rarely mutated genes *U2AF1*, *SF3B1*, *EZH2* and *SETBP1* in 4/231 (1.7%), 4/231 (1.7%), 1/231 (0.4%) and 1/231 (0.4%) samples, respectively (Figure 1).

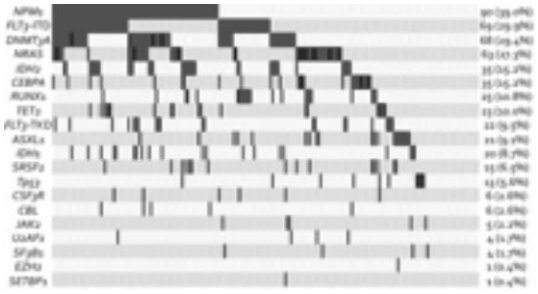

Figure 1. Distribution of gene mutations with VAF ≥2% in AML cohort. Each column represents one patient (n=231). Each row represents one gene described on left, on the right is shown the number of patients with mutation in the gene and its percentage from the total cohort. The color of the squares represents the status of the gene: red – single mutated, blue – double mutated, black – triple mutated, white/grey – no mutation.

Summary/Conclusions: The results of mutational analysis of large cohort of AML patients show high heterogeneity of detected mutations. Surprisingly we have detected high percentage of patients with mutations in gene *NRAS*. Together with sequencing results from the time of remission/relapse/resistance of the disease, the data will enable to get more complex view on the development of AML in time.
Supported by Ministry of Health of the Czech Republic, grant nr. 15-25809A, and by project MUNI/A/1106/2016.

PB1651

INHIBITION OF LIN28B IMPAIRS LEUKEMIA CELL GROWTH AND METABOLISM IN ACUTE MYELOID LEUKEMIA

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Background: 1. Current conventional chemotherapy for acute myeloid leukemia (AML) can achieve remission in over 70% of patients, but a majority of them will relapse within 5 years despite continued treatment. 2. The relapse is postulated to be due to leukemia stem cells (LSCs), which is different from normal hematopoietic stem cells (HSCs). LIN28B is microRNA regulator and stem cell reprogramming factor. 3. Overexpression of LIN28B has been associated with advance human malignancies and cancer stem cells (CSCs), including AML. However, the molecular mechanism by which LIN28B contributes to the development of AML remains largely elusive.
Aims: 1. To study the function role of LIN28B in cell proliferation, cell cycle and colony formation ability of AML cells. 2. To systematically dissect transcriptional signalling mediated by LIN28B on whole genome level. 3. To determine the key targets of LIN28B in AML. 4. To explore the function of LIN28B in AML *in vivo*.
Methods: 1. We modulated LIN28B expression in AML and non-leukemic cells and investigated functional consequences in cell proliferation, cell cycle and colony forming assays. 2. We performed a microarray-based analysis for LIN28B-silencing cells and interrogated gene expression data with different bioinformatic tools. 3. AML mouse xenograft model was used to examine the *in vivo* function of LIN28B.
Results: We first showed that increased LIN28B expression was associated with worse survival in AML patients. We demonstrated that targeting LIN28B in AML cells resulted in cell cycle arrest, inhibition of cell proliferation and colony formation, which was induced by de-repression of let-7a miRNA. On the other hand, overexpression of LIN28B promoted cell proliferation. Mechanistic studies revealed that inhibition of LIN28B induces metabolic changes in AML cells. IGF2BP1 was confirmed to be a novel downstream target of LIN28B via let-7 miRNA in AML. Notably, silencing LIN28B led to slow tumor growth *in vivo*.
Summary/Conclusions: In conclusion, these results uncover a novel mechanism of an important regulatory signaling, LIN28B/let-7/IGF2BP1, in leukemogenesis and provide a rationale to target this pathway as effective therapeutic strategy.

PB1652

Abstract withdrawn.

PB1653

EVALUATION OF MINIMAL RESIDUAL DISEASE IN NPM1-MUTATED AML PATIENTS

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Background: Minimal residual disease (MRD) tests provide early identification of hematologic relapse and timely management of AML patients. About 60% of adult normal karyotype AML has a mutation in exon 12 of *NPM1* gene. This mutation is specific for malignant clone and potentially is a good marker of MRD.
Aims: The aim of the study was to analyze the usefulness of *NPM1* as a marker for MRD quantification in AML during follow-up.
Methods: Retrospective study included 34 patients with mutated-NPM1 and treated with intensive chemotherapy (2009-2015). Bone marrow (188) and peripheral blood (277) samples were analyzed from complete remission (MRD *NPM1* negative). *NPM1* detection was performed by quantitative RT-PCR (*Gorello et al, Leukemia 2006*). Patients were considered positive when presented >1 *NPM1* sample positive or/and one sample NPM1 >0.02%. Cox regression was used for univariate analysis.

Variable	HR (CI 95%)	p
Gender (male/female)	0.9 (0.9-2.9)	0.8
Age (> 55.3)	**5.8 (1.3-27)**	**0.024***
Leukocyte count (>30x103/ul)	**6.7 (1.4-31.2)**	**0.015***
LDH (>499 U/ml)	**7.4 (1.5-35.6)**	**0.013***
FLT3-ITD	1.7 (0.5-5.7)	0.4
IDH1	0.75 (0.1-5.8)	0.8
IDH2	0.04 (0.02-6)	0.3
DNMT3A	0.7 (0.2-2.5)	0.6
Post-induction (PR)	1.2 (0.9-1.4)	0.1
Post-induction (MFC > 0.1)	0.6 (0.1-3.1)	0.6
Post-consolidation (PR)	-	
Post-consolidation (MFC > 0.1)	1.3 (0.3-6.1)	0.7
MRD NPM1 positive	**8.9 (1.4-32)***	**0.029***

Univariate analysis

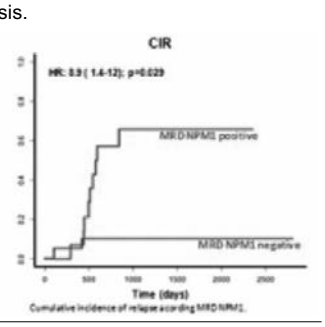

Figure 1.

Results: Patients were segregated in 2 groups: Relapse patients (Group 1: 32.2%, 11/ 34) and no relapse patients (Group 2: 67.6%, 23/34). Group 1 presented MRD *NPM1* positive in 9/11 (82%) of patients, the time from *NPM1* to relapse was 4.6 months (1.6-24), *NPM1* mean was 1.7 (0.03-9). Group 2 presented MRD *NPM1* negative (<0.02% y/ or 1 determination) in 21/23 (91%) patients. Univariate analysis was performed and our results show that age, leukocyte, LDH and MRD *NPM1* are prognostic factors for cumulative incidence of relapse (Figure 1).

Summary/Conclusions: *NPM1* is a useful marker for MRD quantification in AML patients undergoing intensive therapy. *NPM1* positive during follow-up is associated with a higher probability of relapse.

PB1654

AT101 ELIMINATES AML STEM CELLS VIA ACTIVATION OF INTRINSI C APOPTOTIC PATHWAY AND PARTICIPATION IN DNA DAMAGE RESPONSE

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Background: Leukemia stem cells (LSCs) are considered as the main reason for treatment failure and relapse in acute myeloid leukemia. Overexpression of Bcl-2 anti-apoptotic proteins is associated with the survival and self-renewal of LSCs
Aims: To observe the effect for AT101 to eliminate AML stem cells and its underlying mechanism.
Methods: Use $CD34^+CD38^-CD123^+KG1\alpha$ and primary AML $CD34^+$ cells as research object.
Results: In this study, we demonstrated that AT101, a BH3 mimetic pan-Bcl-2 inhibitor, was significantly and effectively cytotoxic towards $CD34^+CD38^-CD123^+KG1\alpha$ and primary AML $CD34^+$ cells, with slight effect on $CD34^+$ normal hematopoietic cells. And the mechanism was closely associated with activation of intrinsic apoptotic pathway, such as loss of mitochondrial membrane potential and caspase activation, along with disturbance of DNA damage response. Further analysis on AML patients' clinical characteristics revealed that the *ex vivo* efficacy of AT101 in primary samples was significantly correlated to hyperleukocytosis or *FLT3-ITD* mutation. Besides, AT101 exhibited exciting effect on $CD34^+$ blasts from patients who are old or cannot achieve CR after induction therapy.
Summary/Conclusions: In conclusion, Together, these findings provides potentiality for the use of AT101 to treat relapse and refractory AML as alternative salvage regime in the future, including those clinically characterized by one or more adverse prognostic abnormalities.

PB1655

COOPERATIVE EFFECT OF CHIDAMIDE AND CHEMOTHERAPEUTIC DRUGS INDUCE APOPTOSIS BY DNA DAMAGE ACCUMULATION AND REPAIR DEFECTS IN ACUTE MYELOID LEUKEMIA STEM AND PROGENITOR CELLS

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Background: Lots of conventional chemotherapeutic drugs are confirmed to take participate in DNA damage generation and initiation of DNA damage response, ultimately leading to apoptosis. However, they fail to completely eliminate leukemia stem cells (LSCs) on account of higher DNA repair capacity of cancer stem cells than bulk cancer cells, which become the root of resistance and recurrence. Thus, new strategy to eliminate LSCs in AML is urgently needed.
Aims: To observe the effect of low dose chidamide in combination with chemotherapeutic agents on eliminating AML stem cells.
Methods: we uesed a novel benzamide-type HDAC inhibitors, chidamide,in combination with DNA-damaging agents (daunorubicin, idarubicin and cytarabine) to treat CD34+CD38- KG1α cells and primary refractory or relapsed AML CD34+ cells.
Results: Here, we report that low dose chidamide, a novel benzamide-type HDAC inhibitors, which selectively targeted HDAC 1, 2, 3, 10, could enhances cytotoxicity of DNA-damaging agents (daunorubicin, idarubicin and cytarabine) in CD34+CD38- KG1α cells and primary refractory or relapsed AML CD34+ cells, reflected by inhibition of cell proliferation and induction of apoptosis *in vitro*. Mechanistically, these events were associated with DNA damage accumulation and repair defects. Co-treatment with chidamide and DNA-damaging agents IDA gave rise to production of γH2A.X, inhibited ATM, BRCA1, checkpoint kinase 1 (Chk1) and 2 (Chk2) phosphorylation. Finally, the combination initiated caspase-3 and PARP cleavage and ultimately induced $CD34^+CD38^-$ KG1α cells apoptosis. Further analysis on AML patients' clinical characteristics revealed that the *ex vivo* efficacy of chidamide in combination with IDA in primary CD34+ samples was significantly correlated to peripheral blood WBC counts at diagnosis, while status, LDH level, karyotype had no effect, indicating that the combination regimen of chidamide and IDA could rapidly diminish tumor burden in a patient with R/R AML.
Summary/Conclusions: these findings provide preclinical evidence for low dose chidamide in combination with chemotherapeutic agents to treat recurrent/resistant AML as an alternative salvage regimen, especially those possessed stem and progenitor cells.

PB1656

Abstract withdrawn.

PB1657

NEW CANDIDATE GENES USEFUL TO PREDICT THE RISK OF RELAPSE IN ACUTE PROMYELOCYTIC LEUKEMIA

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Background: Nowadays, Acute Promyelocytic Leukemia (APL) is a disease entity with a very high rate of cure and an estimated 2-year overall survival of 97%. Early death, rather than resistant disease so common in all other subtypes of AML, has emerged as the major cause of treatment failure, and relapse is a very rare occurrence.
Aims: APL relapse is a very rare entity, and it is announced to become rarer with the advances in first line therapy. Molecular characteristics are hard to analyze without an effort to collect and bank samples together from multiple institutions. Since relapses, especially relapses out of follow-up period, represent a sudden life-treating condition for patients, to predict patients at higher risk of relapse we selected two candidate genes that could be involved in pathways favoring relapse.
Methods: We collected data of all the APL referred to our institution from 2014. Within 23 patients, we encountered 20 new diagnosis and 2 relapse of APL. We analyzed blasts in samples obtained from Bone Marrow with Single Nucleotide Polymorphisms Array Cytoscan HD.
Results: We compared copy number alterations in both relapsed patients with alterations detected in the pool of 20 newly diagnosed APL and we found specific signatures of CN*Vs* for each patient. There were several copy number alterations related to each patient: the first patient presented gain of *ROBO2*, *GRIP1*, *CTNNB1*, *SOX6*, *PBX1*, *GRIK2*, *CDKAL1* and loss *FAF1*, *CREBBP*, *SBF1*; the second patient presented gain of *ROBO1*, *MAPK10*, *CADPS2*, *APBA1* and loss of *GRIP1* and *MYB*. Subsequently we focused our attention on *ROBO* and *GRIP1*genes because they were alterated in both relapsed patients: ROBO proteins are associated to K channels while GRIP1 is involved in various critical functions, for example in androgen receptor binding, beta-catenin binding, glucocorticoid receptor binding, and it is also a regulator of glutamate metabolism, a well-known pathway in Leukemic Stem Cells.
Summary/Conclusions: By the analysis of ROBO 1-2 and GRIP1 at the diagnosis of APL we could establish a different and strict follow-up program for patients with these alterations.
Acknowledgement: ELN, AIL, AIRC, prog. Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL project, HARMONY.

PB1658

THE EXPRESSION OF SALL4 AND BMI-1 GENES IN MYELOID LEUKEMIA

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Background: Sal-like protein 4 (SALL4) and B-cell specific moloney murine leukemia virus integration site-1 (BMI-1) genes are stem cell genes that modulate stem cell pluripotency and may play a role in leukemogenesis. Leukemic stem cells (LSCs) have been implicated in being the origin of the leukemic blast, therapy resistance and relapse.
Aims: The current study aimed at characterizing the expression pattern of SALL4 and BMI-1 genes in acute myeloid leukemia (AML) and chronic myeloid leukemia (CML), in patients who have achieved complete remission (CR), and in CML disease progression.
Methods: Real-time polymerase chain reaction was used to assess the gene expression patterns in 106 myeloid leukemia patients; 54 de novo AML (43 at time of diagnosis, 11 in CR), and 52 CML (31 in chronic phase (CP), 11 in deep molecular response (MR^4) & 10 in accelerated/blastic phase (AP/BP), and in 21 non malignant bone marrow samples.
Results: SALL4 gene expression was increased in AML patients, AML-CR, & CML-CP (median= 5.180, 4.604 & 14.125 respectively). No significant difference was observed between de novo AML and AML in CR patients. CML-CP patients showed a significantly higher percentage of patients with a high SALL4 expression as compared to both CML- MR^4 and CML-AP/BP (p=0.033). BMI-1 gene expression was not found to be increased in any of the patient groups.
Summary/Conclusions: Our data describe altered SALL4 gene expression in different phases of myeloid leukemia. The role of BMI-1 gene needs further delineation to determine its significance.

PB1659

AN INVESTIGATION INTO THE ROLE OF S100A8 AND S100A9 IN ACUTE MYELOID LEUKAEMIA

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Background: Acute myeloid leukemia (AML) is the a haematological malignancy characterised by the over proliferation and block in differentiation of clonal stem cells. In leukaemia potential biomarkers such as S100A8 could assesses the progression and remission of AML.
Aims: S100A8 and S100A9 (Ca2+ binding helix E-loop-helix-F hand), are inflammatory markers which are also suggested to promote chemoresistance by stimulation of autophagy. Microarray data from the Chevassut lab shows that both S100A8 and S100A9 transcripts are downregulated by the BET-bromodomain inhibitor JQ1 in AML cell lines. We aimed to investigate this response in AML patient bone marrow samples and cell lines.
Methods: We used AML cell line including OCI-AML2, OCI-AML3 and THP-1 in addition to AML patient bone marrow samples and healthy volunteer samples. We carried out RT-qPCR and immunocytochemistry and western blotting techniques to look at levels of S100A8 and S100A9 in samples.
Results: Here we show that levels of S100A8 and S100A9 mRNA levels are suppressed in response to JQ1 in the AML cells lines OCI-AML2, OCI-AML3 and THP-1. We find also that protein levels of S100A8 and S100A9 are downregulated in response to JQ1 in OCI-AML3. In bone marrow samples of 17 AML patients with different cytogenetic profiles, the relative expression of S100A8 and S100A9 was found to be variable amongst the samples but also in comparison to OCI-AML3 cell line. In further experiments using AML patient bone marrow samples, treatment with JQ1 showed suppression of S100A8 and S100A9 in some patient samples but enhanced expression in other bone marrows. In peripheral blood samples of healthy volunteers, we found that treatment with JQ1 showed notable suppression of both S100A8 and S100A9; with a greater suppression being observed in the monocyte fraction of the samples.
Summary/Conclusions: Our data suggests that JQ1 regulates the expression of S100A8 and S100A9 in AML. The variability of the response seen amongst AML patient samples and AML cell lines may be reflective of the genetic profiles driving the leukaemogenic process in these samples Further work may give more detailed insight into the mechanisms of action and potential use of S100A8 and S100A9 in AML prognostic markers.

PB1660

SUCCESSFUL COVERAGE OF DIFFICULT TO SEQUENCE GENES (CALR, CEBPA, AND FLT3) ASSOCIATED WITH MYELOID DISORDERS USING A HYBRIDISATION-BASED ENRICHMENT APPROACH PRIOR TO NEXT-GENERATION SEQUENCING

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Background: The application of short read NGS for research into myeloid disorders such as myeloproliferative neoplasms (MPNs) and acute myeloid leukaemia (AML) has been hampered by the inability to sequence certain genes. These genes can harbour key mutations so it is desirable to ensure suitable sequencing coverage is obtained. These genes amongst others include: *CALR* exon 9 insertions and deletions (up to 52 bp), *CEBPA* single nucleotide variants (SNVs) and *FLT3* Internal Tandem Duplications (ITDs) and SN*v*s Each of these regions contain certain challenging DNA sequences that can impact the quality of the data generated, *e.g.* large indels and low complexity regions (*CALR*), high GC content (75% on average for the whole gene with specific regions at 100%) and repetitive regions (*CEBPA*), and complex repetitive elements (*FLT3*).
Aims: To determine whether a hybridisation-based enrichment approach overcomes the difficulties associated with these genes, and permits the generation of high quality (sufficient de-duplicated depth) data to allow these targets to be accurately interrogated.
Methods: We utilised a hybridisation-based enrichment approach for library preparation in combination with a SureSeq myPanel™ NGS Custom AML panel. The library was then sequenced using a 2x150 bp read length protocol on an Illumina MiSeq®.
Results: Here we present the coverage and variants generated from numerous research samples for each of these difficult to sequence genes. The results clearly show that this approach can reliably detect and accurately size (including low allele frequency) insertions and deletions of up 52 bp in *CALR* (exon 9), SN*V*s and deletions in *CEBPA* with a de-duplicated depth in excess of 2000x as well as ITDs of between 24 and 201 bp in *FLT3*.
Summary/Conclusions: This approach is suitable for the analysis by NGS of these difficult genes and therefore removes the requirements for supplementary approaches to analyse these difficult genes, such as Sanger sequencing (*CEBPA*) and fragment analysis (*CALR* and *FLT3*).

PB1661

ASSOCIATION OF MIRNA EXPRESSION PROFILES WITH FUNCTIONAL AND MOLECULAR ACUTE MYELOID LEUKEMIA CATEGORIES

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Background: Development of high-throughput technologies such as Next Generation Sequencing (NGS) allowed the identification of recurrent mutated genes in Acute Myeloid Leukemia (AML), and new molecular markers which help refine patients' classification in different risk groups.
Epigenetic alterations such as aberrantly expressed microRNAs (miRNAs) also play an important role on the pathogenesis of AML. miRNAs control processes such as cell development, differentiation, proliferation and apoptosis. Therefore, aberrant miRNA expression can affect signaling and metabolic pathways, directing cancer cell biological behavior.
Recently, several studies have classified AML according to different criteria. TCGA proposed in 2013 a new classification where genes are grouped according to their biological function. Moreover, Papaemmanuil *et al*. suggested in 2016 a new classification based on molecular markers with not overlapping categories.
Aims: Our aim is to explore the miRNA profile of NK-AML and to find expression profiles associated with the categories proposed by TCGA and Papaemmanuil *et al*. Associations of miRNA expression profiles with altered categories could help understand the molecular mechanisms that lead to leukemogenesis.
Methods: CD34+ cord blood progenitor cells from 5 healthy donors and 7 CD34+ NK-AML samples with >70% blasts were obtained. Total RNA from these samples were hybridized onto an Array miRNA 3.0 chip (*Affymetrix*) in order to identify deregulated miRNAs. The most deregulated miRNAs were validated by qRT-PCR (*miScript*) in an independent cohort of 73 patients. Mutational analysis was performed by Next Generation Sequencing using the AML Community Panel with the Ion Torrent System (*Life Technologies*). Mann-Whitney U test was used to determine which miRNAs were differentially expressed among categories.
Results: We found a profile of 6 miRNAs up-regulated and 61 miRNAs down-regulated in NK-AML *vs* CD34+ cells. Validation by qRT-PCR confirmed that miR-494 (p=0.028) and miR-4507 (p=0.035) were up-regulated and miR-27b (p=0.022), miR-99a (p=0.001), miR-146b (p=0.031), miR-151b (p=0.006) and miR-20b (p=0.001) were down-regulated in NK-AML. Interestingly, some of the deregulated miRNAs were significantly associated to a functional category according to the TCGA classification. Therefore miR-146b was down-regulated in AML with mutations in myeloid transcription factors (p=0.025). Low expression of this miRNA causes the activation of the kB factor signaling pathway, which increases transcription. miR-4668 was down-regulated in AML with mutations in activation pathways genes (p=0.004), several target predictors propose *RASGEF1A* and *BRAF* as targets of this miRNA. Thus, under-expression of this miRNA could cooperate with mutations leading to the activation of signaling pathways. Regarding to Papaemmanuil´s molecular classification, miR-494 was up-regulated in IDH2-R172 category (p=0.009). High levels of this miRNA are associated with lower expression of *TET*, specially *TET1*. Therefore, high levels of miR-494 could contribute to the hypermethylation signature of IDH mutations.
Summary/Conclusions: In conclusion, the mutational landscape of significant functional and molecular groups in AML is accompanied by miRNA deregulation, which could cooperate in the development of this hematologic malignancy.

PB1662

PROTEOMIC APPROACH TO IDENTIFY MOLECULAR TARGETS OF HALOFUGINONE IN ACUTE MYELOID LEUKEMIA

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Background: Halofuginone (HF) is a halogenated derivative of Febrifugine, which is a molecule isolated from the plant Dichroa febrifuga. It has been demonstrated that Halofuginone exhibits anti-fibrotic, anti-cancerogenic, anti-inflammatory and pro-apoptotic effects. Previously, we have reported that treatment with HF has anti-leukemic properties in-vitro and in-vivo in acute promyelocytic leukemia (APL), reducing tumor growth through the induction of apoptosis and by stimulating the synthesis of the TGF-β protein and activating its downstream targets. In addition, HF presented anti-angiogenic effects by modulating the level of pro and anti-angiogenic factors including VEGF. However, it is unknown whether HF has anti-leukemic activity against other subtypes of acute myeloid leukemia (AML) and HF targets were not determined yet.
Aims: Evaluate the anti-leukemic effect of HF on other AML subtypes than APL and investigate its targets using a proteomic approach.

Methods: AML cell lines Kasumi-1, THP-1, MV4-11, U937 and OCI-AML3 were treated in- vitro with HF at concentrations ranging from 25 to 1000 ng/ml. The% of apoptotic cells, the distribution of cells in different cell cycle phases, and the HF IC_{50} was determined for each cell line. We used the Proteome Profiler ™ Array – HumanPhospho-Kinase Array to verify the possible tyrosine kinases and signaling pathways that could be modulated by HF. To analyze the in-vivo effect of HF, we transplanted the cell lines Kasumi-1 and THP-1 into NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (NSG) mice, which were then treated by intraperitoneal injections of HF at a dosage of 150 mg/Kg daily for 14 days. The leukemic infiltration of the peripheral blood was quantified by flow cytometry every 2 weeks (using a anti-human CD45+).
Results: HF IC50 values ranged from 125.58 ng/ml in Kasumi-1 to 786.15 ng/ml in THP-1 cells. Kasumi-1 cells halted in the S phase of the cell cycle when treated with HF, displaying a significant decrease in proliferation, while no effect was observed for THP-1 cells. Corroborating our in-vitro observation indicating resistant of THP-1 cells towards HF, we did not detect significant differences in the overall survival (OS) of NSG mice transplanted with THP-1 cells treated with vehicle or HF (mean OS of 70,5 and 68 days, respectively; p= 0,24). In contrast, the mean OS for NSG mice transplanted with Kasumi-1 cells treated with HF was significantly prolonged compared to the control group (144 *versus* 94.5 days; p= 0.007). The proteomic analysis identified significant decrease upon treatment with HF of four phosphorylated-proteins in both cell lines: Phospholipase C gamma 1 (PLCγ1), Proline-rich tyrosine kinase 2 (PYK2), Endothelial nitric oxied syntase (eNos) and Signal transducer and activator of transcription 3 (STAT3 Y705), thus suggesting that these proteins are primary targets of HF. In addition, the protein target of rapamycin (TOR) was down-regulated only in THP-1, while the levels of STAT3 S727 and STAT5a/b were significantly decreased by HF treatment only in Kasumi-1 cells. This comparative analysis suggests that the sensitivity to HF may be dependent on inhibition of STAT3/5 pathway.
Summary/Conclusions: In summary, our results suggest that HF may be effective against core binding factor leukemias and, that the methodology based on a Phospho-Kinase Array is useful to identify drug molecular targets.

PB1663

DNA METHYLATION AND HYDROXYMETHYLATION PROFILING IS CAPABLE TO DISTINGUISH AML SAMPLES WITH DISTINCT MUTATIONS IN DNA METHYLATION REGULATORY GENES

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Background: Aberrant DNA methylation as well as hydroxymethylation is a hallmark of acute myeloid leukemia (AML). Mutations of DNA methylation regulatory genes (DNMT3A, IDH1, IDH2 and TET2) are present in approximately 40-50% of AML. These mutations are often present together with the exception of TET2 and IDH1/2 as well as IDH1 and IDH2, which are usually mutually exclusive.
Aims: We aimed to perform DNA methylation, hydroxymethylation and gene expression profiling in clearly defined subgroups of AML patients with distinct mutations in DNA methylation regulatory genes to see whether there is a clear epigenetic and gene expression signature.
Methods: We accomplished DNA hydroxymethylation and methylation profiling in 12 AML samples at diagnosis and in CD34+ cells of 3 healthy controls by MethylationEPIC array (Illumina) covering aprox. 850 000 CpGs. AML samples were chosen based on their mutational status and divided into 4 groups: DNMT3A+ (n=3), IDH1+ (n=3), DNMT3A+/IDH1+ (n=3) and IDH2+ (n=3). The remaining DNA methylation regulatory genes as well as CEBPA were unmutated. 1 µg of genomic DNA was treated with TrueMethyl Seq kit (CEGX) to convert DNA through oxidative bisulfite (oxBS) and bisulfite (BS) treatment. This approach allows us to determine whether CpG is methylated or rather hydroxymethylated. We also performed gene expression profiling on the same samples by HumanHT-12 v4 Expression array (Illumina).
Results: We performed hierarchical clustering analysis of oxBS β-values (corresponding to DNA methylation levels) of 830 304 CpGs (with detection P<0.05) and observed clear separation of 4 groups according to mutational status – DNMT3A+, IDH1+, IDH2+ and CD34+ normals. Interestingly, double positive DNMT3A+IDH1+ (n=3) samples clustered each into different group (DNMT3A+, IDH1+, CD34+ normal) strongly suggesting that there is a cumulative effect of these two opposing mutations (Figure 1). We found out that genes hypermethylated in IDH1+ samples are enriched for genes from HOX gene family (P<0.0001) and that these genes are often hypomethylated in DNMT3A+ patients. In addition, we detected a subgroup of CpGs assigned to HOXA2, HOXA4, HOXA10, HOXB3, HOXC4 and HOXD3 genes that are hypermethylated in IDH1+, hypomethylated in DNMT3A+ and normally methylated in DNMT3+IDH1+ samples relative to CD34+ normals. Clustering of DNA hydroxymethylation values (resulting from subtraction of oxBS β-values from BS β-values) resulted into the same 4 main clusters as shown for DNA methylation data. DNMT3A+ patients displayed the lowest hydroxymethylation levels from all patients. Genes hydroxymethylated in IDH1+ patients were enriched for genes involved JNK cascade (comprising of evolutionarily conserved MAP kinases). The gene expression data did not reveal any cluster coherent with mutational subgroups, only CD34+ normals clustered together.

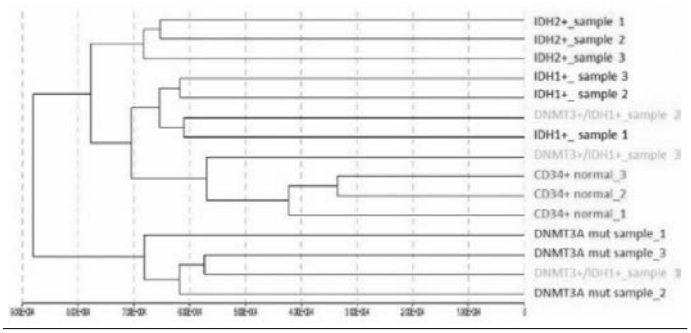

Figure 1.

Summary/Conclusions: We explored that AML patients with clearly defined mutational background exhibit distinct DNA methylation as well as hydroxymethylation profiles. The presence of two mutations that have the opposing effect on DNA methylation pattern (DNMT3A and IDH1) is linked to mixed DNA methylation patterns, which prevents unambiguous assignment to one cluster. Further, our data support that IDH1+ and IDH2+ represent distinct biological entities. On the contrary, gene expression profile did not support separation of samples into different mutational subgroups. We plan to enlarge the patients' cohort and validate the most promising genes involved in selected pathways.
Supported by Ministry of Health of the Czech Republic, grant nr. 15-25809A, and by the project for conceptual development of research organization (00023736) from the Ministry of Health of the Czech Republic.

PB1664

RNA-MEDIATED CORRECTION OF ABERRANT DNA METHYLATION AT THE P15 LOCUS

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Background: *P15* (a.k.a cell cycle dependent kinase inhibitor 2B; CDKN2B; INK4B) is a methylation sensitive gene located on chromosome 9p21 and commonly found silenced during Myelodysplastic Syndrome (MDS) progression to Acute Myeloid Leukemia (AML). *P15* encodes for a cyclin-dependent kinase inhibitor increasingly expressed during granulomonocytic maturation (Teofili *et al.*, *Exp Hematol* 2000). *P15* deletion or promoter methylation has been shown to independently correlate with disease progression and poor patient prognosis (Tien *et al.*, *Br J Hematol* 2001). Additionally, *P15* expression was also sensitive to regulation by myeloid-specific transcription factor PU.1 (Schmidt *Blood* 2004). As MDS evolution to AML includes both myeloid proliferation and blocked differentiation stages, restoration of the natural P15 transcript will provide not only valuable information regarding disease progression but may also alleviate some of their characteristic symptoms.
Aims: Currently available demethylating agents approved for therapeutic applications, *e.g.* 5-azacytidine and decitabine, have major side effects of high toxicity and non-specific DNA methylation that limit their clinical application. Therefore, the aim of this study is to achieve RNA-mediated correction of the aberrantly methylated *P15* locus using small activating RNAs (saRNAs; Li et al PNAS 2006).
Methods: Myeloid Leukemia cell lines HL-60, KG1a, and K562 were screened for basal p15 expression by western blotting and qRT-PCR. As the *P15* locus is also often deleted, deletion of the locus was assayed for by PCR and by Fluorescent *In Situ* Hybridization. Because the methylation status of *P15* was shown to be inversely correlated with ANRIL (Antisense Non-coding RNA in the INK4 Locus) expression (Kotake et al *Oncogene* 2010), p15 and ANRIL gene expression were measured in parallel. HEK293 cells serve as positive control in all studies. SaRNAs were designed against the proximal promoter, first exon, and intron regions of the *P15* gene body. SaRNAs were introduced to cell lines through electroporation, and re-activation of the locus was measured at the transcript level by qRT-PCR and protein level by western blotting. Changes in *P15* promoter level methylation were determined by Methylation Specific PCR.
Results: Transfection of saRNAs into the HL60 cell line showed upregulated p15 expression 24 and 48 hrs post-transfection. Analysis of ANRIL after saRNA-transfection showed no concomitant changes, suggesting locus-specific activity of the saRNAs. Future experiments will elucidate the mechanisms of saRNA activation of P15 gene expression and genome-scale specificity of saRNA-mediated methylation changes.
Summary/Conclusions: There is much interest in using RNA molecules as a therapeutic tool (Kole *et al.*, *Nat Rev Drug Discovery* 2012; Reebye *et al.*, *Hepatology* 2014). Introduction of such an approach offers greater advantages over

existing hypomethylating-based protocols: a) high gene specificity b) lower cytotoxicity and c) absence of drug based off-target side-effects. In the short term, this research can lead to the identification of novel key regulators of leukemogenesis and new targets for therapeutic treatments; in the long term pave the way for development of RNA-based gene demethylating agents for cancer treatment.

PB1665

JQ1 AND CURCUMIN COMBINED TREATMENT SHOWS SYNERGIC EFFECTS IN MLL-REARRANGED LEUKEMIA CELL LINES

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Background: MLL-rearranged leukemia accounts for □70% of infant and □10% adult acute leukemias, featuring a particularly poor prognosis and high risk of relapse. Our main field of study is AML, in which nearly 50% of total cases accounts for t(9;11) translocation, the remaining 50% predominantly includes t(6;11)(q27;q23), t(10;11)(p12;q23), t(11;19)(q23;p13.1) and t(11;19)(q23;p13.3). A 2% of AML total cases, however, is characterized by t(4;11) translocation, which is a marker of bad prognosis and it's, so far, poorly characterized. A key feature of MLL-rearranged leukemia is cMyc overexpression, a well-known oncogene involved in several types of cancer. JQ1 is a novel molecule, which prevents cMYC expression binding an important bromodomain protein, BRD4. Moreover, Curcumin, a natural compound, inhibits HATs enzymes preventing lysine 14 acetylation on histone H3 (AcH3K14), a particular residue which is bind by BRD4 to exert its function.
Aims: We would like to explore a potential synergic effect between JQ1 and Curcumin molecules in the attempt to develop a novel therapeutic alternative to standard chemotherapy and to deeply investigate features underlying the molecular pathogenesis in pediatric MLL-rearranged pediatric AML.
Methods: Four human leukemia cell lines with MLL fusion protein have been employed in this study: RS4:11, MV4:11 expressing MLL-AF4 and THP1, MOLM13 expressing MLL-AF9 fusion genes. 5μM and 10μM Curcumin were used to treat MLL-AF4 and MLL-AF9 cell lines respectively, while 250nM JQ1 was used to treat all the cells lines. After 2 days of treatment, either with single and combined drugs, cell number quantification, based on metabolic activity, was detected through XTT assay. In order to assess the cMYC, CDKN1A, BCL2 transcripts levels and mir-99a expression a quantitative RT-PCR analysis was carried out, while we used western blotting to detect the expression of cMYC, PARP, Caspase3 and AcH3K14. Apoptosis and cell cycle were evaluated by flow cytometric analysis.

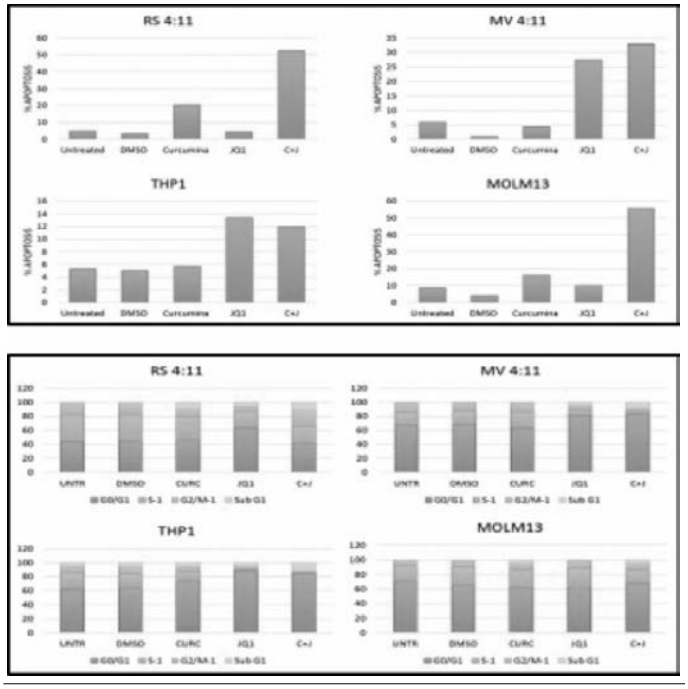

Figure 1.

Results: In apoptosis analysis, a synergic effect was detected for all 4 cell lines, similarly cell cycle evaluation showed a significant accumulation of cells at SubG1 phase (2-8 fold) (Figure 1). XTT metabolic assay showed a reduction in proliferation percentage: 65±5 for curcumin and JQ1 single treatment and 59±5 for combination of drugs in both MLL-AF4 cell lines, meanwhile in MOLM13 cells it was 64±2 and 87±2 for curcumin and JQ1, respectively and 76±2 for their combination (P<0.005). The THP1 cells did not shown any significant modulation in the proliferation. We decided to focus our study on t(4:11) translocated cells, considering the more intense effect of the combined drugs on previous analysis. qRT-PCR and western blot experiments revealed a synergic effect of the 2 experimental drugs on both apoptosis and proliferation gene related (bcl2, caspase3, Parp, cdkn1a) as well as on the direct targets of the drugs (cMyc, AcH3K14). Finally, in MLL-AF4 cell lines, curcumin and JQ1 together induced a significant decrease in mir-99a expression.
Summary/Conclusions: Our data demonstrated that curcumin and JQ1, inhibiting HATs and BRD4 respectively, exert a more intense synergic effect on MLL-AF4 than in MLL-AF9 cells. Increased apoptosis together with a reduced proliferation rate, prompted us to investigate on molecular pathway in which targets of these drugs are involved. Intriguingly, we found a significant decrease in cMyc, bcl2 and AcH3K14 expression, confirming that both curcumin and JQ1 have a synergic effect. Additionally, we revealed a significant reduced expression of mir-99a, a well know oncomiR reported to act as negative regulator of differentiation and involved in drug-resistance, typically upregulated in pediatric AML and ALL.

PB1666

TP53B AND TP53Γ EXPRESSION LEVELS IN RELATION TO NPM1 AND CEBPA MUTATIONS.

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Background: Acute myeloid leukemia (AML) is a heterogeneous clonal disorder with the presence of diverse genetic abnormalities in hematopoietic stem cells. The most frequent alterations in normal karyotype AML (NK AML) are mutations in exon 12 of nucleophosmin gene (*NPM1*). Until now 56 different mutations of *NPM1* exon 12 have been described, mostly insertions. The NPM protein plays an important role in cell cycle and apoptosis control. It cooperates with several proteins, among them with p53 and ARF. The median levels of functional nuclear p53 protein are reduced in *NPM1* and *FLT3 ITD* mutant samples. *TP53* encodes a tumor suppressor protein which consists of transactivation, DNA-binding and oligomerization domains. Due to alternative splicing it may exist in 13 different isoforms. Alternative splicing of intron 9 leads to production of 2 different proteins, p53β and p53γ, without oligomerization domain (stop codon is localized in exon 9b). These isoforms can be present in acute myeloid leukemia (AML) cells. p53β binds to *BAX* promoter and can induce apoptosis independent from p53 wt. p53 has influence on activation of *CEBPA* which is associated with cell cycle regulation, especially cell cycle arrest and plays also role in cell differentiation. Generally, it is a transcription factor expressed during myeloid lineage development, from progenitor cells to mature granulocytes. Various mutations of *CEBPA* gene are described. Among them N-terminal and C-terminal mutations, mostly insertions and deletions, are often present.
Aims: The goal of the study was to assess mutational status of *NPM1, CEBPA* and *FLT3* in association with *TP53*beta and *TP53*gamma expression evels.
Methods: 75 NK AML patients were included in the study. *NPM1, CEBPA* and *FLT3* gene mutations were analyzed by direct sequencing. *TP53*β and *TP53*γ expression levels were assessed with real time PCR. Expression levels were analyzed with ΔΔCt method, with *ABL* as a control gene and K562 cell line as a calibrator.
Results: In all 75 cases, *TP53*β and *TP53*γ transcripts were detected. 36 patients had *NPM1* mutations, 25 had *CEBPA* mutations or known polymorphisms, and 25 had *FLT3* ITD mutation. Assessed median expression level of *TP53*β was much higher (ΔΔCt 43,11) than *TP53*γ (ΔΔCt 10,85; p<0,05). Furthermore, expression level of *TP53*γ in *CEBPA* mutated group (ΔΔCt 11,4) was significantly lower than in *CEBPA* wt group (ΔΔCt 17,7) (p=0,03). We have not found any other important correlation between mutations of studied genes and *TP53*γ or *TP53*β expression. We also classified patients, according to median expression value of *TP53,* to two groups: with overexpression or with low expression. Haematological and clinical features, such as white blood cells count (WBC), blasts count in bone marrow or patient age did not depend on *TP53* isoform expressions. However, statistical analysis showed important difference between WBC count in *NPM1*mutated and *NPM1*wt groups.
Summary/Conclusions: Obtained results may suggest a clinical importance of simultaneous analysis of *TP53* isoform expression and mutations in *CEBPA* gene. It may be hypothesized that a changed sequence of the latter gene might influence *TP53* isoform expression and in consequence regulate the cell cycle.

PB1667

EXPRESSION PROFILE OF EPIGENETIC MODULATORS IN ACUTE MYELOID LEUKEMIA OF INTERMEDIATE RISK

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Background: Whole-genome sequencing has revealed acute myeloid leukemia (AML) as a very complex and dynamic disease.Epigenetic modulation is among the functional categories of the mutational landscape in AML.According to recent reports, suppression of the epigenetic reader BRD4 with small-molecule inhibitors (BET-i) results in antileukemic activity. Clinical trials are being developed, however, so far, identification of those patients that may benefit from this therapy is not possible as changes in mRNA BRD4 levels seem to be very subtle. It has been recently suggested that antileukemic effect of BET-i could be due to c-myc suppression and also that high Bcl-2 levels may target those patients that would benefit of BET-i. We believe that establishing the expression profile of epigenetic modulators in AML may help in the identification of patients that could benefit from BET-i.
Aims: We wanted to get a better insight regarding the expression profile of epigenetic modulator in AML of intermediate risk by studying: 1)expression levels of EZH2, ASXL1, BRD4, c-myc and Bcl-2 in a consecutive series of AML patients; 2) correlation between mRNA and protein levels; 3) Determining BRD4 binding to the c-myc promoter through chromatin immunoprecipitation (CHIP).
Methods: Our series consisted of 104 consecutive patients with a mean age of 55.8 years (range 15-79 years) diagnosed and treated between 2005-2016 at the Hospital Universitario de Gran Canaria Dr. Negrín with a median follow up of 12 months. Gene expression analysis was carried out through real time PCR in a LightCycler 480 Instrument II (Roche) using GUS a control gene.Results were normalized with a cDNA pool from bone marrow of 10 healthy donors which was introduced as internal control in each experiment. Western blot were performed to determine protein levels for BRD4, c-myc and Bcl2. CHIP studies for BRD4 were carried out in HL60 cell line. For statistical analysis the SPSS (v.15.0) software was used.
Results: ASXL1 levels were positively associated with EZH2 (Spearman's= 0.285, p=0.021) and BRD4 withc-myc (Spearman's coefficient=0.420, p<0.001), Bcl2 (Spearman's= 0.471, p<0.001) EZH2 (Spearman's= 0.4565, p=0.008) and ASXL1 (Spearman's=0.949, p<0.001). Survival analysis considering 50th percentile as a cut-off value for BRD4 expression indicated that patients with higher levels shows better overall survival (median overall survival, OS, of 27 months, 95% IC 15.1-38.9) compared to those with low expression (median OS 12 months, 95% IC 0.4-23.7), although the association was not statistically significant (p=0.196) probably due to the limited series size. Protein levels of Bcl2 and c-myc correlated with those of mRNA, but not for BRD4, although other antibodies should be tested in order to confirm these results. CHIP analysis in HL60 cell lines confirmed the binding of BRD4 to c-myc promoter.
Summary/Conclusions: The positive association observed between EZH2 and ASXL1 agrees with the fact that both cooperate in the epigenetic repressive complex PRC2.The association of BRD4 expression levels with c-myc and Bcl2 is in accordance to the reported binding of BRD4 to the c-myc and Bcl2super-enhancer regions and our CHIP analysis also support so. Further studies in a larger series are necessary to confirm the relationship between higher BRD4 levels and better overall survival. Finally, future analysis should be done to determine whether patients with higher BRD4 expression levels determine a subgroup with better response to BET-i.

PB1668

FLOW CYTOMETRY IMMUNOPHENOTYPING IN CEBPA-DM *DE NOVO* AML. BIOLOGIC AND PROGNOSTIC RELEVANCE.

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Background: CEBPA is a transcriptional co-factor of RUNX1 which play a major role in the fate decissions associated with physiologic myelopoiesis. Biallelic CEBPA mutations (dm) define an homogenous molecular subgroup which is associated with a favorable outcome. CEBPA mutations may be transmitted in the germ line giving rise to clusters of familial leukemias.
Aims: To analyze the immunophenotypic findings assessed by multiparametric flow cytometry in a consecutive series of *de novo* CEBPAdm AML.
Methods: Thirty-nine adult patients with *de novo* AML and CEBPAdm who where enrolled on the AML-03 and AML-12 protocols of the Spanish CETLAM cooperative group were included in this study The immunophenotypic analysis was performed on erythrocyte-lysed bone marrow (BM) samples obtained at diagnosis. Antigenic expression of leukemic cells was systematically analyzed by multiparametric flow cytometry using four-color staining. The antigens studied were: CD45, CD34, HLA-DR, CD10, CD20, CD19, CD2, CD33, CD7, CD117, CD66, CD13, CD64, CD36, CD56, CD14, CD123, CD61, CD42b, glycophorin, CD71, CD11b, myeloperoxidase, CD79a, CD3, TdT, lysozyme and lactoferrin. At least 10.000 events/tube were measured. Analytical gates were established according to CD45 reactivity and to FSC/SSC pattern.Positivity thershold was established at 20%. The FACS-DIVA,Paint-a-Gate and Infinicyt software programs were employed for analysis. Amplification of overlapping PCR products covering the whole CEBPA coding sequence followed by Sanger sequencing were used to investigate CEBPA mutations. FLT3-ITD, NPM1, MLL-PTD, WT1 and GATA2 mutations were also investigated by conventional PCR-based molecular methods.
Results: Antigen reactivity was as follows: CD45 (39/39,100%), CD15 (35/39, 90%), CD34 (36/39,92%), HLA-DR (39/39,100%), CD33(39/39,100%), CD2 (2/39,5%), CD7 (36/39,92%),CD117(39/39,100%), CD13(37/39,95%), CD56 (6/39,15%), CD36 (6/39, 15%), CD123(39/39, 100%), CD14 (1/39,0.02%), CD71(38/39,97%), myeloperoxidase (38/39, 97%).In nine cases CD36 and/or CD56 expression on leukemic blasts was greater than 20% Those CD36/CD56+ cases had a shorter overall survival and leukemia free survival (see graph). Four out five tested CD36/CD56+ cases also showed GATA 2 mutations. An additional CD36/CD56+ case had a FLT3-ITD. In three out 39 cases (7%) a population showing cytoplasmic CD79a reactivity was detected (8%, 11%,14% of the neoplastic population, respectively). Two of those cases had also a FLT3-ITD.

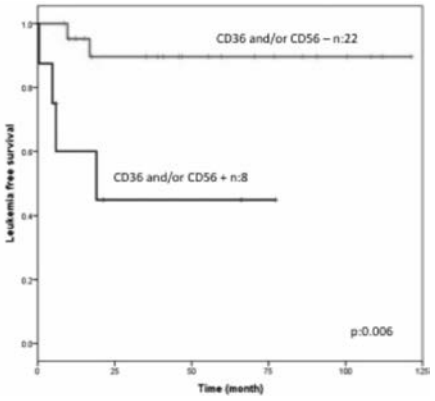

Figure 1.

Summary/Conclusions: CEBPAdm cases showed an homogeneous immunophenotype with positivity for CD45, CD7,CD34,CD123,CD117, HLA-DR, CD71,CD33,CD13 and CD15. CD36 and/or CD56 overexpression was detected in a subgroup of cases (9/39, 23%) with an adverse outcome. The current findings suggest that CD36 and CD56 reactivity should be investigated in larger series of CEBPAdm AML cases. Small leukemic populations with B-cell markers are not uncommon in CEBPAdm AML (3/39, 7%).

PB1669

PROTEOME CHANGES IN ACUTE MYELOID LEUKEMIA PATIENTS BEFORE AND AFTER INDUCTION TREATMENT

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Background: Acute myeloid leukemia (AML) is a malignant disorder of hematopoietic stem and progenitor cells (HSPCs), characterized by the accumulation of immature blasts in the bone marrow and peripheral blood (PB) of affected patients. Standard induction therapy, based on cytarabine and an anthracycline, leads to complete remission in approximately 50% to 75% of patients, depending on prognostic factors, such as age or the presence of certain gene or chromosomal changes. In spite of favorable primary response rates, only approximately 20% to 30% of the patients enjoy long-term disease survival.
Aims: Our aim was to compare the protein expression profile of peripheral blood mononuclear cells (PBMCs) of AML patients at time of diagnosis and after induction therapy.
Methods: PB samples were taken from seven AML patients in Medellin-Colombia at time of diagnosis and after concluding the induction therapy. Informed consent was obtained prior to sample collection. PBMCs were isolated from the 14 blood samples using a Histopaque-1077 solution. Cells were resuspended in lysis buffer (0.5% Triton x-100, 50 mM Tris-HCL pH 8.0, 150 mM NaCL, 1 mM EDTA, protease inhibitor) and proteins precipitated with trichloroacetic acid. Proteins were separated by 2D SDS-PAGE (pI 3–10 NL), and stained with SYPRO®Ruby. The proteomes were compared using PDQuest™ Advanced 8.0.1 Software. Protein spots of interest were those with a fold change of +/- 1.5 and p <0.05.

Results: Image analysis revealed an average of 464 protein spots in PB samples taken at time of diagnosis, and an average of 346 spots in PB taken after induction therapy, reflecting changes in protein expression due to treatment. Comparing the proteomes, we found 11 spots that differed significantly (fold change of +/- 1.5 and p <0.05). Of these, seven proteins were up-regulated and four were down-regulated at time of diagnosis (before treatment) compared to after induction treatment. Nine of these spots correspond to low molecular weight proteins (<40 kDa) and 2 spots have a molecular weight between 40-60 kDa. Based on the molecular weight and isoelectric point information of these spots we were able to search for proteins reportedly involved in leukemia, in order to propose possible identities (see Table 1). In terms of biological process, four proteins (eIF5B, HSP27, 14-3-3 protein zeta/delta, and GST-P) are involved in the regulation of apoptosis. The F-actin-capping protein subunit beta could also be of interest, as reorganization of F-actin reflects unique characteristics of the differentiation process in promyelocytic leukemia cells. RuvB-like 2 is a positive regulator of histone acetylation and DNA repair. GRBP2 is a protein involved in the MAPK cascade and regulation of PI3K signaling, pathways regulating diverse cellular functions altered in leukemogenesis such as proliferation, differentiation, and apoptosis. Alpha-enolase is a key glycolytic enzyme; however, it has been shown to be a multifunctional protein involved in cancer. It promotes cell proliferation by also regulating the MAPK and PI3K pathways. Transaldolase is part of the pentose-phosphate pathway. Annexin II acts in angiogenesis and has multifaceted role in human health and disease.

Table 1.

Possible protein identity of spots with differential expression

Spot	Possible Identity	Uniprot AC	Experimental		Theoretical		Fold change	GO - Biological process
			MW	pI	MW	pI		
1	Eukaryotic translation initiation factor 5A-1 isoform B	P63241	16,48	4,43	17	5,08	2,6	Regulation of apoptotic process.
2	HSP27	P04792	22,74	3,39	22	5,98	4,0	Negative regulation of apoptotic process.
3	14-3-3 protein zeta/delta	P63104	27,01	4,2	27	4.73	-5,6	Negative regulation of apoptotic process.
4	Glutathione S-transferase P	P09211	24,46	5,39	23	5.43	2,7	Negative regulation of apoptotic process.
5	F-actin capping protein subunit beta	P47756	30,27	5,19	30	5,69	-2,0	Actin cytoskeleton organization, regulation of cell morphogenesis.
6	RuvB-like 2	Q9Y230	51,81	5,44	51	5.49	2,4	Positive regulation of histone acetylation, DNA repair.
7	Growth factor receptor bound protein 2	P62993	26,32	6,12	25	5.89	1,8	MAPK cascade, regulation of PI3K signaling.
8	Transaldolase	P37837	37,33	6,94	37	6.36	1,8	Carbohydrate metabolic process, pentose-phosphate shunt.
9	Annexin II	P07355	38,02	8,01	38	7.57	-3,9	Angiogenesis, positive regulation of fibroblast proliferation.
11			38,2	8,23			-3,3	
10	α- enolase	P06733	48,14	7,4	47	7.01	4,6	Negative regulation of cell growth, negative regulation of transcription.

Summary/Conclusions: The protein expression profile of AML patients changes after induction treatment. We found 11 spots that differed significantly, and propose possible identities for these. Further analyses are pending in order to experimentally establish the identities and correlate with response to treatment.

PB1670

AMP-ACTIVATED PROTEIN KINASE ACTIVITY INTERFERES WITH OVEREXPRESSION OF NUCLEOPHOSMIN IN CYTARABINE-INDUCED CHEMORESISTANT AML CELLS

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Background: Cytarabine is a chemotherapeutic drug used alone or in combination with other anticancer drugs to treat acute myeloid leukemia (AML). New treatment strategies are emerging to enhance the anti-cancer effect and decrease the toxiciteis. Nucleophosmin (NPM1 or B23) is a ribosomal protein located mainly in nucleolus, and multifunctional enzyme in cancer cell growth and protein synthesis. AMP-activated protein kinase (AMPK) is a critical energy sensor to regulate homeostasis and plays a potential role for anti-cell proliferating activity.
Aims: We investigated the effects of AMPK activation on the cell death (apoptosis) and NPM1 expression in AML cells treated with low or high dose of cytarabine, an anti-leukemic drug, to predict the mechanisms responsible for AML cells chemoresistance.
Methods: The HL-60 (FAB M2) cells were exposed to the different drug combinations including cytarabine and AMPK activators. The molecular mechanisms of drug synergism detected by western blot assay and RT-qPCR. Cell viability and apoptosis were assessed using cell counting kit - 8 assay and flow cytometry.
Results: We found that cell apoptosis (36.27 ~ 42.11%) showed little dependence on cytarabine concentrations (10, 100, and 1000 mM), while the overexpression of NPM1 increased proportionally with drug dependence, indicating the drug-induced cell resistance. In the same point, cytarabine also inhibited the phosphor-activity (Thr172) and expression level of AMPK, which has mTOR-p70S6K pathway-repressor activity. As expected, single cytarabine treatment increased the ratio of p-mTOR/mTOR and p-p70S6K/p70S6K. Co-treatment of AMPK activator (phenformin or AICAR) in cytarabine-treated HL-60 AML cells inhibited significantly the induction of NPM1 overexpression level with the decrease of phosphor-activities of mTOR and its substrate p70S6K, resulted in the accelerated cell apoptosis.
Summary/Conclusions: Our results suggest that the higher concentration of cytarabine induces NPM1 overexpression, and that AMPK activation might be used to sensitize AML cells to cytarabine with the control of NPM1 expression levels. These modulatoins to standard therapeutic strategies could actually enable the reduction of the chemotherapeutic dose, therefore reducing their toxicity and adverse effects.
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PB1671

QUERCETIN REGULATES TELOMERE-BINDING PROTEINS EXPRESSION OF POT1, TRF1, TRF2 TO INHIBIT PROLIFERATION AND INDUCE APOPTOSIS IN AML THP-1 CELLS

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Background: Leukemia cells are limitless cell sources for initiation and maintenance of leukemia. Telomere-binding proteins are key regulatory factors for various diseases, including leukemia. Therefore, targeting telomere-binding proteins is considered as a promising therapeutic strategy for treatment of leukemia.
Aims: We aimed to explore whether quercetin, a natural flavonoids, could regulate telomere-binding proteins expression to inhibit proliferation and induce apoptosis in acute myeloid leukemia(AML) THP-1 cells.
Methods: 1. *In vitro*: (1) We cultured human AML THP-1 cells. (2) The cells were treated with different concentration of quercetin for 24/48 h, and the cell viability was measured by cell counting kit-8(CCK-8) to determine the IC50 of quercetin. (3) The cell cycle distribution and apoptotic rate were measured by Annexin V-FITC/PI double staining flow cytometry(FCM). (4) The protein expression levels of POT1, TRF1, TRF2 were measured by western-blotting. (5) The mRNA expression levels of POT1, TRF1, TRF2 were measured by real-time fluorescent quantitative polymerase chain reaction(RT-qPCR). 2 *In vivo*: (1) Established AML-NOD/SCID model based on THP-1 cell line in NOD/SCID mice, and treated with optimal quercetin concentration 40mg/(kg*d) for 4 weeks by tail vein injection. (2)We observed the changes of mice survival status, peripheral blood and bone marrow cell morphology and organ histopathology by microscopy before and after treatment with quercetin. (3) The cell cycle distribution and apoptotic rate of spleen cells were measured by Annexin V-FITC/PI double staining FCM. The protein expression levels of POT1, TRF1, TRF2 were measured by immunohistochemistry(IHC) staining.
Results: In this study, we found that quercetin significantly suppressed THP-1 cells proliferation in dose- and time-dependent manner. Treatment with quercetin significantly increased THP-1 cells apoptotic rate and G1 phase arrest rate. Furthermore, the protein expression levels of POT1 and TRF1 increased and the protein expression level of TRF2 decreased. The mRNA expression levels of POT1, TRF1, TRF2 were consistent with their protein expression levels, respectively.
Summary/Conclusions: Our results demonstrate that quercetin has anti-leukemia activity. It is mediated by regulating telomere-binding proteins expression of POT1, TRF1 and TRF2. Taken together, our findings support the concept that quercetin is a promising therapeutic strategy for treatment of leukemia.

PB1672

PPARΓ AGONISTS INHIBIT ADHESION SIGNAL TO ENDOTHELIAL CELLS IN THE DIFFERENTIATION INDUCTION OF HL-60 ACUTE PROMYELOCYTIC LEUKEMIA CELLS.

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Background: All-*trans* retinoic acid (ATRA) has successfully been used in the treatment of acute promyelocytic leukemia (APL) patients, with a remission rate of greater than 90% .
Despite the high cure rates, induction mortality is a still a problem in APL. One of the most common causes of death was the differentiation syndrome (DS) . The early administration of high-dose dexamethasone at the onset of the first

signs or symptoms of DS is crucial, however specific biological therapies to counteract the syndrome are still not available.
Peroxisome proliferator activated receptor gamma (PPARγ) is a ligand-dependent transcription factor and a member of the nuclear receptor superfamily, which is expressed in normal monocytes, various leukemias, and epithelial malignancies. PPARγ is highly induced in differentiating myeloid cells and subsequently contributes to their differentiation.
Differentiation induction of APL cells is associated with increased expression of specific adhesion molecules and inflammatory cytokines, which may promote activation, migration, and adhesion of these cells.
Aims: Here, we studied the effect of PPARγ agonists on the adhesion of a human leukemia cell line (HL-60) to endothelial cells.
Methods: Differentiation was determined by an increase in reactivity with the CD11b antibody. For the adhesion assay, the Matrigel transwell system was used.
Results: HL-60 cells were differentiated into macrophage-like cells by a PKC activator, 12-*O*-Tetradecanoylphorbol-13-acetate (TPA). During the differentiation of HL-60 cells, PPARγ agonists acitvate TPA-induced CD11b expression. However, PPARγ agonists completely blocked TPA-induced ICAM-1 expression of endothelial cells, which resulted in the inhibition of adhesion of HL-60 cells to endothelial cells. These responses also were reversed by PPARγ antagonist (GW9662), indicating that PPARγ agonists inhibits the adhesion of the HL-60 cells to endothelial cells through a PPARγ dependent mechanism.
Summary/Conclusions: These results suggest that PPARγ agonists inhibit TPA-induced adhesion signal in the between HL-60 cells and endothelial cells, and may control differentiation syndrome in APL patients.

Acute myeloid leukemia - Clinical

PB1673

IN VITRO DRUG SENSITIVITY TEST IN THE INDIVIDUALIZED ANTI-LEUKEMIA CHEMOTHERAPY FOR THE NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA

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Background: The biological properties, genetic abnormalities of leukemic cells influence on their sensitivity to chemotherapeutic drugs. It is widely known that there can be significant differences both in genetic features as well as in drug resistance profile of individual tumors with the same phenotype.
Aims: The purpose of this study was to analyze the relationship between *in vitro* chemosensitivity test results using the Cell Titer-Glo assay and clinical response on chemotherapy, and to find the possibility of optimizing the treatment for individual patients according to their actual drug resistance.
Methods: For The Cell Titer-Glo assay, we obtained bone marrow aspirates or peripheral blood samples from 68 patients with newly diagnosed acute myeloid leukemia at the time of initial diagnosis. The following drugs were tested: cytarabine arabinoside, daunorubicin, idarubicin, fludarabine, etoposide, and methotrexate. We evaluated clinical response and survival outcome according to chemosensitivity of drugs and protein expression.
Results: In this study, *in vitro* chemosensitivity test with the Cell Titer-Glo assay showed the relationship between chemosensitivity and survival outcome significantly. The 5-year overall survival rates with dichotomized chemosensitivity of idarubicin (64.6% *vs* 33.3%, p=0.046), cytarabine (63.1% *vs* 43.3%, p=0.0291), and fludarabine (80.1% *vs* 37.5%, p= 0.020) were higher in low centration level than in high concentration level. There was a tendency of higher relapse-free survival rate at 4-year in the patients with low level IC_{50} than in the high level IC_{50}. However, cytotoxic effect of testing drugs *in vitro* by the Cell Titer-Glo assay did not show a relationship with complete remission rate after induction and leukemia recurrence rate.
Summary/Conclusions: Although the Cell Titer-Glo assay did not provide the prediction of clinical response of induction treatment, it can be a useful tool in individually optimizing the chemotherapy of patients with newly diagnosed acute myeloid leukemia.

PB1674

PROGNOSTIC IMPACT OF P53 EXPRESSION IN BONE MARROW BIOPSY OF PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Several studies have shown that the presence of the *TP53* mutation is related to an unfavorable prognosis in patients with acute myeloid leukemia (AML). However there are few reports on the evaluation of its expression by immunohistochemistry in bone marrow (BM) biopsy.
Aims: To evaluate the expression of p53 in BM biopsy of AML patients at diagnosis and its impact on survival.
Methods: This retrospective analysis included 85 patients with *de novo* AML diagnosed from January 2005 to December 2015 submitted to BM biopsy at diagnosis. p53 expression was detected by immunohistochemistry, and staining was evaluated using the H-score (range 0-300). The t-test and Mann-Whitney U test were used to detect differences in the distribution of continuous parametric and nonparametric variables, respectively. Overall survival (OS), disease-free survival (DFS) and event-free survival (EFS) were calculated using the Kaplan-Meier method. The log-rank test was used for comparison of survival curves. The interaction between the examined prognostic variables was tested with univariate and multivariate Cox regression analysis.
Results: Median age was 60 years (17-81). There was a predominance of patients >60 years (54.1%) and males (56.5%). The median H-score for p53 was 11.8 (0.4-161.1), with no significant correlation with age or cytogenetic risk. p53 expression was significantly higher in patients with a complex karyotype (p=0.0031) and high risk by European Leukemia Net (ELN) criteria (p=0.047). There was a positive correlation with complex karyotype and prognostic risk by ELN. Excluding early deaths (<30 days from induction), patients younger than 60 years with H-score >60 showed worse overall survival when compared with patients with H-score <60 (0% *vs* 14.6%, respectively) (p=0.048). There was no statistical difference in disease-free survival and event-free survival. In the Cox univariate analysis including all cases, peripheral leukocyte counts at diagnosis (p=0.014), cytogenetic risk groups (p=0.07), ELN risk categories (p=0.023) and H-score (p=0.025) were significant. In a multivariate model including leukocytes, ELN risk and p53, all variables remained in the model.

Summary/Conclusions: Expression of p53 assessed by immunohistochemistry is a fast, objective and promptly available tool for prognostic evaluation of AML. A high expression of p53 (H-score >60) was related to a lower overall survival in *de novo* AML.

PB1675

Abstract withdrawn.

PB1676

LONG-TERM FOLLOW-UP OF SALVAGE TREATMENT FOR RELAPSED AML WITH CLADRIBINE, HIGH DOSE CYTARABINE AND IDARUBICIN

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Background: Despite improving response rates in induction treatment for AML during the last years the outcome for relapsed or refractory AML is still poor. Currently, no standard therapy exists for patients with relapsed AML. Furthermore, CR rates are lower than in newly diagnosed patients and range between 15% and 50%. There is evidence that cladribine (2CdA) has single drug activity in AML as well as an enhancing effect on other cytostatic drugs such as cytarabine (AraC) and thus may help to overcome resistance mechanisms.
Aims: Therefore, testing the combination of 2CdA, AraC and idarubicin (CAI) seems reasonable. Here we present the final analysis from our single-center phase II trial evaluating the safety and efficacy of CAI in relapsed AML patients after a follow-up of 5 years.
Methods: Patients with relapsed AML after at least 6 months of remission and ECOG 0-2 were included. Chemotherapy regime consisted of two courses of 2CdA 5 mg/m²/12 h, d 1-3, AraC 1000 mg/m²/12 h, d1-3 and Idarubicin 8 mg/m²/d, d1-3. After 8 patients, the prolonged duration of neutropenia especially in course 2 prompted us to change the protocol by 1) application of growth factors from day 15 onwards, and 2) omission of idarubicin from the 2nd course. The primary endpoint was the overall remission rate and safety of CAI.
Results: Because of slow recruitment the study was stopped after 20 patients. The median age was 63 years, 40% were female. 19/20 (95%) patients were included in the first relapse after at least 6 months of CR following 1st line therapy for AML. 1/20 (5%) patient was included with a second relapse. In 14/20 patients cytogenetic data at the timing of relapse were available, according to the ELN-risk-group 2017 3/14 (22%) had favourable cytogenetic changes, 9/14 (64%) intermediate and 2/14 (14%) belonged to the adverse cytogenetic group. The performance status was good in most patients (ECOG 0 in 10%, ECOG 1 in 80%), but reduced (ECOG 2) in 2 (20%) patients. After the first course, CR/CRi was achieved in 60% and PR in 10% of patients. Median duration of neutropenia was 24 days (range 18-41d). The main grade 3 or 4 non-haematologic toxicity was infection seen in 85% of courses. Nausea occurred in 30%, hepatotoxicity, mucositis and diarrhea in 11% of courses. Cardiac or renal toxicities grade 3/4 were not observed. Two patients (10%) died due to infection. Six patients received a second course of CAI/CA. Altogether, 6 patients were refractory. Nine patients (48%) proceeded to allogeneic stem cell transplantation after induction therapy with CAI. Of those, 4 patients are still alive and free of leukemia and one patient died in CR 88 months after salvage-therapy accounting for a 5-year survival rate of 55%.
Summary/Conclusions: Combination therapy with CAI in relapsed AML patients is feasible and induces good response rates. Combined with allogeneic stem cell transplantation, long-term survival can be achieved. However, infection rates are a serious complication warranting intensive supportive care.

PB1677

HIGH EVI1 EXPRESSION PREDICTS POOR OUTCOMES IN ADULT ACUTE MYELOID LEUKEMIA PATIENTS WITH INTERMEDIATE CYTOGENETIC RISK RECEIVING CHEMOTHERAPY ONLY

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Background: Nearly half of acute myeloid leukemia (AML) patients are defined as an intermediate cytogenetic risk, however the patients in this group have greatly varied outcomes and need to be stratified. Apart from gene mutation, abnormal gene expression might also be prognostic, and ecotropic viral integration site 1 (EVI1) expression is a representative. To date, the poor prognostic impact of EVI1 expression in AML has been reported, but almost all studies have been undertaken by European researchers. EVI1 prognostic significance in AML remains to be confirmed in other populations. Furthermore, because the detection protocol and cutoff value selection methodologies differed among studies, the threshold for defining EVI1 high expression remains obscure, which hinders its clinical routine application.
Aims: We investigated the prognostic impact of EVI1 transcript levels in Chinese adult intermediate cytogenetic risk AML (ICR-AML) patients who received chemotherapy only in a single center. The appropriate cutoff values for grouping EVI1 expression were also evaluated.
Methods: A total of 191 adult patients receiving chemotherapy only were included in this study. They were diagnosed as ICR-AML according to morphology, immunophenotyping, cytogenetics and molecular biology. Their bone marrow samples were collected at diagnosis. Real-time quantitative PCR was performed to test EVI1, MLL partial tandem duplicate (MLL-PTD) and WT1 transcripts, and their transcript levels were calculated as the percentage of target transcript copies/ABL copies. NPM1 mutations and FLT3 internal tandem duplication (FLT3-ITD) were individually screened by real-time quantitative PCR and qualitative PCR. 27 normal bone marrow (NBM) samples form volunteers were simultaneously tested EVI1, MLL-PTD and WT1 transcripts. All participants provided written informed consent in accordance with the Declaration of Helsinki.
Results: The upper limit of EVI1 transcript levels in 27 NBM samples was 8.0%. Receiver operating characteristic curve analysis showed that 1.0% (a 0.9-log reduction from the normal limit) was the EVI1 optimal diagnostic cutoff value for significantly differentiating relapse (P=0.049). A total of 23 patients (12%) had EVI1 levels ≥1.0%. EVI1≥1.0% had no impact on complete remission achievement. EVI1≥1.0% was significantly associated with lower 2-year relapse-free survival (RFS), disease-free survival (DFS) and overall survival (OS) rates in the entire cohort (P=0.0003, 0.0017 and 0.0009), patients with normal karyotypes (n=148, P=0.0032, 0.0047 and 0.0007) and FLT3-ITD (-) patients (n=150, all P<0.0001). Multivariate analysis showed that EVI1≥1.0% and FLT3-ITD (+) were independent adverse prognostic factors for RFS (Table 1), DFS and OS in the entire cohort. In addition, patients with EVI1 between 1.0% and 8.0% had 2-year RFS rates similar to those with EVI1≥8.0% (P=0.16), and both patient groups had significantly higher RFS rates than those with EVI1<1.0%.

Table 1.

	HR (95%CI)	P value
EVI1 expression		
≥1.0%	4.0 (2.1-7.7)	<0.0001
FLT3-ITD		
(+)	3.4 (1.9-6.0)	<0.0001
PLT count		
<100×10E9/L	2.1 (1.1-4.3)	0.030
Blast percentage in BM		
>65%	2.1 (1.1-3.6)	0.017

Summary/Conclusions: EVI1 transcript levels at diagnosis could further stratify adult ICR-AML, and high EVI1 expression predicts poor outcomes in patients receiving chemotherapy only. The optimal cutoff value which best differentiates patients is different from the normal upper limit.
Grant support: The Nature Science Foundation of China (81370637, 81370639 and 81570130).

PB1678

EFFICACY AND SAFETY OF DECITABINE IN ELDERLY AML PATIENTS: A REAL LIFE MULTICENTER EXPERIENCE OF THE NETWORK RETE EMATOLOGICA LOMBARDA

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Background: The optimal treatment decision in older patients (pts) with AML remains controversial, especially in patients pts with comorbidities, non-fit to intensive therapy or with AML adverse biologic features. Recently decitabine was approved in Italy in AML pts unfit to chemotherapy aged >65 years (y) and could be adopted in a population based setting.
Aims: To evaluate efficacy and toxicity of decitabine in a consecutive series of elderly AML pts (no M3), considered unfit to chemotherapy (CT) according to Ferrara et al (Leukemia, 2013) and treated at 6 centers of the Hematological Lombardy Network (REL).
Methods: Between Dec 2015 and Dec 2016, 46 (F/M: 22/24) newly diagnosed AML pts received decitabine (20mg/mq/daily for 5 days every 4 weeks) as outpatients. Median age was 76 y (69-85), ECOG performance status (PS) was ≥3 in 10.8%. According to "fitness", 41 pts (89.1%) were defined unfit to intensive CT, 1 frail and 4 fit. Unfitness causes were age >75y (58.5%), PS ECOG≥3 unrelated to leukemia (12.2%), and comorbidities (29.3%). AML was "de novo" in 25 pts (54.3%), therapy-related in 3 and secondary to antecedent hematological disorders in 18 pts. WBC count at diagnosis was 4.4 x10^3/mL (0.46 to 63), marrow blasts were 51% (<30% in 19.5% of pts). Karyotype (K) was normal (NK) in 43%, t(8;21) in 4.5%, intermediate in 20.5%, adverse (adv) in 32% of

pts, according to ELN (Doehner, 2017). In 2 pts it was not evaluable. Molecular analysis was available in 17/19 NK, NPM1 was mutated in 5 cases, with (2) or without (3) FLT3-ITD mutation.
Results: The total number of cycles administered was 231 (median 3.5; range 1-20). In 37/46 evaluable pts (2 ongoing, 1 early and 6 aplastic deaths), overall response rate (ORR) and complete remission (CR) rate were 51.% and 32%, respectively. Partial response and hematological improvement were achieved in 5.5% and in 13.6%, stable disease in 29.9% and failure in 19% of pts, respectively. Median time to best response was 3.5 months (range 1-8.5). Median response duration was 5.3 months (1-18+ ms). Relapse/disease progression was observed in 42% of responders. ORR was 21.4%, 47.3% and 77% in adv, NK and intermediate K, respectively (P=0.0289). After a median follow-up of 6.5 months, median survival was 8.4 months and projected OS at 1 y and 2 y was 43%+/-9 (SEM) and 30%+/-12% (SEM). Treatment was fairly well tolerated except for a high incidence of infections (46 episodes in 231 cycles) particularly during the first 3 cycles (29% *vs* 11%) (p 0.0072). Pneumonia was the most frequent infection (46%), followed by sepsis (28%). It was more frequent during the first 3 cycles [14% *vs* 4% : p 0.012)] when 44% of cases were of suspected fungal origin (3 probable aspergillosis and 4 possible IFI). Death occurred in 24 pts (52.2%): 12 (50%) of disease progression, 1 of early CNS hemorrhage and 11 (45.8%) of infection. In the first 3 months, infections were responsible for 46.7% of deaths. Pulmonary IFI were fatal in 57% of cases. These figures are higher than those reported by Cashen (JCO 2010) where the frequency of pneumonia was 11%.
Summary/Conclusions: These preliminary data confirm, in a population based setting, the high efficacy of decitabine and its longer time to response (more than 3 cycles) compared to CT. However infections complications were more frequent than expected and often fatal, particularly early during treatment. Since pneumonia, especially IFI, was the major cause of death, the adoption of routine antimicrobial prophylaxis may be considered in order to reduce early mortality and further improve the results.

PB1679

CLOFARABINE, CYTARABINE AND MITOXANTRONE FOR RELAPSED OR REFRACTORY ACUTE MYELOID LEUKAEMIA – INTERIM RESULTS OF A PROSPECTIVE PHASE 2 STUDY
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Background: In unselected patients with acute myeloid leukaemia (AML) in first relapse or refractory to primary daunorubicin / cytarabine therapy, complete response (CR) rate is merely 20 - 30%. In patients <60-years old, CR rates of about 55% may be achieved.
Aims: We tested in a multicenter prospective phase 2 study the efficacy and safety of clofarabine, cytarabine and mitoxantrone (CLAM) in AML patients in first relapse or refractory to first-line daunorubicin / cytarabine induction therapy.
Methods: Consenting patients aged 18 to 65 years in first relapse or refractory to first-line dose-intensified daunorubicin / cytarabine were recruited. Bone marrow pathology and karyotype at diagnosis and relapse were centrally reviewed. Next-generation sequencing of a myeloid panel of 67 genes was performed. Re-induction CLAM comprised clofarabine (40mg/m^2/day, days 1-5), cytarabine (750mg/m^2/day, days 1-5) and mitoxantrone (12mg/m^2/day, days 3-5). Bone marrow assessment was done on day 28 using standard criteria. Treatment toxicity was evaluated using the Eastern Cooperative Oncology Group Common Toxicity Criteria (ECOG-CTC). Survivals were determined using Kaplan Meier method. The primary outcome was the response on day 28. Secondary outcomes included treatment toxicity, leukaemia-free and overall survivals.
Results: In this interim analysis, 24 patients (14 men, 10 women) with a median age of 44.5 (19-66) years were treated. Karyotypic and genetic profiles were: normal karyotype (N=8) (*NPM1* mutant, N=1; *FLT3*-WT, N=8), t(8;21)(q22;22) (N=4) (*KIT D816V* mutant, N=1), inv(16)(p13.2;q22)/t(16;16)(p13.2;q22) (N=1) (*KIT D816V* mutant, N=1), inv(3)(q21;q26) (N=5), del (11)(q23) (N=2), t(9;11)(p21;q23) (N=1), trisomy 13 (N=1), near-tetraploidy (N=1), and complex karyotype (N=1). Twenty patients (83.3%) responded (CR, N=16; CR with incomplete hematopoietic recovery, N=4). Eight responding patients underwent allogeneic haematopoietic stem cell transplantation. Grade 3/4 haematologic toxicity was seen in all patients. Grade 1/2 and grade 3/4 hepatotoxicity was observed in 17 (70.8%) and 2 (8.3%) patients respectively. Grade 1/2 rash was observed in 4 patients (20%). Cardiotoxicity or treatment-related mortality was not seen. With a median of follow-up of 4 (1-32) months, 6 patients relapsed. The 12-month overall and leukaemia-free survivals were 81.7% and 66.8% respectively.
Summary/Conclusions: CLAM resulted in a high CR rate for AML in first relapse or refractory to first-line induction therapy, which was associated with an acceptable toxicity profile.

PB1680

FATAL EVOLUTION IN THE FIRST 96 HOURS OF PATIENTS DIAGNOSED WITH ACUTE LEUKEMIA: ANALYSIS OF A SERIES OF 346 CONSECUTIVE CASES OF ACUTE LEUKEMIA IN A SINGLE CENTER
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Background: The very early death of a newly diagnosed acute leukemia (AL) patient is very frustrating, and there are very few published works (except for the case of acute promyelocitic leukemia, APL) analyzing this circumstance and the features of these patients.
Aims: Our objective was to study the main characteristics of patients with acute leukemia who died within the first 96 hours after diagnosis in our centre in the last 15 years.
Methods: We studied all cases of acute leukemia diagnosed in our institution between April 2002 and January 2017, focusing on the analysis of those who died within the first 96 hours after diagnosis. In this subset of patients, we collected data concerning clinical presentation, hemogram, biochemical parameters, coagulation status, performance of a bone marrow aspirate, acute leukemia subtype, started therapy, initiation or not of induction chemotherapy, time elapsed from diagnosis to death (hours), and cause of death, among others.
Results: A total of 346 consecutive cases of acute leukemia were recorded in this period of time: 222 of acute myeloid leukemia (AML, 64%) and 124 of acute lymphoblastic leukemia (ALL, 36%). Thirty-three patients were diagnosed of acute promyelocytic leukemia (15% of all AML). Those patients who died in the first four days after the diagnosis were only seven (2%), with a median of 45 hours of life (range 21-96). Main clinical and analytical findings are shown in the Table 1. They were 5 men and 2 women with a median of 57 years (range 22-91). Two of the seven patients had an APL (6% of all diagnosed APL). All patients showed leukocytosis, but hyperleukocytosis was only recorded in 2/7 patients, and severe thrombocytopenia (Plt ≤ 20 x10^9/L) in 3/7. There was possibility of bone marrow aspiration only in 4/7 cases. Coagulopathy was detected in four of six patients, including criteria for disseminated intravascular coagulation (DIC) in three cases. The exitus took place in the Intensive Care Unit in 5 cases, while it occurred in the Hematology facility in two.

Table 1.

Main features of the 7 patients who died in the first 4 days after diagnosis of AL.

	Case #1	Case #2	Case #3	Case #4	Case #5	Case #6	Case #7
Age (sex)	22 (M)	49 (F)	91 (F)	74 (M)	54 (M)	67 (M)	57 (M)
Type of AL	Monoblastic	Promyelocytic	T-ALL	Myelo-monocytic	Myeloid therapy-related	Secondary to myelofibrosis	Promyelocytic
PB WBC (x10^9/L)	222	13	23	66	41	164	16
PB Hemog. (g/L)	8,0	9,4	8,9	14,5	11,9	16,0	7,5
PB Plt (x10^9/L)	61	30	18	60	7	55	20
PB blasts (%)	78	73	90	56	32	97	82
Bone marrow aspirate	No	Yes	No	Yes	Yes	No	Yes
Serum LDH	Increased	Increased	Increased	Increased	Increased	Increased	Increased
Serum creatinin	Normal	Increased	Normal	Increased	Increased	Increased	Normal
Coagulopathy	Yes	Yes	No	Yes	No	Unknown	Yes
DIC	Yes	Yes	No	No	No	Unknown	Yes
Started therapy	Leukapheresis + hydroxiurea	Induction chemothrapy	Supportive	No	No	Hydroxiurea	Induction chemotherapy
Cause of death	Intracranial hemorrhage	Multiorgan failiure	Urinary sepsis	Multiorgan failiure	Tumor lysis	Pulmonary leukostasis	Multiorgan failiure
Hours from diagnosis	45	38	32	24	48	21	96

Abbreviations: PB, peripheral blood.

Summary/Conclusions: In our experience, about 2% of patients with acute leukemia die within the first 96 hours after diagnosis (including 6% of APL). Clinical and analytical feautures of this subset of patients are very heterogeneous, although AML clearly predominate on ALL. More extensive and multicenter studies are needed to deepen into the circumstances conditioning this early fatal course of the disease.

PB1681

PRIMARY POSACONAZOLE PROPHYLAXIS IN ACUTE MYELOID LEUKEMIA - A SINGLE CENTER REAL LIFE EXPERIENCE
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Background: Invasive fungal infections (IFI) are a major cause of mortality and morbidity in acute myeloid leukemia (AML) patients receiving remission induction therapy, and relapsed/refractory AML patients. Posaconazole prophylaxis has shown the greatest benefit in preventing IFI in AML .
Aims: We present the data of our real-life experience in AML patients under PP.
Methods: We have retrospectively reviewed the data from 82 AML patients

receiving 105 cycles of chemotherapy between June 2012 and December 2016 in Marmara University Pendik Research and Training Hospital. Median patient age was 50 years (18-73); and there was no significant gender difference (38 female *vs* 44 male (46% *vs* 54%)). All patients had active disease, 78 (74.3%) of them received 3+7 (idarubicine - ara-c), 25 (23.8%) of them FLAG-Ida, 1 patient received EMA and 1 patient received CLARA chemotherapy protocol. Acute promyelocytic leukemia was excluded from the analysis. All patients received posaconazole as oral suspension at the dose of 200 miligrams three times daily starting on the first day of chemotherapy. Prophylaxis was continued until marrow regeneration, or occurrence of IFI, or onset of adverse events, or discontinuation due to other reasons. All fungal infections were classified as possible, probable, or proven according to European Organization for the Research and Treatment of Cancer (EORTC) and the Mycoses Study Group (MSG) consensus criteria.

Results: Mean posaconazole prophylaxis duration was 20±13 (1-68) days. This duration was 29.7 days (16-50) in patients receiving prophylaxis until marrow recovery, 18.9 (9-34) days in patients developing IFI under prophylaxis, and 12.7 days (1-68) in prophylaxis discontinuations due to adverse events and other reasons. Posaconazole prophylaxis was administered until marrow recovery without IFI (clinical success rate) in 42 of 105 (40%) chemotherapy cycles. In 18 cycles prophylaxis was stopped after diagnosis of IFI (%17.1). Discontinuations were due to adverse events in 6 cycles (5.7%), and due to other reasons (diarrhea, intolerance of oral medication, recurrent high grade fever, death) in 39 cycles (37.1%). IFI incidence under effective posaconazole prophylaxis was 28.1% (18/64). Total clinical failure rate was 60% (63/105). IFI was diagnosed with pulmonary nodules in 12 of 18 patients (66.6%; EORTC-MSG: possible), with galactomannan positivity in 3 patients (16.6%; EORTC-MSG: probable), and with fungal culture in 3 patients (16.6%; EORTC-MSG: proven). Data from 70 patients were available for mortality analysis. In patients receiving effective posaconazole prophylaxis, all-cause mortality rate at day 100 was (9/44; 20.4%) significantly lower than patients unable to continue posaconazole prophylaxis (12/26; 46.1%) (p:0.023). In the subset of patients receiving prophylaxis as planned; there was no statistically significant difference in IFI incidence between previously untreated AML (13/46; 28.2%) and relapsed/refractory AML (5/18; 27.7%).

Summary/Conclusions: In our real-life experience, we have demonstrated early survival benefit in patients receiving effective posaconazole prophylaxis. Although our IFI rate was comparable to other real-life data, our clinical failure rate was slightly higher. This is probably due to compliance issues, since in many chemotherapy cycles (37.1%) posaconazole was discontinued due to "other reasons" such as drug intolerance. Although not as effective as in the clinical trials; our data still supports the use of posaconazole prophylaxis in high risk AML patients.

PB1682

CLINICAL AND PROGNOSTIC VALUE OF FLT3 MUTATIONS IN ACUTE MYELOID LEUKEMIA PATIENTS IN ROUTINE CLINICAL PRACTICE – SINGLE CENTER EXPERIENCE

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Background: Detection of FLT3 gene mutations in acute myeloid leukemia (AML) now recognized as an unfavorable factor that affects the disease course, emerging the risk of relapses and overall survival (OS) shortening. Although about 30% of AML patients harbor one of the FLT3 gene lesion, at present there are no internationally standardized assays to quantify FLT3 mutation burden and no results of randomized clinical trials intended to individualize AML treatment based on FLT3 status. Some hematologists advocate to allo-SCT as consolidation in FLT3 ITD+ patients, but this way could be hard in frail and old patients and in countries with low access to transplant techniques. On the other hand, the development of target drug therapy – FLT3-kinase inhibitors gives us a new hope for improvement in the treatment results of such poor-prognosis subset of AML patients.

Aims: To assess the frequency of FLT3 gene mutations and its impact on clinical parameters and overall survival of the patients with acute myeloid leukemia (AML) in routine clinical practice.

Methods: We have analyzed FLT3 gene mutation frequencies, complete blood count (CBC) parameters, karyotype and survival outcomes per FLT3-mutation status in 199 patients with AML (83 male / 116 female). The median age at diagnosis was 52 years (20-86 years). To determine FLT3 gene mutations we used the method of polymerase chain reaction (PCR) with subsequent restriction. FLT3 gene mutations were classified as internal tandem duplication (FLT3-ITD) and point mutation in the "A-loop" (FLT3-TKD). Statistical analysis was included Kruskall-Wallis ANOVA and Kaplan-Meyer curves.

Results: We observed next FLT3 gene mutations rates: FLT3-ITD - 22.6% (45/199), FLT3-TKD 5.5% (11/199), FLT3-ITD and FLT3-TKD in combination 1.0% (2/199), other 70.8% (141/199) patients had no mutations (FLT3-). CBC data at the time of diagnosis were as follows (median (max-min)): · FLT3-ITD: Hb 9.7 (3.7-13.0) g/dl, WBC 40.3 (0.6-400.0) x 10^9/l, blasts 80% (21-100), platelets 60 (2-140) x 10^9/L; · FLT3-TKD: Hb 10.2 (5.8-12.8) g/dl, WBC 62.4 (1.7-362.0) x 10^9/l, blasts 68% (23-100), platelets 55 (12-115) x 10^9/L; · FLT3-ITD+TKD: Hb 5.8, 8.4 g/dl, WBC 37.0, 157.0 x 10^9/l, blasts 65%, 86%, platelets 38, 186 x 10^9/L; · FLT3-: Hb 9.0 (2.8-14.0) g/dl, WBC 12.9 (1.0-260.0) x 10^9/l, blasts 64% (20-103), platelets 63 (1-334) x 10^9/L; Significant differences across the groups were seen only in WBC and blasts. Chromosomal aberrations were revealed in 38% of FLT3-ITD, 64% of FLT3-TKD, none of FLT3-ITD+TKD and 51% of FLT3- patients. All patients received chemotherapy (7+3, 5+2, HAM). Transplantation of hematopoietic stem cells (SCT) was performed in 28 (allo/auto 17/11) (14%) patients: FLT3-ITD allo-3; FLT3-TKD allo-1, auto-1; FLT3- allo-13, auto-10. We found significant (p=0.00024) differences regarding to OS between FLT3-ITD, FLT3-TKD and FLT3- patients (Figure 1). Median survival times were: 5.1 months for FLT3-ITD, 7.1 months for FLT3-TKD and 13.0 months for FLT3- patients.

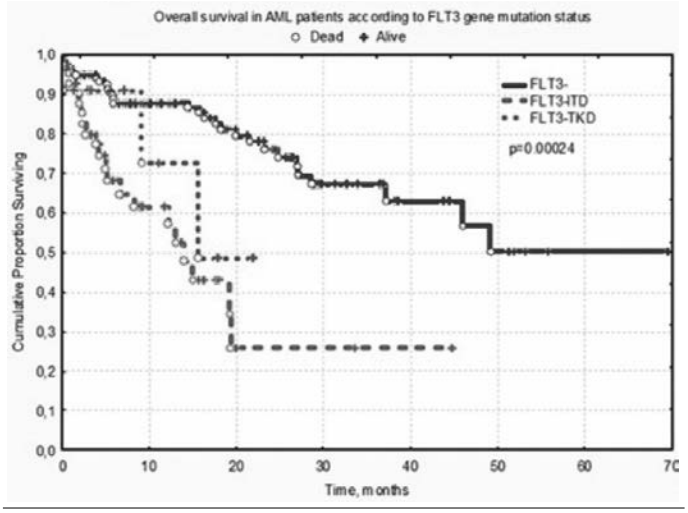

Figure 1.

Summary/Conclusions: We confirmed the role of FLT3 gene mutations as an unfavorable factor for AML patients in routine clinical practice by own experience. The investigation of qualitative assessment potential and target therapy value especially in SCT ineligible FLT3 gene mutations positive patients has of great value for AML management.

PB1683

TARGETING ENDOTHELIAL DYSFUNCTION FOR PROTECTION FROM ANTHRACYCLINE-INDUCED CARDIOTOXICITY IN PATIENTS WITH ACUTE LEUKEMIA AND CO-MORBID ISCHEMIC HEART DISEASE

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Background: Cardiotoxicity of chemotherapeutic drugs, in particular anthracycline antibiotics (AA), is one of the biggest problems in treatment of patients with acute leukemia (AL). Chemotherapy with AA is accompanied by systemic endothelial dysfunction, increasing the cardiovascular toxicity risk and promoting vascular complications. Patients with co-morbid ischemic heart disease (IHD) are at extremely high risk of myocardial injury and in need of anthracycline cardiotoxicity (AC) prevention.

Aims: To assess the effectiveness of L-arginine in the prevention of endothelial dysfunction as a predictor of acute AC in patients with AL and co-morbid ischemic heart disease.

Methods: A total of 66 patients with newly diagnosed acute leukemia (acute lymphoid leukemia – 7 patients, acute myeloid leukemia – 59 patients) and co-morbid ischemic heart disease were included in the study. The cohort consisted of 34 (51.5%) males and 32 (48.5%) females, age of 54–72 years, ECOG I-II. The duration of IHD ranged from 3 to 15 years. Chemotherapy (CT) schemes included AA (doxorubicin). The evaluation of endothelial dysfunction was performed by determining the stable metabolites of nitric oxide – nitrite anions $[NO_2]^-$ and activity of total NO-synthase in serum of patients before the CT and upon reaching a cumulative dose of AA from 100 to 200 mg/m^2 by doxorubicin. The mean total cumulative dose of AA reached 162,04±24,65 mg/m^{2}and 166,49±27,34 mg/m^{2}in groups I and II respectively. The study was approved by the local ethical committee and all patients gave a written consent before they were included in the study. Patients were divided into two groups: (n=36) – AL patients treated with CT; II (n=30) – AL patients, whom during the CT in order for prevention of acute AC were given L-arginine hydrochloride 4.2% 100 ml IV the day before and during administration of AA, followed by oral L-arginine aspartate for a month.

Results: In the debut of AL prior to the CT in all 66 (100%) patients the increased activity of total NOS in 3.8 times compared with the norm (p<0,001) was noted, with simultaneously reduced concentration of $[NO_2]^-$ in 1.5 times relatively normal values (p<0,05) (Table 1). As a result of two CT courses of remission induction in patients of group I the tendency to reduce the total NOS activity compared with its level before treatment was observed. At the same time the significant decrease of $[NO_2]^-$ in 1.8 times relatively normal values (p <0.01) and a trend to lower their content in 1.2 times compared with the data before treatment (p>0.05) was noted. These changes constitute the violation of NO-dependent vasodilation mechanism and endothelial dysfunction intensification. Provided achieving low cumulative dose of AA in patients of group II on the background of AC prevention with L-arginine showed a significant decrease in 1.9 times the total NOS activity (p <0.001) with a simultaneous tendency to increase concentration of $[NO_2]^-$ in 1.3 times (p>0.05) compared to that before treatment.

Table 1.

Indicators of total NOS and $[NO_2]^-$ in AL patients with co-morbid IHD in the CT dynamics.

Research groups		$[NO_2]^-$, μmol/L	NOS, μmol/L*min
Practically healthy (n=18)		3,2±0,38	0,61±0,08
pts of group I (n=36)	before CT	2,18±0,31*	2,3±0,14*
	after CT	1,8±0,21*	1,97±0,13*
pts of group II (n=30)	before CT	2,14±0,29*	2,1±0,14*
	after CT	2,4±0,33	1,2±0,11*√δ

Note: significant differences * – between indicators of healthy persons and in the groups (p<0.05); √ - between indicators before CT and upon reaching cumulative dose of AA 100-200 mg/m² (p<0.05), δ - between indicators of groups I and II in reaching cumulative dose of AA 100-200 mg/m² (p<0.05).

Summary/Conclusions: Thus, during the CT with the inclusion of AA without L-arginine in patients with AL and co-morbid IHD we observed the depletion of NO substrate production, accompanied by endothelial dysfunction impairment. The additional appointment of L-arginine on the background of CT can restore synthesis of NO and, respectively, the mechanism of NO-dependent vasodilation, thus reducing the risk of early anthracycline cardiotoxicity development.

PB1684

CLINICAL CHARACTERISTICS AND SURVIVAL OUTCOMES IN ACUTE ERYTHROID LEUKEMIA (AML-M6): AML/MDS WORKING PARTY STUDY OF KOREAN SOCIETY OF HEMATOLOGY

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Background: Acute erythroid leukemia is a morphologically distinct and rare entity designated as M6 in FAB classification. In Korea, patients with AML-M6 have been treated as acute myeloid leukemia with intensive chemotherapy whenever possible rather than as myelodysplastic syndrome. The 2016 revision of the WHO reclassified erythroid/myeloid subtype (a case with ≥50% BM erythroid precursors and ≥20% myeloblasts among non-erythroid cells) to MDS category based on the close biological and genetic relationships between them.
Aims: The aims of this multi-center study were to characterize clinical characteristics and treatment outcomes in patients with newly diagnosed acute erythroid leukemia.
Methods: Clinical data from newly diagnosed M6-AML patients between 2002 and 2012 at 11 academic centers were retrieved from the electronic registry data of AML/MDS working party of Korean Society of Hematology. Conventional cytogenetic analysis was performed on metaphase cells prepared from bone marrow aspirate by G-banding technique. Patients were classified according to the UK MRC cytogenetic risk criteria and the International Prognostic Scoring System (IPSS) risk groups for MDS based on karyotypes. Survival curves were analyzed using the Kaplan-Meier method and compared with a log-rank test. A p-value <0.05 was considered statistically significant.
Results: A total of 84 patients with AEL (M6-AML) as defined by 2008 WHO classification criteria were included in this study. The median age at diagnosis was 55 years with following distribution: age ≤ 49, 34 patients (40.5%); age 50 – 59, 17 (20.2%) patients; 60 – 69, 19 (22.6%) patients; age ≥70, 14 (16.7%) patients. There were 50 (59.5%) males and 34 (40.5%) females. Median hemoglobin, white blood cell count, and platelet count were 8 g/dL, 3.69 × 10^9/L, and 58 × 10^9/L, respectively. Peripheral blood blasts were observed in 55 (65.5%) patients. Cytogenetic results were available in 80 patients. Among them, karyotype was normal in 43 (53.8%) and complex in 13 (15.5%) patients, respectively. Trisomy 8 was observed in ten (12.5%) patients. Monosomies of chromosome 5 and 7 were observed in five (6.2%) and four (5.0%) patients, respectively. Four (5.0%) patients had t(9;22)(q34;q11.2). Cytogenetic risk groups according to UKMRC criteria were intermediate in 63 (78.8%) patients, and poor in 17 (21.2%) patients. Seventy-two (85.7%) patients received induction chemotherapy and 55 patients (76.4%) achieved complete remission. Nineteen patients received two or three cycles of induction chemotherapy. Thirty-eight patients (45.2%) underwent allogeneic hematopoietic stem cell transplantation (HSCT): 8 patients, matched-sibling donor; 15 patients, matched-unrelated donor; 5 patients, alternative donor were used. Treatment-related mortality of HSCT was observed in five (17.9%) patients. Fourteen (16.7%) among the study patients relapsed. The median overall survival (OS) of total 84 study patients was 21 months. Patients with intermediate risk karyotype showed better median OS than those with poor risk karyotype (22 months *vs* 7 months, P=0.020). The median OS was similar in patients with good and intermediate IPSS, but significantly worse in patients with poor IPSS (21 months, 27 months, 7 months, respectively, P=0.026) (Figure 1).

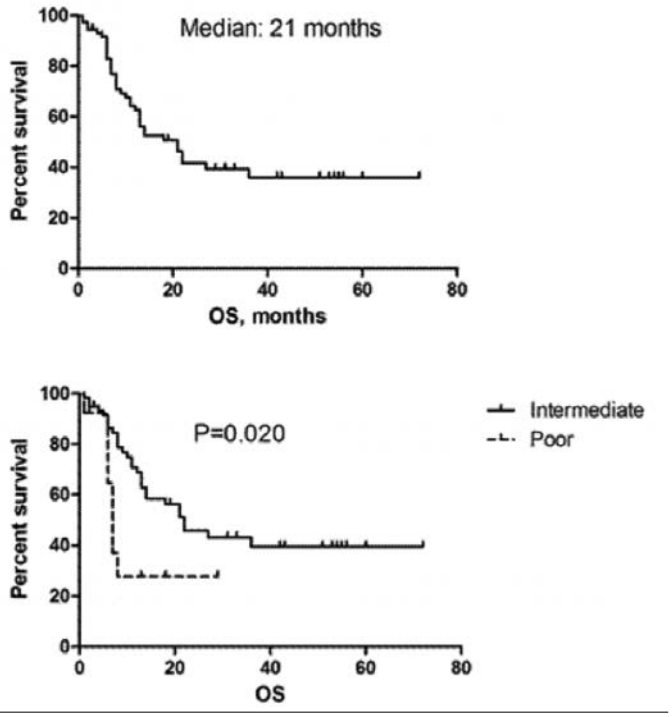

Figure 1.

Summary/Conclusions: Patients in this study were younger than previous studies. The most common aberrations in chromosomes are trisomy 8, followed by numerical changes in chromosome 5 and 7. The median in total patients was 21 months with many patients received intensive treatment, including HSCT in 45.2% of patients. We also confirmed that patients with poor-risk karyotypes had very poor median OS of 7 months. Therefore, we suggest that although erythroid/myeloid subtype is similar to the MDS with excess blast, treatment decision might be carefully considered according to the karyotype risk.

PB1685

PREGNANCY ACCUMULATES UNFAVORABLE MOLECULAR GENETIC AML AND SHOULD BE CONSIDERED AS A POOR PROGNOSTIC FACTOR

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Background: Acute myeloid leukemia (AML) during pregnancy – is a rare clin-

ical condition that limits the possibility to conduct large prospective clinical studies. All publications present small retrospective data and case reports. Most of them conclude that pregnancy doesn't affect the prognosis of acute leukemia.
Aims: To assess the pregnancy, as independent prognostic factor, in non APL AML-patients (pts), prospectively treated within Russian AML multicenter studies.
Methods: From 1990 to 2017 yy the Russian Acute Leukemia study group has treated 33 with *de novo* AML pregnant women (Me-27 (21-42) yrs). AML was diagnosed in the Ist trimester in 1 woman (3%), in the IInd– 15 (45,5%), in the IIIrd- 17 (51,5%). Molecular genetic risk group was estimated in 27/33 pts: 52% (n=14) were referred to the intermediate risk group and 48% (n=13) to the poor prognosis. High risk group comprised complex karyotype (n=5), -7/del7 (n=4), translocations involving gene MLL (n=2), 1 pt - inv3/-7 and 1 pt - AML with myelodysplasia-related changes, normal karyotype and FLT-3+.
In 1 pt at the 1st trimester medical abortion was conducted and 11 women delivered at the gestation age of 34-40 weeks before chemotherapy (CT). 21 pregnant women received CT, that was started at 23 (14-32nd) weeks of gestation. Classical 7+3 was applied in all of pts: either with daunorubicin (45-60 mg/m^2), or mitoxantrone (10 mg/m^2), or idarubicin (12 mg/m^2) regarding the treatment study-protocol.
Results: As our data show, AML in pregnancy is characterized by high prevalence of unfavorable cytogenetic abnormalities (48%), that is substantially different from AML in non-pregnant women of the same age (11,5%) (p=0,006) [Blood 2016,128;22,p.5171]. 1 pt died before CT due to septic shock, 2 pts – in induction CT now. 2 pregnant women died due to severe infections in aplasia during induction (5,7%). So, induction results were evaluated in 30/33 pts: CR rate - 73,3% (22/30): after the 1st course CT - in 16 and after the 2nd– in 6 pts. In pts, with available cytogenetic data, CR was received in 100% (9/9) from the intermediate and in 80,0% (8/10) from the poor prognostic group. Primary resistance was registered in 6/30 pts (20%). Antenatal fetal mortality was registered in 2 cases at the 21stand 32nd weeks during induction. 29 children were born. Allogenic bone marrow transplantation (allo-BMT) was done in 10 of 28 (35,7%) AML-pts who had survived induction therapy at a median of 6 months after CR. 4 pts relapsed after allo-BMT and 1 woman remained with refractory AML after allo-BMT. Our results demonstrated rather low 10-y OS and DFS (10,48% and 10,46%) in women, whom AML was diagnosed during pregnancy. In order to evaluated the role of allo-BMT, we performed a landmark analysis (landmark=6 months of CR), that has shown better OS and DFS only in pts after allo-BMT (Pic) (Figure 1).

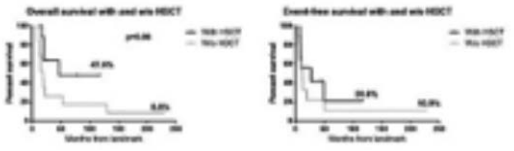

Figure 1.

Summary/Conclusions: Our results demonstrate: almost half of women, who were AML diagnosed during pregnancy, are referred to the poor molecular genetic prognostic group; they demonstrated very low OS and DFS whit their improvement after allo-BMT.

PB1686

CLOFARABINE IN RELAPSED-REFRACTORY ACUTE MYELOGENOUS LEUKEMIA: A SINGLE CENTRE EXPERIENCE

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Background: Clofarabine has been shown to be effective in AML patients, mainly in combination with high dose cytarabine
Aims: On the basis of these reports, we tested clofarabine in association with high dose cytarabine in relapsed/refractory AML patients, selecting cases of primary refractory to at least two induction therapies, relapsed but refractory to a standard re-induction treatment, or very early relapsed.
Methods: Betweeen 2008 and 2016 we treated 67 patients with a regimen including clofarabine at 22,5 mg/m^2 daily on days 1-5, followed after three hours by cytarabine at 1 gr/m^2 daily on days 1-5.
Among the 67 patients, 24 were in first relapse, 29 in second or third relapse, 14 with resistant disease. The mean age was 54 years (range .36-77 years).
Results: 20/67 patients achieved a complete remission (29,9%), 4/67 a partial response (6%) ,38/67 had resistant disease (56,7%), 3/67 died of complications during the aplastic phase (4,4%). The most frequent non haematologic adverse events were: transient liver toxicity (41% grade 1-2, 11% grade 3-4) skin rash (33%), vomiting (28%), diarrhea (15%). Comparing with other salvage strategies, in this cohort of patients we did not observe a significant delay in bone marrow recovery (median time to ANC recovery 21 days). Febrile neutropenia was observed in 58 cases (85%), with bacterial infections microbiologically documented in 20 patients (29%) and 2 cases (3%) of fungal infections. The median overall survival of the whole cohort was 115 days , with a median event free survival of 111 days. Among the responding patients, 16 (24%). underwent allogeneic bone marrow transplantation; in these selected patients, median overall survival was 185 days.
Summary/Conclusions: These results suggest that the clofarabine-ARA-C regimen was able to induce a response in about one third of this particularly poor prognosis category of patients, with safety data consistent with previously reported salvage therapies. Nevertheless, long term results remain still and completely unsatisfactory. Further studies, with different combinations or in more selecting conditions, are warranted.

PB1687

PRESENCE OF MULTIPLE DRIVERS IN THE SELECTION OF HIGH AND LOW INTENSITY CHEMOTHERAPY IN AML

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Background: Data on the key drivers of initial treatment choice for patients diagnosed with acute myeloid leukemia (AML) in the United States is limited. The use of age as a selection driver of induction therapy is well established; however, there is limited data and a knowledge gap about additional factors driving treatment selection.
Aims: This analysis explored the key physician drivers, which led to the selection of high- and low-intensity induction therapy in AML patients.
Methods: Data from the Adelphi AML Disease Specific Programme, a real-world, cross-sectional survey conducted between February–May 2015, was analyzed. A total of 61 hematologists/oncologists provided attitudinal information about their management and treatment choices for AML patients via survey. Each physician was provided a pre-specified list of 16 patient characteristics. Via two separate questions, they were asked to select those considered important when choosing high and low intensity chemotherapy for their AML patients. Characteristics were analysed descriptively and ranked based on the frequency of mention from highest to lowest.
Results: The top three drivers for decision making when selecting high and low intensity treatment were: patient age, performance status and presence of comorbidities. More than 60% of physicians would prescribe high-intensity treatment to patients aged under 65, with a good performance status or with no comorbid conditions. Over half of physicians would consider those who are eligible for a stem cell transplant or have a mutation in the CEBPA gene to be eligible for high-intensity chemotherapy (Table 1). Low-intensity chemotherapy was considered by more than 60% of physicians as being the most appropriate treatment for patients aged ≥65, with a poor performance status or increased number of comorbid conditions. A total of 38% of physicians would likely consider low-intensity chemotherapy if the patient was ineligible for a stem cell transplant or had had previous cancers or exposure to radiation/chemotherapy in the past.

Table 1. Top 5 patient characteristics considered by physicians when choosing high- or low-intensity treatment in AML.

Top 5 drivers of selection	Total Physicians (N=61)
High-intensity chemotherapy	
Patients aged <65 years	41 (67%)
Good performance status (ECOG score 0-1)	39 (64%)
Patients without comorbidities	37 (61%)
Patients eligible for stem cell transplant	33 (54%)
Patients with mutation in the CEBPA gene	33 (54%)
Low-intensity chemotherapy	
Patients aged ≥65 years	41 (67%)
Very poor / poor performance status (ECOG score 2+)	38 (62%)
Patients with comorbidities	38 (62%)
Patients ineligible for stem cell transplant	23 (38%)
Patients with prior cancers / previous to radiation therapy or chemotherapy	23 (38%)

Summary/Conclusions: Irrespective of treatment intensity, patient age, performance status and the presence of comorbidities are the top three drivers of treatment selection for physicians. In addition to patient age, identification of the other key drivers for therapy selection and the physician awareness of them is critical to ensure patients receive the most appropriate therapy. This improved awareness could also lead to better communication tools for patients and improve shared decision-making.

PB1688

IRAIN LONG NON CODING RNA ARE DOWN-REGULATED IN POOR PROGNOSIS AML PATIENTS

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Background: *IRAIN* which is produced from the insulin-like growth factor type I receptor (IGF1R) imprinted locus is a newly identified lncRNA. There are very little knowledge about the specific role of this lncRNA in tumorogenesis presses. Recent studies were revealed that *IRAIN* is down-regulated in leukemia cell lines and viral expression of the *IRAIN* lncNRA inhibits tumor cell migration, suggesting a tumor suppressor function for this transcript.
Aims: In this study, we attempted to examine the expression level of *IRAIN* in different cytogenetic subtypes of AML patients.
Methods: Using quantitative polymerase chain reaction (qPCR) the expression level of *IRAIN* were analyzed in bone marrow specimen of AML patients (n=76) and healthy individuals (n=18).
Results: IRAIN expression was found to be remarkably decreased in AML patients compared with healthy individuals (p= 0.02). Significant IRAIN down-regulation was observed in all FAB types except for the M3 (p= 0.11). When we analyzed the expression level of *IRAIN in* different cytogenetic subtypes of AML patients the statistically down-regulation of IRAIN was observed only in poor prognosis AML patients (p= 0.008).
Summary/Conclusions: Our results suggest that down-regulation of IRAIN lncNRA might play a role in the AML development and hence may be a potential prognostic factor and serve as therapeutic target for AML treatment.

PB1689

PERFORMANCE OF THE LEUKOSTRAT® CDX FLT3 MUTATION SIGNAL RATIO ASSAY TO DETECT INTERNAL TANDEM DUPLICATION AND TYROSINE KINASE DOMAIN MUTATIONS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) in general has a poor prognosis. Assessment of the mutation status of the *FLT3* (fms related tyrosine kinase 3) receptor gene in AML is the most important prognostic indicator of disease outcome, which is often substantial, as many studies in AML have shown that the presence of *FLT3* activating mutations portends a poor prognosis. The LeukoStrat® CDx *FLT3* Mutation Assay targets regions of the *FLT3* gene to identify internal tandem duplication (ITD) mutations and tyrosine kinase domain (TKD) mutations. Since this assay is a signal ratio (SR) assay with a validated cutoff of 0.05, demonstration of international harmonization of results is paramount. *FLT3* ITD mutations are caused by duplication and insertion of a portion of the *FLT3* gene that includes the region in and around the juxtamembrane region of the *FLT3* gene. These mutations vary in both the location and the length of the inserted duplicated DNA sequence. ITD mutations result in constitutive autophosphorylation and activation of *FLT3. FLT3* TKD mutations are caused by nucleic acid substitutions and/or deletions that result in a change in the amino acid sequence in this highly-conserved catalytic center. TKD mutations, such as D835 and I836 substitutions and deletions, result in constitutive autophosphorylation and activation of *FLT3.*
Aims: To assess the performance of the Invivoscribe® LeukoStrat® CDx *FLT3* Mutation Assay.
Methods: White blood cells were removed from peripheral blood after 30 minutes of centrifugation at 2000 xg to create leukocyte depleted blood (LDB). Various ratios of four ITD positive cell lines, with insert sizes from 21 bp to 279 bp, and one TKD positive cell line, with a D835 substitution mutation, were created over a wide range of signal ratios (0.02 to 1.83) and added to the LDB. Mononuclear cells were isolated from the contrived LDB samples. DNA was extracted and amplified via PCR. The amplicons were analyzed via capillary electrophoresis. The assay measured the ratio of signals from mutation against a background of signal from wild type. A *FLT3* mutation was detected (and reported as positive) if the mutant:WT type SR met or exceeded the clinical cut-off of 0.05. Proprietary software calculated the SR and reported positive or negative. Clinical specimens were de-identified by LabPMM in San Diego. DNA from twenty specimens was tested by three laboratories: LabPMM LLC in San Diego, LabPMM GmbH in Germany and LabPMM Gk in Japan.
Results: The analytical performance of the LeukoStrat® CDx *FLT3* Mutation Assay was evaluated using contrived LDB samples, with known *FLT3* mutations. For limit of blank (LoB), the SR was 0.00 in the ITD assay and 0.00 to 0.01 in the TKD assay, which is well below the clinical cutoff SR of 0.05. The limit of detection in the ITD assay detected allelic ratios of 0.03, 0.05, and 0.33 above the LoB SR in more than 95% of samples for insertions sized at 30 bp, 126 bp and 279 bp, respectively. The limit of detection in the TKD assay detected an allelic ratio of 0.05 above the LoB. For precision and reproducibility, the SR%CV was within 3-14% across ITD and TKD mutation types regardless of reagent lots, equipment, or operator.
There was 100% agreement between all three clinical LabPMM laboratory sites.
Summary/Conclusions: This robust assay produced a SR%CV less than 15% regardless of reagent lot, equipment or operator. The high reproducibility between the three laboratories on three different continents provides evidence that the Invivoscribe® LeukoStrat® CDx *FLT3* Mutation Assay is an internationally standardized assay.

PB1690

CLINICAL FEATURES AND OUTCOME OF PATIENTS WITH CORE BINDING FACTOR ACUTE MYELOID LEUKEMIA

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Background: Acute Myeloid leukemia is classified into different prognostic groups according to their cytogenetic profil; AML with t (8; 21) or inv16/ t (16; 16) called AML CBF belong to a prognostic group of low-risk; they represent 15% to 20% of the AML.
Aims: The aim of this study is to present clinical , cytogentic features and outcome of this group of patients (pts) in an emerging country.
Methods: Cytologic diagnosis of AML CBF is completed by immunophenotyping and cytogenetic analysis:
t(8; 21), inversion 16/t (16.16) and del 16q22. Induction treatment: Daunorubicin 45 to 90 mg/m² day (d1-d3)+Cytarabine 100 mg/m² (d1- d7) (with progressive doses if major leukocytosis). Assessment between d 21 and d 28 by bone marrow analysis ; If failure a second course induction is performed by Cytarabine high dose 3 g m²/12 h d1, d3, d5. Central nervous system prophylaxis for patients (pts) with AML4, M5 and hyperleukocytosis forms. Consolidation: Cytarabine high dose: 2 to 3 cures; low dose of chemotherapy for pts older than 55 years or pts presented severe toxicity at the first induction. Research of Kit, FLT3 and residual disease is not available in our laboratory, hematological stem cell transplantation (HSCT) was proposed for all pts; if no compatible donor, 3 courses of Cytarabine high doses was instituted.
Results: From 2010 to 2016, cytogenetic analysis was performed in all cases of AML of which 58 cases (18,6%) of LAM - CBF were diagnosed. The male to female ratio was 0.5 ; average age: 37 years (16-72); t (8,21) was found in 28 pts (16 M,12F); inversion (16)(p13.11;q22.1), t(16,16)(p13.11;q22.1) and del 16q22 were found in 30 pts (12M,18F), respectively in 27 pts, 2 pts and 1 pt. Four cases of del(16)(p13) were associated with inv(16). For inv(16), FAB subtypes were AML4 (26), AML2 (1) and 3 AML; For t(8;21), there was 26 AML2 and 2 AML4. Evaluation of induction: not evaluable: 13 cases, Complete Remission (CR): 38 cases (65,5%); for 7 cases in failure , a second induction was proposed, we obtained 2 CR. 15 pts were transplanted. Outcome: 27 pts are alive in CR of which 12 transplanted . 31 pts died of which 18 toxic deaths (15 after induction treatment and 3 after engraftment). Median overal survival for inv(16) : 11 months *vs* 15 months for t(8;21) (p=0,87).
Summary/Conclusions: In our study, the frequency of the CBF AML is closer than those described in another Algerian study and literature: 18,6% *vs* 15.4% and 20% respectively; a slight predominance of the inv 16 or t (16; 16) identical to that reported by the SWOG study. The RC rate is under to that reported by the CALGB study. We noted less relapse compared with literature. Relapses were observed in pts with poor prognostic factors: age, leukocytosis and failure to first induction. Regarding the favorable prognosis of AML CBF, our results are bad because the high rate of toxic deaths. The CBF AML are characterized by a better prognosis, but with a 30% relapse rate essentially when associate poor prognosis factors such as a kit mutation that increases the risk to 70%, mutation FLT3, advanced age, the leukocytosis, severe thrombocytopenia and additional cytogenetic abnormalities.

PB1691

FLOW CYTOMETRY ANALYSIS SOFTWARE FOR REMOTELY LOCATED HAEMATOLOGISTS

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Background: Current flow cytometry software packages are unsuitable in cases where the interpreter of the data isn't physically located at the computer with the software installed. This is particularly disadvantageous in urgent situations, such as in the diagnosis of acute leukemia.
Aims: Develop a tool to allow haematologists to analyse flow cytometry data from anywhere on any internet-enabled device *e.g.* tablet, smartphone, laptop, PC.
Methods: We came up with principles a new software package should adhere to: 1. should be accessible from any Internet-enabled device *e.g.* iPad, Android phone, Blackberry, laptop; 2. should not require installation; 3. FCS data should be anonymised; 4. data transfer should be secure and encrypted; 5. software must include all basic functionality of flow cytometry software *e.g.* dot plot graphs, histogram graphs and gating 6. should put collaboration to the forefront *e.g.* analysis can be instantly linked to via a web URL.
Results: The resulting software package is a web app which is accessible from any Internet-enabled device *e.g.* smartphone, tablet, laptop or PC. On mobile devices such as an iPad, touch is used for drawing of gates, selection of quadrants, selections of parameters etc. On laptop's and PCs, these are drawn via

the mouse or keypad. The software utilises the latest strides made in web technologies to respond to the varying screen sizes of devices, and display suitably sized graphs and gating information accordingly. Collaboration between parties is facilitated - a lab technician running the sample can upload the sample and instantly shared with other parties with the required permissions. Analysis, such as gating, can take place immediately and can then be instantly shared via a web URL. No sensitive file data is displayed within the platform. All data transfer happens via SSL encryption.
Web app is available at https://www.redmatterapp.com

Figure 1.

Summary/Conclusions: The latest web technologies can be effectively harnessed to enhance flow cytometry analysis and allow for faster, more accessible and more collaborative analysis. Within the field of haematology in particular, this opens up the option of remote diagnosis - a haematologist need to be in the lab, or even in the same country, to deliver a diagnosis.

PB1692

FLAG-IDA IN THE TREATMENT OF ACUTE LEUKEMIA: SINGLE-CENTER EXPERIENCE

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Background: A variety of different treatment regimens have been studied in an effort to improve outcomes of patients with relapsed or refractory acute myeloid leukemia (RR-AML), there appears to be no single superior approach. Spanish groups usually use the FLAG-IDA protocol (Fludarabine 30 mg/m2 days 1-4, Idarubicine 12 mg/m2 days 1-3, ara-C 2 mg/m2 days 1-5) in these patients.
Aims: To evaluate our response rates and the survival with FLAG-IDA protocol.
Methods: Descriptive study of a case series of patients with acute leukemia who received intensive induction chemotherapy with FLAG-IDA protocol at our hospital between January 2007 and December 2016. Biodemographic, histopathological, cytogenetic and molecular results and previous treatment were recorded. We analyzed the response rate, the 30-day mortality rate and the overall survival.

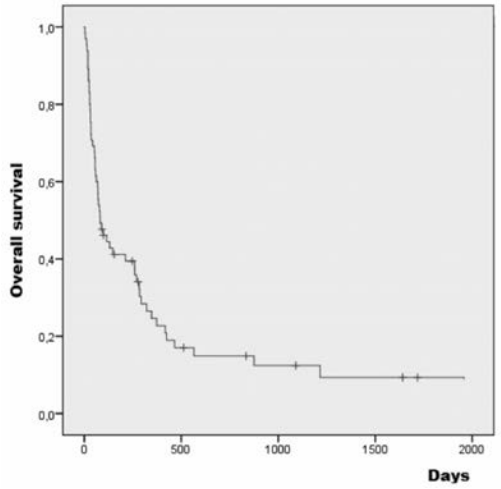

Figure 1.

Results: 65 patients received treatment with FLAG-IDA protocol between 2007-2016, 36 of them female, with and average age of 53.4 years (DS+/-23.3). We treated with this protocol mostly patients with relapsed or refractory acute myeloid leukemia (RR-AML) (primary refractory or resistant AML as defined by not achieving complete remission after 1 cycle of intense induction therapy); 60% (n=38) of patients had a RR-AML, 37% (n=23) of them were relapsed AML and 23% (n=15) refractory AML. Based on European Prognostic Index Score (EPI-SCORE) for patients with RR-AML, 61% of them had a poor prognosis (10-14 points), 36% had an intermediate prognosis (7-9 points) and only 3% had a favorable prognosis (1-6 points). The next important group, 25% (n=17) were MDS patients transformed to AML. We had 9% (n=6) patients with treatment related AML and 6% with other acute leukemia (3 cases of refractory ALL and 1 case of biphenotypic leukemia). We observed a global response rate of 63%: 51% (n=33) of patients had a complete response (CR) and 12% (n=8) partial response, 17% (n=11) did not have a response and 20% of patients were not evaluated after to receive the treatment because they had a early dead. The 30-days mortality rate was 21.5% (n=14), similar to the rate of no evaluated patients. We can see in the overall survival curve (picture 1) that most patients died first months after treatment, after that patients remain alive and we achieve a plateau. The median overall survival was 82 days (standard deviation: 25 days). 10 patients were alive when we analyzed the data (Figure 1).
Summary/Conclusions: Most AML patients ultimately die from their disease. In our case serie none died by any other cause. We had a similar response rate, mortality and overall survival that other groups in our country. Despite a variety of salvage therapy options, like FLAG-IDA protocol, prognosis in patients with RR-AML is generally poor and treatment is very complex.

PB1693

BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASMS - UNUSUAL PRESENTATIONS AND UNFAVOURABLE OUTCOMES

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Background: Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare hematological malignancy with an aggressive clinical course. Most patients (pts) with BPDCN have skin lesions and involvement of the peripheral blood, bone marrow, and lymph nodes. Very few cases have been described with lack of skin and or bone marrow manifestations at the time of diagnosis.
Aims: To characterise the clinical presentation and clinical outcomes of a cohort of consecutive patients with a rare blastic plasmacytoid dendritic cell neoplasm in a single institution.
Methods: Patients diagnosed with BPDCN at the National Hematology Hospital between 2010 and 2016 were retrieved from the database. The diagnosis was confirmed by morphology and immunophenotying by flow cytometry and/or immunohistochemistry, according to 2008 WHO Classification of Hematopoietic Neoplasms. The relevant clinicopathologic features were reviewed.
Results: We identified 8 adult patients at a median age of 70 years (range: 37-84 years) with a male:female ratio of 6:2 (75%:25%) and only 1 child. Mean values of blood cell counts were as follows: WBC 5.10^9/L; hemoglobin 99 g/L; platelets 116.10^9/L. LDH was generally elevated with a mean of 962.8 U/L. At diagnosis the skin was invoved in 5/9 patients. Five patients developed leukemic presentation with 40-95% of bone marrow infiltration. Interestingly, in 4 pts (50% of adult pts) the initial presentation affected other tissues and organs such as testis, bronchial wall, stomach and periorbital soft tissues, however, only the latter one case presented with a leukemic picture. Biopsies revealed diffuse, monomorphous infiltrate of medium-sized blast cells with irregular nuclei, fine chromatin with ≥1 nucleoli, scant and agranular cytoplasm, without angioinvasion or coagulation necrosis. Immunophenotype generally demonstrated CD45+, CD4+, CD56+, CD123+. No standard therapies were applied. Patients received CHOP or HyperCVAD or AML-induction therapy. However, response rates in adult patients were low and the mean OS was 2.6 months (ranging from early deaths before any treatment could be initiated to 10 months).
Summary/Conclusions: BPDCN is a rare aggressive disease that typically affects elderly patients. The most commonly affected non-hematopoietic organ is the skin, however any other organ or tissues can also be involved. Response to therapy if any is relatively short and long-term prognosis is poor despite of the site of presentation. Larger scale studies are warranted to understand the pathophysiology of the disease and to find optimal management.
Acknowledgements: Partial support by the National Science Fund.

PB1694

PREDICTIVE RELEVANCE OF CLINICAL CHARACTERISTICS IN PEDIATRIC PATIENTS WITH RELAPSED ACUTE MYELOID LEUKEMIA TREATED AT SINGLE INSTITUTION– REPORT OF AN OUTCOME ANALYSIS

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Background: Western hospitals have achieved First Complete Remission (CR-1) and Overall Survival (OS) rates of 90% and 60% for children with Acute Myeloid Leukemia (AML). Intensified regimens of standard chemotherapy along with precise risk classification and improvements in supportive care are mainly attributed to this achievement.
Aims: We analyzed clinical data of our pediatric AML patients treated at KFSH&RC from 2005 to 2015 in order to assess the outcome of our treatment efforts including Hematopoietic Stem Cell Transplantation (HSCT).
Methods: A total of 155 pediatric patients with AML were registered at our institution from 2005 to 2015. 55.5% (86) were boys with a F:M ratio 1:1.2 and median age at diagnosis 5.5 years (Min: 1.3months, Max: 13.8 years). 12 patients were excluded from further analysis for not being able to complete induction at our institution. 7% (10 of 143) had Down 's syndrome, 7.7% (11 of 143) had concomitant malignancies. 85.7% (120 of 140) were CNS-1, 27.4% (20 of 73) had MLL Gene rearrangement, 21.2% (14 of 66) were positive for TEL AML/RUNX1/RUNX1T1 and 22% (13 of 59) had PML/RAR (+). Trisomy 4, 10 or 17 was not seen among any of 13 patients tested. Most commonly observed FAB classification was M-5 (23.5%, 24 of 102) followed by M-2 (18.6%). 27.3% (39) were Low Risk, 43.4% (62) Intermediate and 29.4%(42) High Risk. 43.3% (58 of 134) received HSCT.
Results: Our CR-1 rate was 93.7% (134 of 143) with 100% in Low Risk, 95.2% Intermediate Risk and 85.7% in High Risk patients (P-Value: 0.023), requiring 1-3 cycles of chemotherapy with a median time of 1.3 months. Treatment Failure was observed in 6.3% (9 of 143). Relapse rates was 38.8%(52 of 134). Most common site of relapse was bone marrow (75%, 39 of 52). PML/RAR (P-Value: 0.044), Post-Induction BM Classification M-3 (P-Value: 0.034) and AML High Risk (P-Value: 0.003) were found to be significantly associated with Relapse. Age at diagnosis, or Time to CR-1 were not found to have any association with relapse. 51.9% (27 of 52) who relapsed, went for HSCT. With a median follow-up of 68.8 months, five year overall survival for our cohort of patients was (0.567±0.046); significantly poor (P-Value: 0.001) in relapsed (n=52, 0.137±0.051) compared to non-relapsed (n=82, 0.863±0.041); resulting in a five year event free survival of 0.472±0.044. Among relapsed group (n=52), five year overall survival was significantly better (0.160±0.073) for those who received HSCT (27) than who did not (n=25, 0.114±0.073, P-Value: 0.029). Five year overall survival was also significantly better for Non-Relapse group (n=31, 0.828±0.070) compared to relapsed patients (n=27, 0.160±0.073, P-Value=0.001) for whom HSCT was administered (n=58)
Summary/Conclusions: The results of our treatment efforts are in conformity with the western literature. Precise risk classification can be a vital predictor in planning for first line and salvage therapies including HSCT for pediatric patients with AML.

PB1695

IS HIF-2 ALPHA A POOR PROGNOSIS FACTOR IN HUMAN ACUTE MYELOID LEUKEMIA? A SINGLE CENTER ANALYSIS - PRELIMINARY RESULTS

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Background: Hypoxia-inducible transcription factors (HIF) are well known regulators of cellular response to hypoxia. HIFs control functional, metabolic and vascular adaptation to hypoxia on transcriptional level. HIF-1 alpha has been also described to be responsible for solid tumors chemoresistance, invasion, metastasis and relapse. The role of HIFs in leukemias has not been established well so far. First reports of poor outcomes of antileukemic treatment linked with overexpression of HIF-1α has been published. Moreover another HIF subunit - HIF-2 alpha - has been described in mouse model as increasing myeloid preleukemic cell proliferation and accelerating disease progression with reduced survival. On this background, we found interesting if HIF-s alpha expression in acute myeloid leukemia cells (AML) influences the prognosis in human. We have tried to find a connection between AML cells percentage expressing HIF-2 alpha and first line chemotherapy results.
Aims: The aim of the study was to determine the roole of HIF-2 alpha in human AML.
Methods: We analyzed a 26 primary AML patients group (median age 54.5 (21-77), F/M – 13/13). The group consisted of 21 AML-NOS cases, 2 AML cases with inv(16), one case with t(6;9) and one with t(9;11) according WHO classification. ELN cytogenetic risk stratification divided the group into intermdiate-1, intermediate-2 and adverse cases in 10, 12 and 4 patients respectively. All patients were treated with Daunorubicine, Cytarabine and Cladribine based first line chemotherapy. We collect bone marrow and blood samples before chemotherapy and blood samples alone 48 hours after chemotherapy start. In all samples leukemic blasts were counted and determined by flow cytometry and the subpopulation of HIF-2 alpha positive blasts was estimated as well. Volunteer bone marrow donors were the control group in this study and the CD34+HIF-2α+ subpopulation was assessed in their bone marrow samples during the routine harvest procedure. The study was approved by the local Ethics Committee.
Results: After the first line chemotherapy 15 patients achieved complete remission (CR group) and 11 did not (NR group). We did not find significant differences between the groups regarding patients age, the mean percentage of blasts in bone marrow and blood before the treatment, the percentage of HIF-2 alpha positive blasts in BM and blood before and 48 hours after the treatment start (data not shown). But the analysis of the percentage of HIF-2 alpha positive blasts in blood before and 48 hours separately in CR and NR groups revealed quite different dynamics. In CR group the mean percentage of HIF-2 alpha positive blasts was 14.65 (±33.32) and 8.48 (±11.63) before and after chemotherapy respectively (p=NS); in NR group the values were 11.74 (±22.6) and 24.01 (±33.68) respectively (p=0.007) (Figure 1). The Cox analysis revealed HIF-2 alpha positive blasts in blood after chemotherapy to be proportional to death probability (p=0.0036) (Figure 2).

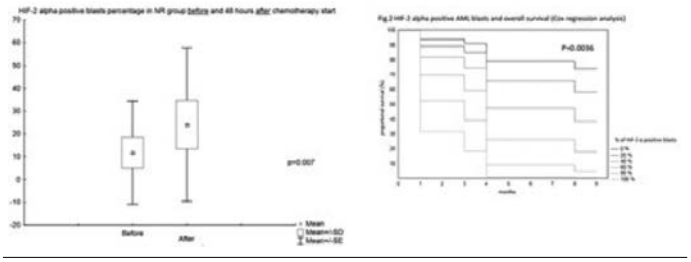

Figure and 2.

Summary/Conclusions: We are aware our results are preliminary. But if they are confirmed it will be very interesting to determine the role of HIF-2 alpha inhibitors in improving the prognosis and survival in human AML.

PB1696

RARE BCR/ABL FUSION PROTEINS AND THEIR CLINICAL SIGNIFICANCE INTO PH+ ACUTE MYELOID LEUKEMIAS

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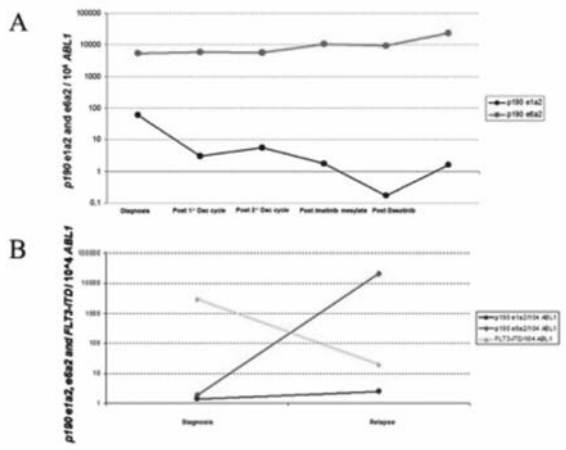
Background: The Philadelphia (Ph) t(9;22)(q34;q11) results in an oncogenic *BCR/ABL* gene fusion, representing the hallmark of chronic myeloid leukemia (CML), although it has been also described in acute lymphoblatic (ALL) and myeloid (AML) leukemia. Three main different transcripts have been described (p210, p190 and p230), but rare atypical *BCR* breakpoints outside the cluster regions have been also reported and their clinical significance is under investigation. Atypical transcript p190 e6a2 is a rare fusion protein associated with aggressive phenotype and dismal prognosis. The breakpoint in *BCR* intron 6 is responsible for increased kinase activity and greater transforming potential because of the partial loss of the Guanine Exchange Factor (GEF)/dbl-like domain, completely absent in p190 proteins. This truncation could increase the BCR/ABL oncogenic activity.
Aims: In this report we describe 2 rare cases of Ph+ AML patients with the atypical p190 e6a2 isoform.
Methods: Routine morphologic, immunophenotypic and genetic analyses were carried out in all samples at diagnosis. cDNA extracted from bone marrow was synthesized from 1 µg of total RNA. Most common AML genetic alterations were investigated and a quantitative RT-PCR (qRT-PCR) for p190 transcripts was performed. qRT-PCR assay for *FLT3*-ITD and p190 e6a2 transcript were developed.
Results: Case 1. A 78-years old male was admitted at our hospital with clinical and laboratory features allowing to make the diagnosis of AML. No evidence of a preceding CML (splenomegaly or basophilia) was found. The karyotype on G-banded metaphases was 46,XY, t(9;22)(q34;q11). While the molecular analysis was ongoing, the patient started treatment based on decitabine. The molecular biology analysis revealed the simultaneous presence of the common p190 e1a2 and the rare e6a2 isoforms (Figure 1A). Because of persisted pancytopenia and presence of blasts, according to the molecular data, he was then switched to TKIs treatment. Nevertheless, after 2 months, the patient was still refractory to second line treatment dying because of a pulmonary infection. Case 2. A 61-years old male was admitted with a diagnosis of AML 46XY, *FLT3*-ITD post MDS. Immediately, after cytoreduction, chemotherapy was started, obtaining the complete remission. Because of infectious complications, the

consolidation chemotherapy was postponed, relapsing without reach the already planned allogeneic transplantation. At the relapse karyotype was 46XY, t(9;22)(q34;q11) and the molecular biology showed the presence of p190 e1a2 and e6a2 isoforms and *FLT3*-ITD mutations with a low mutant allelic burden (Figure 1B). Salvage chemotherapy was then performed, allowing at this time to obtain disease remission and further allogeneic transplantation. Nevertheless, the patient died 5 months later for transplant complications. qRT-PCR assays performed in diagnosis sample showed the main clone *FLT3*-ITD accompanied by subclones with p190 e1a2 and e6a2 isoforms. These data indicate a clonal selection process and the expansion of a resistant clone with p190 e6a2.



Kinetic's comparison of P190 e1a2, e6a2 and *FLT3*-ITD transcripts in (A) UPN1 and (B) UPN2. Dac: Decitabine

Figure 1.

Summary/Conclusions: The atypical p190 e6a2 transcript seems to be associated in AML with aggressive disease. TKI therapy alone does not seem to control the disease. Prompt observations on these patients carrying rare *BCR/ABL* transcripts may allow help to establish optimal treatment approaches on these aggressive BCR/ABL phenotypes.

PB1697

HYPOMETILATING AGENTS AS SALVAGE THERAPY IN RELAPSED OR REFRACTORY AML: A 2-CENTERS RETROSPECTIVE STUDY

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Background: 5-azacytidine and decitabine have been widely studied as first line chemotherapy in acute myeloid leukemia (AML) patients not eligible for allogenic stem cell transplantation, but data on their use as salvage chemotherapy are limited.
Aims: To define efficacy and feasibility of hypometilating agents (HMA) as salvage chemotherapy in patients without previous allogenic stem cell transplantation.
Methods: We retrospectively reviewed clinical records of 15 patients treated with HMA as salvage therapy in our institutions since their introduction in clinical practice for AML patients.
Results: Median age was 66 years. Six patients were men and 9 women. One patient was AML with t(8;21), 7 were AML MRC, 1 was therapy related AML, 6 were AML NOS. Two patients were favorable risk sec ELN 2010, 11 were intermediate I and II and 2 were adverse risk. 67% of patients received HMA as second line therapy for their disease, 27% as third line and 6% were beyond the third line. Seven patients were treated with decitabine and 8 with azacitidine. Five patients reached CR or CRi after HMA. All patients underwent intensive chemotherapy (*i.e.* FLAI like or 3+7 like) as first line induction, and we excluded patients who had a HMA as first line chemotherapy and another one as second line. Median number of hospitalization days during HMA therapy was 16; median number of HMA cycles was 2 (range 1-31). 26% of patients underwent allogenic stem cell transplantation after HMA therapy. Median OS was 197 days from the starting of HMA and median EFS was 70 days. Median OS in patients with refractory disease was 91 days and median OS in relapsed patients was 331 days (p=0,0049). Median EFS in patients with refractory disease was 57 days and median EFS in patients with relapsed disease was 198 days (p=0,039). We did not find significant differences between transfusion needs before and after salvage therapy but this could be due to the small size of our sample.
Summary/Conclusions: HMA showed efficacy and a considerable OS in our patients. In our cohort refractory patients were almost all refractory to HMA too, and their OS was dismal. So HMA could be a good clinical option in a selected population of relapsed patients, especially in those not suitable for allogenic bone marrow transplantation, in whom the prognosis is generally extremely poor. Further studies are needed to determine which are the cytogenetic subsets of patients who could benefit from such a salvage chemotherapy.

PB1698

OMITTING CYTARABINE FROM CHEMOTHERAPY FOR ACUTE PROMYELOCYTIC LEUKEMIA REDUCES TOXICITY AND NOT EFFICACY

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Background: The introduction of retinoic acid (ATRA) has changed the treatment paradigm in Acute Promyelocytic Leukemia (APL). Combination of ATRA plus anthracycline-based induction (AIDA) without cytarabine (AraC) has shown high efficacy in Spanish and Italian studies. However, early mortality resulting from coagulation disorders remains high. Furthermore, AraC administration during consolidation is questioned and often limited to high-risk patients.
Aims: We aim to compare the efficacy, tolerance and toxicity between 2 consecutive treatment protocols that differed in AraC administration during consolidation.
Methods: We studied clinical characteristics, prognostic factors, response to treatment, tolerance, toxicity and outcomes in APL patients treated in our Department during the last decade. All patients received induction with AIDA (Idarubicin x4, ATRA until remission) and 2-year maintenance therapy. Protocol 1 included 2 cycles of consolidation with anthracyclines/AraC. Protocol 2 was implemented the last 5 years and included 3 cycles of anthracyclines and AraC only in high-risk patients (PETHEMA LPA2005).
Results: APL was diagnosed in 35 patients, of whom 2 patients older than 80 years did not receive treatment and were not included in the analysis. The rest 18 male: 15 female patients aged 37 (10-75) years old presented at diagnosis with: thrombocytopenia (32), leukopenia (22), leukocytosis (6), impaired performance status/PS >2 (10), lactate dehydrogenase >400 IU (17), increased d-dimers (33), low fibrinogen (11), fibirinogen <1 mg/dl (5). Five patients died during induction from severe differentiation syndrome (2), bleeding (2) and infection (1). In the multivariate analysis, these patients had significantly impaired PS (3, p=0.005), older age (median of 59 years, p=0.014) and lower fibrinogen (median of 0.9 mg/dl, p=0.05). Among 28 patients eligible for the comparison, all patients achieved complete remission (CR=100%). Protocol 1 (AraC) was applied to 16 patients and 2 to 12 patients. Complete molecular remission was achieved after a median of 2 chemotherapies (1-3). Efficacy could not be compared between protocols because there was only 1 relapse in Protocol 2, refractory to chemotherapy, ATRA, arsenic trioxide and allogeneic transplantation. However, there were significant differences in tolerance and toxicity. Patients in Protocol 1 had significantly higher transfusion needs compared to Protocol 2(p<0.001): 9(2-15) *versus* 1(0-17) red blood cell and 11(3-32) *versus* 2(0-10) platelet transfusion. Duration of grade 4 leukopenia was significantly higher in Protocol 1 [16(5-19) *versus* 9(0-18) days, p=0.002]. The same was true for neutropenia (p=0.04) and resulted to higher infection rates in Protocol 1 (58% *versus* 17%, p=0.03), including 2 aspergilloses and 1 fatal sepsis. 10-year overall survival probability was 73.1%, with no difference between Protocols.
Summary/Conclusions: Our study confirms that early mortality is a significant issue in APL, in particular for older patients. AraC can be safely omitted from treatment of low- and intermediate-risk patients, resulting in significantly reduced toxicity.

PB1699

DISEASE CHARACTERISTICS AND TREATMENT PATTERNS OF AML PATIENTS <60 YEARS OLD *VERSUS* ≥60 YEARS OLD

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Background: There is limited real-world data in patients with acute myeloid leukemia (AML) that looks at presenting disease characteristics and subsequent treatment decisions made for patients <60 and ≥60 years of age in the United States (US).
Aims: This analysis examined the characteristics of patients <60 years of age and ≥60 years of age at the point of AML diagnosis and further investigated subsequent treatments.
Methods: Data from the Adelphi AML Disease Specific Programme, a real-world, cross-sectional survey conducted between February–May 2015, were analyzed. A total of 61 hematologist/oncologists provided data on their 457 AML patients treated at various stages of AML. Disease characteristics upon

initial AML diagnosis including symptoms, performance status, and physician-determined prognostic category were taken from physician-completed patient record forms. Details about subsequently prescribed AML treatment were also taken from this data source. Treatments for n=15 (3.3%) patients were reassigned as high or low intensity following evaluation of physician treatment selection. Post-hoc T-tests and Chi-Squared/Fisher's exact tests were used to determine differences between groups.

Results: Table 1 shows key presenting characteristics of AML patients <60 and ≥60 years old. According to physicians, those patients <60 years of age were significantly more likely than those ≥60 years of age to have de novo AML, a performance score of 0 *versus* ≥1 at diagnosis, more tests conducted to establish the diagnosis and a more favorable prognosis at baseline, according to physician perception. Following initial diagnosis, patients <60 years of age were 1.65 times more likely than patients ≥60 years of age to be initiated on high-intensity induction treatment: 67% (n=143) of patients <60 years of age compared to 40% (n=98) of patients ≥60 years of age (high *versus* low intensity by age group *P* <0.001). All other patients received low intensity treatment. Irrespective of age, the most common high intensity treatment given was a cytarabine-based regimen and the most common low intensity treatments were low dose cytarabine-, decitabine- or azacitidine-based regimens.

Table 1. Disease characteristics of patients <60 and ≥60 years of age at diagnosis of AML.

Disease characteristics		<60 years old (n=214)	≥60 years old (n=243)	*P*-value
Gender	Male	128 (60%)	127 (52%)	0.106
Pathophysiology	De novo	206 (96%)	209 (86%)	<0.001
Symptoms	No. of symptoms at diagnosis [mean (SD)]	3.5 (3.3)	3.2 (2.9)	0.307
Performance status	ECOG score at diagnosis – 0 (Fully active, able to carry on all pre-disease performance without restriction)	81 (38%)	43 (18%)	<0.001
Diagnostic tests	No. of tests used to establish AML diagnosis [mean (SD)]	5.5 (3.6)	4.7 (3.6)	0.025
Physician-defined prognostic category	Favorable	101 (47%)	54 (22%)	<0.001
	Intermediate	84 (39%)	122 (50%)	
	Poor	22 (10%)	55 (23%)	
	Not determined	7 (3%)	12 (5%)	

Summary/Conclusions: The age of an AML patient at initial diagnosis appeared to play a significant role in the diagnostic, prognostic and treatment intensity decisions made by AML-treating physicians in the US. The estimated performance and prognostic status' tend to be considerably better for younger patients and consequently, they were more likely to receive the most aggressive yet more effective high intensity treatments currently available to treat AML.

PB1700

FLT3, NPM1, CEBPA AND TP53 MUTATIONS AT ACUTE PROMYELOCYTIC LEUKEMIA: PROGNOSTIC FACTORS AND CORRELATION WITH OTHER MARKERS WITHIN THE PATIENTS OF GOMEL REGION IN BELARUS

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Background: Acute Promyelocytic Leukemia (APL) is one of the favourable variant of acute myelocytic leukemias due to the usage of ATRA in the treatment simaltenously with chemotherapy. But relapses occur in 13-33% cases after achievement the remission and there are cases of early death from the bleeding. High leukocytosis, the presence of lymphoid immunophenotypic markers and gene mutations are important prognostic factors.

Aims: To examine prognostic factors in APL

Methods: The materials for research were the samples of whole venous blood and bone marrow of 40 patients with APL treated in the period of 2009-20016 in Hematology department for adults, Gomel. The diagnosis was proved by the presence t(15;17) or PML/RARA. Induction therapy was carried out according to the protocol «7+3» using ATRA. Immunophenotypic analysis was carried out by standard immunofluorescence methods. The method of polymerase chain reaction (PCR) with specific primer and following electrophoretic detection was used for recognition of gene mutations.

Results: Out of 40 examined patients (mean age 48,5),80%(32) achieved remission and 15.6%(5) subsequently relapsed after the first course of chemotherapy. Clinical, laboratory, molecular genetic and immunophenotypic data which could affect remission results and general survival rate were analyzed within all the patients. As a result, mutations were detected in 55% of cases. FLT3-ITD mutations were detected in 32,5%(13), NPM1 mutations in 12,5%(5), combination of FLT3-ITD and NPM1 in 7,5%(3), TP53 and CEBPA mutations were detected in 5%(2) and 12,5%(5) of cases respectively. After achievement of remission after the first course of chemotherapy NPM1 mutation remained at 6.2%(2). Mutations were identified more frequently within the patients with the absence of response to the therapy or with the developed relapse in a while. The worst prognosis had the patients with the combination of FLT3-ITD and NPM1 mutations. There were the patients with high leukocytosis, presence of CD56 and CD2 immunophenotypic markers, who didn't achieve remission or had the recurrence when the treatment was dropped. The presence of leukocytosis was detected in 25% of cases, in 90% (9/10) of cases leukocytosis was combined with FLT3-ITD mutations and 80% of these patients subsequently had the recurrence. Within the patients with the combination of FLT3-ITD and NPM1 mutations who brought into remission after the first course of chemotherapy these mutations were not detected later on. There were the patients who had leukocytosis rate less than 20x10 9 * \ l and didn't have CD56 and CD2(11,5%) at the time of verification. The presence of TP53 mutation was combined with high leukocytosis of the patient and with the absence of effect on the conducted therapy. When analyzing the immunophenotypic markers CD56 and CD2, they were detected in 75% of the patients, but in the absence of gene mutations and leukocytosis, such patients had a favorable prognosis (16,7%(3/18, p=0,046)).

Summary/Conclusions: Our results prove that the presence of only one of the signs is not a factor of high risk. Only combination of clinical, laboratory, molecular-genetic and immunophenotypic markers can include the patients into a high risk group and influence general survival rate.

PB1701

A UNIQUE PRESENTATION OF ACUTE PROMYELOCYTIC LEUKEMIA: AORTOILIAC OCCLUSIVE DISEASE (LERICHE SYNDROME)

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Background: Acute promyelocytic leukemia (APL), FAB M3 subgroup of acute myeloid leukemia is known for its association with haemostatic disorders. Compared to bleeding thrombosis is a less commonly encountered complication of APL. Thrombosis of major arteries is a rare form of presentation.

Aims: A case, who applied with acute lower limb ischemia and diagnosed with APL and aortoiliac occlusive disease (Leriche syndrome), is presented.

Methods: A 53-year-old female patient presented with weakness, loss of appetite and pain in the lower extremities. She had diabetes mellitus (DM) regulated with metformin, hyperlipidemia (HL), and smoking history. Physical examination revealed general paleness and ischemia around big toe of the right foot. Laboratory studies revealed leukopenia, neutropenia, anemia, thrombocytopenia, elevated D-Dimer. A bone marrow aspiration and biopsy was done to enlighten the etiology of pancytopenia. The pathological examination of the bone marrow revealed abundant granular blasts (78%) and Auer rodes. The patient was diagnosed with APL, hypergranular classical form. t(15;17) was positive with fluorescence *in situ* hybridization. All-trans retinoic acid (ATRA) plus idarubicin treatment was started. In few days findings of ischemia progressed and encompassed 2nd, 4th and 5th toes together with the big toe (Figure 1 on the left). Monophasic flow pattern (proximal stenosis?) was detected in bilateral common femoral arteries in lower extremity venous doppler ultrasonography. On CT angiography, abdominal aorta and bilateral common iliac arteries were observed to be occluded from L3 vertebra level till 1.5 cm after aortic bifurcation (Figure 1 on the right). Low-molecular-weight heparin therapy was started. According to rheumatological tests and tests for lupus anticoagulant, anticardiolipin and antiphospholipid antibodies, anti-beta-2 glycoprotein-1, protein C-S, Antithrombin III and homocysteine levels, methylenetetrahydrofolate reductase, Factor V Leiden and prothrombin gene mutations no cause of tendency to thrombophilia could be determined. Echocardiography was normal. The patient was transferred to Cardiovascular Surgery Department for axillofemoral bypass operation.

Figure 1.

Results: In APL 80% of thrombotic events occur before treatment or during induction. Acute lower limb ischemia as an initial feature of APL is very rare

which makes our case unique. Thrombotic risk factors in APL include high leukocyte count, presence of coagulation disorder, ATRA+chemotherapy+ antifibrinolytic therapy and ATRA syndrome. None of these were seen in the presented case. The effects of known predisposing risk factors to thrombosis meaning DM, HL and smoking cannot be ruled out. But development of acute thrombosis concomitant with APL diagnosis points out to the relationship between these two entities.

Summary/Conclusions: Current literature knowledge is based on case reports and 9 patients with APL who presented with acute lower limb ischemia were reported yet. As far as we know our case is the first APL case presenting with aortoiliac occlusive disease (Leriche syndrome).

PB1702

A CASE OF THERAPY-RELATED ACUTE LEUKEMIA WITH MIXED PHENOTYPE WITH BCR-ABL1 AFTER TREATMENT OF DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Although therapy-related acute leukemia (tAL) is a well-recognized clinical syndrome and is increasing owing to the prolonged survival of patients treated with chemoradiotherapy, tAL with mixed phenotype is extremely rare.

Aims: Here, we report a rare case of tAL with mixed phenotype with *BCR-ABL1* after achieving complete remission (CR) of Diffuse Large B-Cell Lymphoma (DLBCL).

Methods: A 57-year-old woman was diagnosed as DLBCL. The patient received six cycles of R-CHOP regimen with G-CSF injected after each cycle and achieved CR. The patient was readmitted to the hospital after a follow-up examination revealed the presence of immature cells in the blood.

Results: Her complete blood count findings were as follows: hematocrit, 35.1%; hemoglobin, 116 g/L; platelet count, 129×10^9/L; and white bloodcellcount, 2.41×10^9/L, with 4% blasts, 26% segmented neutrophils, 3% band neutrophils, 39% lymphocytes, and 26% monocytes. Bone marrow aspirations mears revealed 40.7% leukemic blasts with medium cell size, ovaltoround shape, vesicular nuclei, fine chromatin patterns, and basophilic cytoplasm. On cytochemical staining, these blast cells were not positive on PAS and NSE staining, but were weakly positive for MPO staining. Flow cytometric analysis showed that the blasts were positive forboth T-lymphoid and myeloid markers (cytoplasmic CD 3,87%; CD 5,90%; CD 7,96%; cytoplasmic myeloperoxidase, 20%; CD 13,91%; CD 33,87%) and negative for CD2, CD10, CD11b, CD14, CD15, CD19, CD20, CD61, CD117, and TDT. Immuno phenotyping ful filled the diagnostic criteria of T/myeloid biphenotypic leukemia based on the scoring system of the EGIL and WHO classifications. Multiplex reverse transcription PCRusing Hema Visionkit (Bio-Rad Laboratories) revealed the presence of minor *BCR-ABL1* (*e1a2*) fusion transcripts. Chromosome analysis of bone marrow cells failed because of insufficient mitotic cells. Immunoglobulin heavy chain gene rearrangement and TCR gene rearrangement were not detected on bone marrow aspirates.

Summary/Conclusions: Mixed phenotype acute leukemia is an uncommon subtype that comprises 0.5-1% of leukemia. The T/myeloid phenotype is rarer and represents 35% of all MPAL cases The risk of secondary malignancies after lymphoma treatment is relatively increased for leukemia. AML, ALL, MDS, CML and chronic myelomonocytic leukemia are reported secondary hematologic malignancies. Until now, only one case of tAL with mixed phenotype after lymphoma has been reported worldwide. To the best of our knowledge, this is the second case of tAL with mixed phenotype after DLBCL. This case is also unique because the *BCR-ABL1* has not been described in the literature for patients with tAL with mixed phenotype, after hematologic malignancy. According to the 2008 WHO classification, tAL can be attributed to radiation, alkylating agents, or topoisomerase II inhibitors. Our patient did not receive radiation therapy but previously received cyclophosphamide and doxorubicin. Therefore, this is the first case of tAL with mixed phenotype and *BCR-ABL 1* after alkylating agent and topoisomerase II inhibitor therapy for DLBCL.

PB1703

CROSS-SECTIONAL ANALYSIS OF CONCORDANCE RATES BETWEEN KARYOTYPING AND RT-PCR IN ACUTE MYELOID LEUKEMIA; REAL WORLD CHALLENGES

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Background: Translocation and chromosomal anomalies have prognostic implications in acute myeloid leukemia (AML). Cytogenetic analysis assumes great importance in their diagnosis and treatment stratification which are assessed by karyotyping and/or reverse transcriptase polymerase chain reaction (RT-PCR). Given the dependency of karyotyping on sample quality, more and more centers are now relying on RT-PCR to detect specific translocations. Varying rates of concordance between Karyotyping and RT-PCR have been reported and no consensus has prevailed. Given the resource constraint, it is economically non-viable to perform both for prognosis in real world scenarios. In addition, the cost of the extra tests also adds to the burden of healthcare economy.

Aims: In 132 patients of AML, we aimed at determining the incidence of cytogenetic abnormalities and molecular anomalies detected by Karyotyping and RT-PCR respectively. Concordance rates between conventional cytogenetic tests and RT-PCR were also calculated.

Methods: We conducted a retrospective analysis on the medical records of 132 patients of AML at a tertiary health care facility in India, treated during 2010-2017. Results from commercially available molecular assays for detection of specific translocations by RT-PCR and of adequate samples of karyotype analysis were compared.

Results: In AML patients, out of those tested 50.6% had chromosomal aberrations detected by karyotyping while 30% had a positive detection with RT-PCR. The concordance rate in AML was found to be 56.3%. In a large number (31 in AML) karyotyping provided additional information in the form of detection of deletions, additions and hyper-diploidy (Table 1).

Table 1.

Incidence of various translocations in AML as detected by RT-PCR and Karyotyping

Type of Translocation	Incidence using RT-PCR (%) (n = 90)	Incidence using Karyotype (%) (n = 83)
Inv (16)	2.32	1.17
t (8;21)	14.47	9.75
t (15;17)	17.33	8.53

Summary/Conclusions: RT-PCR cannot substitute conventional cytogenetic analysis due to the absence of a broad based application for detection of aberrations other than translocations. However, given its efficiency and reliability it can have a complimentary role in prognosis assessment.

PB1704

CLINICAL, CYTOMORPHOLOGIC AND IMMUNOPHENO/IMMUNOHISTOCHEMICAL CHARACTERISTICS OF PATIENTS WITH BLASTIC PLASMOCYTOID DENDRITIC CELL NEOPLASM - DIAGNOSTIC AND THERAPEUTIC DILEMMA

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Background: Blastic plasmocytoid dendritic cell neoplasm (BPDCN) is a clinically aggressive haematological malignancy that originates from clonal proliferation of plasmocytoid dendritic cells and their precursors. BPDCN is rare, represents less than 1% of acute leukemias. The disease has two patterns of presentations: cutaneus and leukemic. The main histological differential diagnosis includes: cutaneous NK/T-cell lymphoma; cutaneous T-cell lymphoma with co-expression of CD56 and CD56+ acute myeloid leukemia with monocytic differentiation.

Aims: The aim of study was to analyze heterogeneity of BPDCN differential diagnosis, especially with regards to clinical, immunological and cytomorphological characteristics of blastoid cells in terms of the optimal treatment.

Methods: During period 2010-2016. at the Clinic of Hematology, eight patients with BPDCN were diagnosed (M/F 6/2; average age 38 yrs, range 26-60yrs). In the blood count, average concentration of Hb was 108g/l (range 87-154); WBC 6,38x10e9/l (range 2,6-12); Plt 147,8x10e9/l (range 20-282). Hemorrhagic diathesis was registered in 3/8; splenomegaly in 6/8 (average diameter by ultrasound exam 140mm, 110-150mm); and hepatomegaly existed in 3/8 pts (average diameter 166mm, 140-200mm). Cutaneous infiltrations were present in 5/8 pts as livid maculopapular rash along lower extremities in 5 pts, and in 1 female pts in the breast region of 1-4cm diameter. In all 5 pts, immunocytochemistry confirmed BPDCN diagnosis. In the bone marrow aspirates of 7/8 pts, average 75% infiltration (27-89%) with blasts was revealed. Cells were of median size, with high nucleus cytoplasm ratio, with visible oval or slightly imprinted nucleoli. Basic immunophenotype profile was characterised by expression of $CD56^+CD4^+CD123^{+high}$ $CD45RA^+$, and negativity for $CMPO^-$ $cCD79a^-$ cCD3c- in 4 cases. Immunohistochemitry staining in the rest of 4 pts, characterized with dry aspiration, revealed LCA+CD43+CD56+CD4+ CD33+ positivity and MPO- TDt-CD34 -CD117- CD68- HLADR-negativity. Only 1 pts had CD4 negativity. Cytogenetic analysis revealed normal karyotype in 4 pts, while the rest of 4 pts had pathological findings: 1. 92,XX, XY; 2. 80-120,XXYY,+ mar (16)/46XY; 3. 46XY,del 5q/46XY; and 4. 46XX,del 12p, respectively.

Results: Four pts were treated with 3+7 chemotherapy. Complete remission (CR) was achieved in 3 pts, and treatment was continued according to the HIDAC and IDAC protocol. The duration of remission was 3, 8 and 11 months respectively, followed with relapse and letal outcome. One of the pts died within first 0,5 months after BPDCN was diagnosed. Three pts, treated with Hyper-CVAD, are alive and in CR with duration of 1, 3 and 10 months respectively. The continuation of the treatment within the programme of allogeneic stem cell transplantation is planned in 2 pts.
Summary/Conclusions: BPDCN diagnostics is difficult due to the heterogeneity of immunological characteristics of disease. Aggressive course of disease with median survival of 12-18 months, in the view of the unique treatment recommendations indicates necessity of further clinical investigations on larger patients groups.

Aggressive Non-Hodgkin lymphoma - Clinical

PB1705

ECONOMIC IMPACT AND HEALTHCARE UTILIZATION IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA IN ROUTINE CLINICAL CARE IN THE UNITED STATES – A CLAIMS DATABASE STUDY

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Background: DLBCL is the most common histologic subtype of non-Hodgkin lymphoma (NHL), accounting for about 33% of all NHL cases. However, the healthcare burden associated with DLBCL has not been extensively studied in a US population.
Aims: We evaluated the costs of care and healthcare utilization (HCU) of DLBCL patients treated during routine care in the US.
Methods: The Optum claims database was used to identify adult patients (≥18 years old) with newly diagnosed DLBCL between 01/01/08 and 10/31/15. DLBCL diagnosis was based on ≥1 inpatient claim or ≥2 outpatient claims with DLBCL diagnosis codes, with the index date being the first DLBCL claim. Patients were followed from index date until end of continuous enrollment, death, or end of study period (12/31/15) for the assessment of HCU and costs. DLBCL-related and non-DLBCL-related HCU and costs incurred during follow-up were evaluated. DLBCL-related HCU and costs were medical claims with a primary diagnosis of DLBCL or DLBCL-related treatment (chemotherapy, radiation, stem cell transplant [SCT], supportive care) and pharmacy claims for DLBCL treatment. Proportions of patients with HCU were reported. Costs were calculated as per-patient-per-month (PPPM) costs and reported as mean and standard deviation (SD). Patients with a capitated payment plan were excluded from the cost analysis.
Results: 1,267 treated DLBCL patients were identified. Over the follow-up period, 66.0% of patients had ≥1 inpatient admission, with more patients having a non-DLBCL-related than DLBCL-related admission (Table 1). 60.0% of patients had ≥1 emergency room visit over the follow-up period; visits were predominately for non-DLBCL-related. Nearly all patients had ≥1 physician office visit (92.4%) and other outpatient visits (99.6%). The mean PPPM costs incurred during the follow-up period was $11,890 (SD: $11,515) (Table 1), and costs were higher in Year 1 ($14,402, SD: $10,951) than in Year 2 ($4,190, SD: $8,076). About 55% of costs overall were related to DLBCL medical services ($6,532 PPPM, SD: $6,457). DLBCL-related medical PPPM costs decreased substantially from Year 1 ($8,327, SD: $5,925) to Year 2 ($1,443, SD: $4,349). This decrease was driven by the decreases in chemotherapy and supportive care medical services from Year 1 to Year 2. Non-DLBCL-related medical costs accounted for about 42% of the overall PPPM costs ($4,955, SD: $7,210); and a decrease was observed from Year 1 ($5,640, SD: $7,468) to Year 2 ($2,447, SD: $5,456). Inpatient admission was the main component of non-DLBCL-related costs, and associated costs decreased from Year 1 to 2.

Table 1.

Healthcare Cost and Utilization Overall and Year 1 and Year 2 Post-index Date for Treated DLBCL Patients

Healthcare Resource Use, %*	Overall N=1,267		Year 1 N=1,267		Year 2 N=1,267	
Inpatient Visit (all)	836	66.0%	730	57.6%	174	21.0%
DLBCL-related	158	12.5%	149	11.8%	9	1.1%
Non-DLBCL-related	802	63.3%	688	54.3%	173	20.8%
ER Visit (all)	760	60.0%	616	48.6%	221	26.6%
DLBCL-related	40	3.2%	40	3.2%	1	0.1%
Non-DLBCL-related	751	59.3%	605	47.8%	220	26.5%
Physician Office Visit (all)	1,171	92.4%	1,159	91.5%	688	82.9%
DLBCL-related	861	68.0%	828	65.4%	381	45.9%
Non-DLBCL-related	1,161	91.6%	1,148	90.6%	678	81.7%
Other Outpatient Visits (all)	1,262	99.6%	1,260	99.4%	753	90.7%
DLBCL-related	1,018	80.1%	968	76.4%	374	45.1%
Non-DLBCL-related	1,261	99.5%	1,259	99.4%	741	89.3%
Cost, mean $ in PPPM (SD)	N=951		N=951		N=584	
Total	$11,890	$11,515	$14,402	$10,951	$4,190	$8,076
DLBCL-related	$6,691	$6,593	$8,495	$6,061	$1,828	$4,538
Non-DLBCL-related	$5,199	$7,283	$5,903	$7,539	$2,663	$5,561
Medical	$11,487	$11,303	$13,967	$10,717	$3,890	$7,819
DLBCL-related	$6,532	$6,457	$8,327	$5,925	$1,443	$4,349
Non-DLBCL-related	$4,955	$7,210	$5,640	$7,468	$2,447	$5,456
Pharmacy	$403	$1,220	$435	$1,253	$300	$794
Medical Costs Components						
Inpatient	$2,690	$6,629	$2,976	$6,539	$1,012	$4,221
DLBCL-related	$262	$2,018	$332	$2,091	$5	$67
Non-DLBCL-related	$2,428	$5,957	$2,645	$6,183	$1,003	$4,202
ER	$199	$407	$209	$414	$99	$346
DLBCL-related	$4	$48	$5	$53	$0	$0
Non-DLBCL-related	$194	$403	$204	$408	$99	$346
Physician Office	$739	$823	$917	$993	$345	$928
DLBCL-related	$294	$843	$388	$829	$75	$185
Non-DLBCL-related	$445	$535	$532	$613	$272	$488
Other Outpatient	$2,310	$2,987	$2,771	$3,220	$1,178	$2,472
DLBCL-related	$403	$1,138	$511	$1,391	$106	$308
Non-DLBCL-related	$1,907	$2,612	$2,260	$2,766	$1,072	$2,406
Chemotherapy	$3,190	$3,360	$4,121	$3,104	$650	$2,734
Supportive Care	$1,254	$1,351	$1,680	$1,287	$120	$864
Radiation	$913	$3,100	$1,123	$3,308	$257	$1,515
Stem Cell Transplant	$192	$1,441	$170	$1,510	$228	$1,923

*DLBCL-related vs non-DLBCL-related are not mutually exclusive groups; patients could be in both categories, and percentages are relative to the overall "n" in the column as the denominator.
Key: ER = emergency room; DLBCL = diffuse large B-cell lymphoma; PPPM = per patient per month.

Summary/Conclusions: The economic burden associated with the treated DLBCL population is high, with the majority of costs incurred during the first year of diagnosis. Between the first and second year of diagnosis, costs decrease mainly because of the decrease in the DLBCL-related treatment costs. In addition, HCU for DLBCL-related services decreased in Year 1 *vs* Year 2.

PB1706

PHARMACOKINETICS OF RITUXIMAB IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Rituximab dosing is based on evidence from clinical practice rather than from consideration of pharmacokinetics and factors influencing individual exposure. Clinical use of rituximab can be improved through a more individualized treatment.
Aims: The objective of this investigation was to typify rituximab pharmacokinetics in 29 newly diagnosed patients with the diffuse large B-cell lymphoma who received rituximab in combination with cyclophosphamide, doxorubicin, vincristine and methylprednisolone every three weeks. The association of rituximab pharmacokinetics with clinical outcome was also investigated.
Methods: Rituximab serum levels were defined by enzyme-linked immunosorbent assay and assessed by a population pharmacokinetic analysis applying the non-linear mixed effects modelling.
Results: A 2-compartment model comprising linear non-specific clearance of 0.252 (95% CI: 0.227 – 0.279) L/day and time-varying specific clearance of 0.278 (95% CI: 0.181 – 0.390) L/day, corresponding to target-mediated drug disposition of rituximab was recognised to best describe the data. The nonspecific clearance was found to be lower in older patients and those with lower body weight. Additionally, the central compartment volume was higher in males. An unambiguous association of clinical response with rituximab pharmacokinetics has been detected. The rate constant of specific clearance decay was 0.143 day^{-1} (95% CI: 0.0478 – 0.418) in patients with no disease progression, while in patients with disease progression it was 82.2% lower (95% CI: 33.4 – 95.0).
Summary/Conclusions: These results imply that time-changes in clearance could serve as a predictive marker of response to rituximab. Our findings prove the rationale for studies evaluating higher doses of rituximab in selected patients.

PB1707

HOW 18FDG PET/CT CAN IDENTIFY BONE MARROW INFILTRATION IN PATIENTS WITH NON-HODGKIN'S LYMPHOMA

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Background: Bone marrow infiltration (BMI) evaluation plays a key role in lymphoma staging, treatment and prognosis. The role of PET/CT in the assessment of BMI is still controversial, especially in non-Hodgkin's lymphoma (NHL).
Aims: To evaluate the role of 18F-FDG PET/TC in bone marrow infiltration for the diagnosis of Non Hodgkin Lymphoma. We compared 18F-FDG PET/TC visual and quantitative analyses with bone marrow biopsy in NHL patients.
Methods: Fifty patients with newly diagnosed NHL from February 2011 to February 2016 were retrospectively analyzed. Of these, 26 (group A) patients had aggressive NHL and 24 (group B) indolent NHL. To detect BMI on the posterior iliac crest 3 different PET/CT evaluation methods were used: 1) visual analysis, 2) maximal standardized uptake values (SUVmax, cut-off >2.5), and 3) Deauville score (categorical). Each method was applied in the whole patients cohort, in group A and in group B. Images were blindly reviewed separately by 3 nuclear medicine physicians. PET/CT results were compared with the bone marrow biopsy performed after imaging in all patients. Decision-curve analysis was used to evaluate the increment in net benefit (NB) obtained considering the Deauville score over a biopsy-all strategy.
Results: The prevalence of a positive biopsy was 38% in whole cohort, 19% in group A and 58% in group B. In the whole cohort, sensitivity, specificity and accuracy were 21%, 84% and 60% for visual analysis; 58%, 55% and 50% for SUVmax; and 47%, 81% and 68% for Deauville score. In group A, sensitivity, specificity and accuracy were 0%, 76% and 62%, for visual analysis; 40%, 52% and 50% for SUVmax; and 20%, 71% and 62% for Deauville score. In group B, sensitivity, specificity and accuracy were 29%, 100% and 58% for visual analysis; 64%, 60% and 62% for SUVmax; and 57%, 100% and 75% for Deauville score. At probability threshold equal to the prevalence of a positive biopsy, the increase in NB by Deauville score was 0.11 in the whole cohort, 0.02 in group A and 0.33 in group B. In this latter group, biopsying patients on the basis of the Deauville score is a strategy that reduced the biopsy rate by 24%, without missing any BMI.
Summary/Conclusions: FDG-PET/TC visual analysis has a limited value for detecting BMI in patients with NHL, while quantitative analysis by Deauville score provides a higher diagnostic performance. Noteworthy, the high positive predictive value in patients with indolent NHL suggests a potential role of FDG-PET/TC in avoiding bone marrow biopsy in this subtype of lymphoma.

PB1708

LOW ALBUMIN LEVEL CORRELATES WITH POORER SURVIVAL OF PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA: SERBIAN LYMPHOMA GROUP EXPERIENCE

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Background: Current prognostic scores are not sufficient to define high risk patients with diffuse large B cell lymphoma (DLBCL). Besides parameters included in the International Prognostic Index (IPI), other clinical and laboratory parameters have been investigated as potential prognostic markers. However, contradictory data have been reported.
Aims: The aim of this study was to evaluate prognostic significance of clinical and laboratory parameters on the overall survival (OS) of patients with DLBCL.
Methods: A total of 393 patients (188 females/205 males) with the median age of 60 years (range 18-84) were analyzed. All patients were initially treated with rituximab plus CHOP (Cyclophosphamide, Doxorubicine, Vincristine, Prednisone) or CHOP like protocols.
Results: Ann Arbor stage I, II, III and IV had 56 patients (14.2%), 142 (36.1%), 71 (18.1%) and 124 (31.6%), respectively. Bulky disease had 99 patients (25.2%), B symptoms 263 patients (66.9%), and poor performance status according to the European Cooperative Oncology Group (ECOG) ≥2 had 82 (20.9%). Bone marrow involvement was present in 68 patients (17.3%). Low IPI risk was present in 194 patients (49.4%), low intermediate in 86 (21.9%), high intermediate in 77 (19.6%), and high in 36 (9.2%). Median absolute lymphocyte count (ALC) at diagnosis was $1.35x10^9$/L (range 0.07-60.76x10^9/l), absolute monocyte count (AMC) was 0.64x10^9/l (range 0.06-8.58x10^9/l), ALC/AMC was 2.3 (range 0.07-37.0x10^9/l), hemoglobin level was 125g/l (range 57-421g/l), platelet level was 274x10^9/l (range 50-584x10^9/l), C-reactive protein was 10.2 mg/l (range 0.10-438mg/l), erythrocyte sedimentation rate (ESR) was 30mm/h (range 2-636mm/h), and albumin level was 38g/l (range 20-51g/l).
Complete remission (CR) was achieved in 288 patients (73.3%), partial remission (PR) in 58 (14.8%), stable disease (SD) in 5 (1.3%) and progressive disease in 42 (10.7%). Disease relapse was confirmed in 59/346 patients (17.0%). OS was influenced by the presence of B symptoms (p=0.004, 95% CI 1.263-3.549), ECOG≥2 (p<0.0001, 95% CI, 1.827-4.290), Ann Arbor clinical stage (p<0.0001, 95% CI 1.601-3.883), and albumin level (p<0.0001, 95% CI 0.905-0.953). Optimal cut off point for albumin level was 34g/l, and was determined by Receiver operating characteristic (ROC) curve (AUC 0.699, 95% CI, 0.629-0.770, p<0.0001). The prognostic value of IPI was highly statistically significant for OS (p<0.0001, 95% CI, 1.545-2.236). However, other analyzed parameters did not influence OS. Multivariate analysis among significant parameters (presence of B symptoms, IPI, and albumin), has pointed to IPI (HR 1.81, p<0.0001, 95% CI, 1.489-2.222), and albumin level (HR 1.77, 95% CI, 1.164-2.69, p=0.008) as the most important parameters that influenced survival.
Summary/Conclusions: Although IPI is widely used as a prognostic index in DLBCL, it cannot fully recognize high-risk patients. Pretreatment albumin level may represent a useful tool in order to discriminate high-risk patients and is likely to add significant information to the IPI.

PB1709

TREATMENT PATTERNS AND TREATMENT RESPONSE IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA IN ROUTINE CLINICAL CARE IN THE UNITED STATES – A CLAIMS DATABASE STUDY

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Background: DLBCL is the most common histologic subtype of non-Hodgkin lymphoma. Treatment guidelines recommend rituximab in combination with chemotherapy as first-line therapy (1LT). For patients who are refractory or relapse, high-dose chemotherapy with stem cell transplant, combination chemotherapy, or single-agent rituximab are recommended in subsequent lines.
Aims: To compare real-world treatment patterns of patients with newly diagnosed DLBCL to NCCN guideline recommendations.

Methods: The Optum claims database was used to identify adult patients (≥18 years old) with newly diagnosed DLBCL between 01/01/08 and 10/31/15. DLBCL diagnosis was based on ≥1 inpatient claim or ≥2 outpatient claims with DLBCL diagnosis codes, with the index date being the first DLBCL claim. Patients were followed from index date until end of continuous enrollment, death, or end of study period (12/31/15). Treatment patterns and response to treatment were assessed during follow-up. Possible remission was defined as no additional chemotherapy and no supportive care use or receipt of supportive care <30 days after end of line of therapy (LOT) for <30 days. Lack of remission was defined as receipt of supportive care <30 days after end of LOT for >30 days. Progression was defined as initiation of another LOT or evidence of supportive care >30 days after end of a LOT.
Results: Of the 2,216 patients selected into the study, 1,267 (57.2%) initiated 1LT, and median (interquartile range [IQR]) time to therapy was 0.7 (0.4–1.1) months. The majority of patients received combination (87.7%) *vs* single-agent (12.3%) chemotherapy. R-CHOP (60.5%) was the most frequently used combination chemotherapy, while rituximab monotherapy comprised ~67% (8.2%) of single-agent use in 1LT. Median (IQR) duration of 1LT was 4.2 (2.3–4.5) months. At the end of 1LT, 64.0% (n=811) had evidence of remission, 15.0% (n=190) progressed, and 1.2% (n=15) had no evidence of remission. Second-line therapy (2LT) was initiated by 159 patients who progressed after 1LT; 29.6% received a single agent, and 70.4% received combination chemotherapy. In 2LT, rituximab (12.6%) remained the top single agent used, while bendamustine+rituximab (15.7%) and R-CHOP (8.2%) were the most common combinations; 8.2% of patients received stem cell transplant. Median (IQR) duration of 2LT was 2.1 (1.2–3.8) months. Of the 2LT patients, 44.0% (n=70) had evidence of remission, 26.4% (n=42) progressed, and 3.1% (n=5) had no evidence of remission. 34 patients who progressed after 2LT received third-line therapy (3LT); 29.4% received a single agent, while 70.6% received combination chemotherapy. In 3LT, rituximab (5.9%), etoposide (5.9%), and carboplatin (5.9%) were the most common single agents, while bendamustine+rituximab (20.8%) and etoposide+oxaliplatin+rituximab (17.6%) were the most common combinations; 8.8% of patients received stem cell transplant. Median (IQR) duration of 3LT was 3.5 (0.9–5.2) months. Following 3LT, 32.4% (n=11) had evidence of remission, 29.4% (n=10) progressed, and 5.9% (n=2) had no evidence of remission.
Summary/Conclusions: DLBCL treatment in routine clinical care aligns with guidelines, with most patients receiving rituximab in combination with chemotherapy. A small proportion of patients received single-agent chemotherapy in 1LT. As expected, remission rates decreased with subsequent lines of therapy. Some patients were untreated; therefore, subsequent studies should explore reasons for lack of treatment.

PB1710

TP53 GENE MUTATIONS IS A PREDICTOR OF HIGH GRADE B-CELL LYMPHOMA PROGRESSION

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Background: High grade B-cell lymphoma (HGBL) is subdivided on poor prognosis double-hit (DH) and not otherwise specified (NOS) variant, which appears sometimes with primary refractory behavior. Mutations in *TP53* gene (MUT-TP53) lead to blockage of apoptosis in cells and appearance of additional oncogenic events contributing to tumor progression. Correlation between presence of MUT-TP53 and anti-tumor response in patients with HGBL is unclear.
Aims: To evaluate an effect of MUT-TP53 on survival parameters of patients with high grade B-cell lymphoma.
Methods: Since 2005 to 2017 years in FGBU National Research Center for Hematology Ministry of Health Russian Federation diagnosis of high grade B-cell lymphoma were established in 47 patients: 13 – double hit, 34 – not otherwise specified. We had available biologic samples from 32 pts with HGBL: 11 – double-hit and 21 – NOS HGBL. 19 pts underwent courses of intensive treatment according to BL-M-04 [Efficacy and toxicity of a new short-term high intensive protocol BL-M-04 for adult patients with Burkitt lymphoma. Baryakh E. *et al.* Haematologica. 2011; 96 (S2): 391-392], 11 - R-(DA)-EPOCH, 2-R-CHOP-21 treatment. A median follow-up period was 10,8 (0,6-160,9) months. *MYC*-rearrangement (*MYC*-R) without *BCL2* and/or *BCL6* rearrangement was detected in 7 pts with HGBL. Sanger sequencing of DNA extracted from paraffin embedded tumor tissue were performed to reveal mutations in exons 5-8 of *TP53* gene. To evaluate an effect on overall survival (OS) and on time to disease progression (of TTP) such factors as MUT-TP53, *MYC*-R and DH was conducted univariate analysis (test of Kaplan-Meier method, log-rank test) and multivariate variance and Cox regression analysis (STATISTICA 10).
Results: A significant mutations of *TP53* were detected in 9 pts (c.535C>T 45,6% p.H179Y, c.524G>C 15,6% p.R175P, c.770T>A 32,4% p.L257Q, c.743G>A 75,6% p.R247Q, c.487T>A 25,2% p.Y163N, c.824G>A 75% p.C275Y, c.713G>A 87,7% p.C238Y, c.517G>A 22,4% p.V173M, c.517G>A 22,4% p.V173M). Pts and their relatives hadn't a history of primary multiple tumors. Seven from nine pts with MUT-TP53 had MYC-R (3-double hit, 4-single hit lymphoma). Groups of pts with WT-TP53 and MUT-TP53 were comparable for main clinical characteristics. According to results of univariate analysis, patients with MUT-TP53 had lower duration of overall survival a higher probability of disease progression. Thus, median of overall survival in pts with c MUT-TP53 was 7,0 (3,5 - 40,9) *vs* 30,5 (0,6 - 160,9) months in patients with WT-TP53, (p=0,03). Median time to disease progression in pts with c MUT-TP53 was 3,5 (0,3 - 16,1) *vs* 30,5 (0,6 - 160,9) months in patients with WT-TP53, (p=0,00016). In multivariate analysis, MUT-TP53 was an independent factor of early disease progression in HGBL independently of double-hit status (Figure 1).

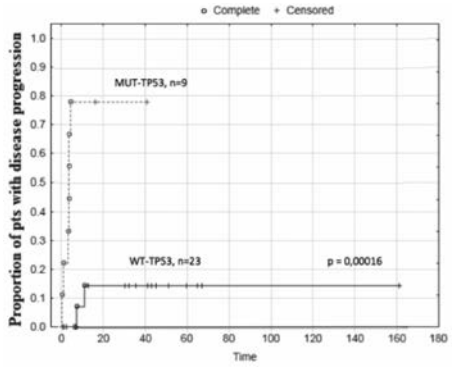

Figure 1.

Summary/Conclusions: Mutations in *TP53* gene - a significant predictive factor of early disease progression in high grade B-cell lymphoma.

PB1711

HTLV-1 INFECTION INCREASED THE RISK OF OTHER MALIGNANCY

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Background: The correlation between HTLV-1 infection and malignant neoplasm other than ATL remains unknown. Some previous studies have indicated that the frequency of primary malignant neoplasms in patients with HTLV-1 seropositive is higher than HTLV-1 seronegative.
Aims: To clarify the correlations between HTLV-1 infection and malignant neoplasms other than ATL.
Methods: We retrospectively analyzed 203 patients with HTLV-1 seropositive who were diagnosed between 2006 and 2015 at Kansai Medical University Hospital.
Results: Among 203 patients (median age 62 years: range 19 to 86 years), 43% was carrier and 57% was diagnosed with ATL. According to clinical subtype, 5% was chronic, 38% was smoldering, 28% was acute, 29% was lymphoma type. Median overall survival was 30 months in carrier, 10 months in acute, 8 months in lymphoma, and smoldering was not available. In all HTLV-1 seropositive patients, the occurrence of primary malignant neoplasm was 32%, they were all carrier or smoldering. Among them, 53% was hematology malignancy (T cell lymphoma; 41%, B cell lymphoma; 29%, MPN; 18%, MDS; 12%). Solid tumor was 47% (lung cancer; 33%, prostate cancer 13%, colon cancer; 13%, renal cell cancer; 13%). Four patients with HTLV-1 carrier who developed primary malignant neoplasm received standard chemotherapy for the neoplasm, and after the chemotherapy they developed 3 acute type and 1 smoldering type ATL.
Summary/Conclusions: In our cohort, the occurrence of primary malignant neoplasm with HTLV-1 seropositive patients was significantly high. Chronic HTLV-1 infection might associate with reduction of cytotoxic T cells and an increased risk of developing other malignancy. Furthermore, cytotoxic chemotherapy for primary malignant neoplasm might reduce cytotoxic T cells for HTLV-1 and exacerbate ATL conditions.

PB1712

Abstract withdrawn.

PB1713

THIOTEPA BUSULFAN CYCLOPHOSPHAMIDE, A TOXIC CONDITIONING FOR AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN CENTRAL NERVOUS SYSTEM LYMPHOMA: REMISSION OR INFECTION?

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Background: CNSL represent 4% of primary central nervous system (PCNSL) and secondary CNS lymphoma (SCNSL) occur in 7% of systemic lymphoma. Overall survival (OS) and progression free survival (PFS) have dramatically increased in PSNCL since the introduction of Methotrexate high doses and ASCT usually conditioning with TBC (Thiotepa, Busulfan and Cyclophosphamide). The studies usually tend to recommend TBC/ASCT in front line for patients under 65 years with CNSL with very few prospective data about this strategy.
Aims: We report in this multicenter retrospective study our experience concerning TBC/ASCT and its main toxicities.
Methods: All patients treated with TBC/ASCT for PCNSL or SCNSL from August 2010 to November 2016 in our centers were researched by using CHIMIO® software. TBC combined Thiotepa (250mg/m²/d from d-9 to d-7), Busulfan (3.2mg/kg/d from d-6 to d-5 and 1.6mg/kg/d on d-4) and Cyclophosphamide (60mg/kg/d on d-3 and -2) followed by ASCT transplantation at d0. Clinical data were extracted from the medical records. We measured OS and PFS from the date of ASCT and transplant related mortality (TRM) (defined by death occured 3 months after ASCT).
Results: 24 patients, without any major co-morbidity, were included. Median age at ASCT was 58 years (23-66). 22 of 24 were DLBCL and 2 follicular lymphoma and there were 15 PCNSL and 9 SCNSL. All but one, received 1 or 2 lines of chemotherapy (with high doses Methotrexate in first or second line) before ASCT. 15 were in complete response (CR) and 9 in partial response (PR) before TBC/ASCT. Median duration of hospitalisation was 33 days (15-78 d) and of aplasia was 14 days (7-37 d). Median follow-up was 10 months (0-73). At the end of follow up 5 patients have died. Among the 3 patients older than 60 years in PR before ACSCT, no one survived. At 1 year, OS and PFS were respectively 78% and 73%. Surprisingly (Table 1), we noted an important rate of toxicity (100% with 66% ≥grade 3) with a TRM=21%. Neurological adverse events (37%: 9 patients with 4 comas) and infections (100% with 41% ≥grade 3) were predominant. We documented 2 CMV reactivations and 5 fungal infections (3 candida, 1 aspergillus and 1 cryptococcus).

Table 1.

WHO criteria	Grade I-II	Grade III	Grade IV	All
Infections	13	2	9	24
Neurologic	4	1	4	9
Mucositis	5	7	3	15
Cutaneous	5	2	0	7
Colitis	18 of 24 (75%)			
Renal dysfunction	2 of 24			
Haemorrhagic cystitis	2 of 24			

We observed 5 deaths (4/5 older than 60 years) in first 3 months due to a septic choc, 4 associated with a persistent coma and 2 with an acute respiratory distress syndrome.
Summary/Conclusions: To our knowledge, here is one of the biggest retrospective cohort concerning TBC/ASCT in CNSL. If TBC seems to give interesting response rates (72% CR), we noted an unacceptable toxicity compared to other used conditionnings (for example TRM with Thiotepa Carmustine is 1%). Our high toxicity rates (66%≥grade 3), especially in elderly patients, with neurological adverse events and infections (with unusual microbiological agents) lead us to disadvise the use of TBC before ASCT.

PB1714

TREATMENT RESULTS IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA FROM HIGH RISK AND HIGH-INTERMEDIATE RISK GROUPS

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Background: Using of Rituximab-containing regimens, as the «gold standard» of treatment of patients with diffuse large B-cell lymphoma (DLBCL), showed significant improvement in the treatment results throughout all prognostic groups. The "real-life" treatment approaches vary depending on financial support of healthcare system in different countries. Unfortunately, treatment results in patients with DLBCL from high and high-intermediate risk groups are still unsatisfying.
Aims: Aim of our study was to compare efficacy and toxicity of different treatment approaches in patients with DLBCL from high risk and high-intermediate risk groups.
Methods: Prospective cohort study was initiated in 2014 in three Ukrainian centers. Patients with newly diagnosed DLBCL and ≥3 risk factors according to International Prognostic Index (IPI) were treated according to "investigators decision" with 6-8 cycles of CHOP-like (first group), R-CHOP (second group) or R-DA-EPOCH regimens (third group). Primary end-point was 2-year progression-free survival (PFS), secondary end-points were 2-year overall survival (OS), overall response rate (ORR), complete response rate (CRR), toxicity rates.
Results: 104 patients were included into analysis in January 2017, 50 males (48,1%), 54 females (51,9%), in the age 23-86 years old, median age 63 years (95% CI 60;65). Observation period was 1-64 months, median – 10.5 months. Patients were divided into three groups according to the treatment regimen. Patients treated with CHOP-like regimens were included into the first group (52 patients, 50.0%). Patients treated with R-CHOP were included into the 2nd group (40 patients, 38.5%) and 12 patients (12.5%) treated with R-DA-EPOCH were included into the 3rd group. Significant difference between the groups was observed by the age (younger patients in the 3rd group, p=0.042) and stages distribution (early stages were more common in the 1st group, p=0.05). ORR was 61.5% in the 1st group, 52.5% in the 2nd group and 83.3% in the 3rd group (p=0.01). CRR was 17.3%, 42.5% and 83.3%, respectively (p<0,001). 2-year PFS was 44,1±9,7% in the 1st group, 74,1±8,8% in the 2nd group and 88,9±10,5% in the 3rd group (p=0,09). 2-year OS was 51,8±8,7%, 54,8±10,2% and 87,5±11,7%, respectively (p=0,197).
The rates of anemia, thrombocytopenia and hepatotoxicity were comparable in three groups. Neutropenia, febrile neutropenia and cardiotoxicity were less common in the group treated with R-DA-EPOCH (p=0.05, p=0.051, p<0,01, respectively). Neurotoxicity was more frequent in this group (p=0.043).
Summary/Conclusions: The level of ORR and CRR was significantly higher in the R-DA-EPOCH group. 2-year PFS was significantly higher in the R-DA-EPOCH group, as well. There was no significant difference in the level of 2-year OS between the groups. Toxicity was acceptable in all groups. Levels of neutropenia, febrile neutropenia and cardiotoxicity were less common and neurotoxicity was more frequent in the R-DA-EPOCH group. Thus, R-DA-EPOCH could be considered as the most efficient treatment regimen in patients with DLBCL from high and high-intermediate risk groups.

PB1715

PROGNOSTIC MODEL WITH NEUTROPHIL-LYMPHOCYTE RATIO AND PERFORMANCE STATUS IN DIFFUSE LARGE B CELL LYMPHOMA TREATED WITH R-CHOP

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Background: Growing evidences suggest the close relationship between inflammation, host immunity, and tumor cells. The neutrophil to lymphocyte ratio (NLR) has been known to predict the prognosis in patients with diffuse large B-cell lymphoma (DLBCL).
Aims: This study was planned to confirm the prognostic and predictive value of NLR and to make a model to predict the prognosis more precisely in patients with DLBCL.
Methods: Data of 192 DLBCL patients treated with R-CHOP from 2004 to 2016 were retrospectively assessed. Patients with NLR ≥4 and <4 were determined as the high and low NLR groups, respectively. Treatment response and survival were compared according to the NLR status and using the model including NLR and other variable interacting with NLR.
Results: High NLR group was associated with old age, poor performance status (PS), elevated lactate dehydrogenase, and more advanced prognostic indices than low NLR group. High NLR group had a low complete response (CR) rate compared to low NLR group (57.5% *vs* 81.4%, p=0.004). However, the role of NLR as prognostic factor was not demonstrated on multivariate analysis, which showed strong interaction between NLR and PS. The model composed of NLR and PS could stratify the patients into low-, intermediate-, and high-risk groups for overall survival (OS). On multivariate analysis, compared to low risk group, the hazard ratios of intermediate and high risk groups on OS were 1.871 (p=0.019) and 2.733 (p=0.004).
Summary/Conclusions: High NLR is associated with poor treatment response and unfavorable clinical features in DLBCL. The prognostic model using NLR and PS can predict more precisely the prognosis of this population and needs to be validated in the independent cohort.

PB1716

HIGH SERUM LEVELS OF SOLUBLE INTERLEUKIN-2 RECEPTOR ARE ASSOCIATED WITH A POOR PROGNOSIS IN CASES OF RELAPSED/REFRACTORY PERIPHERAL T CELL LYMPHOMA, NOT OTHERWISE SPECIFIED

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Background: The prognosis is extremely poor for cases of relapsed/refractory peripheral T cell lymphoma, not otherwise specified (PTCL-NOS), and there

are no established predictors of prognosis. Although serum soluble interleukin-2 receptor (sIL-2R) levels are associated with clinical outcomes in newly diagnosed patients with PTCL-NOS, it remains unclear whether sIL-2R levels can predict prognosis in patients with relapsed/refractory PTCL-NOS.
Aims: This study evaluated whether sIL-2R levels at the time of salvage chemotherapy were associated with prognosis in cases of relapsed/refractory PTCL-NOS.
Methods: We retrospectively analyzed 45 patients with relapsed/refractory PTCL-NOS who received salvage chemotherapy at our institutions (1996–2016). All patients received CHOP or CHOP-like therapy as their initial treatment. The primary outcome was defined as overall survival (OS), which was calculated from the date of the salvage chemotherapy to the date of death from any cause or the last follow-up.
Results: The median age at salvage chemotherapy was 68 years (range: 37–86 years). The median serum sIL-2R level was 3,476 U/mL (range: 280–24,400 U/mL). Receiver operating characteristic curve analysis revealed that the optimal sIL-2R cut-off value for predicting OS was 2,283 U/mL (area under the curve: 0.672, 95% confidence interval [CI]: 0.421–0.923). Thus, we defined patients with serum sIL-2R levels of ≥2,283 U/mL as the high sIL-2R group and the other patients as the low sIL-2R group. The two groups had similar clinical characteristics at the salvage chemotherapy, with the exception of their international prognostic index (secondary IPI) and performance status (PS). The high sIL-2R group had significantly higher secondary IPI (≥H-I) and poorer PS (≥2). Eight patients were alive at the time of the analysis, with a median follow-up of 55 months (range: 2–136 months). The 2-year OS among all patients was 25.1% (95% CI: 13.6–38.5), and the high sIL-2R group had significantly poorer 2-year OS (10.9%, 95% CI: 2.8–25.4 *vs* 50.0%, 95% CI: 24.5–71.0, P <0.001). A multivariate analysis was performed using the following factors: serum sIL-2R levels (high *vs* low), secondary IPI (≥H-I *vs* ????????) (Figure 1).

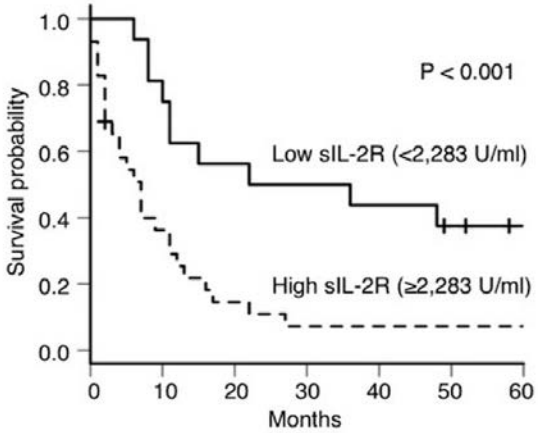

Figure 1. Overall survival according to serum sIL-2R levels.

Summary/Conclusions: Serum sIL-2R levels are a useful predictor of prognosis in cases of relapsed/refractory PTCL-NOS, especially among patients with low secondary IPI risk.

PB1717

AUTOIMMUNE DISEASES ARE NOT ASSOCIATED WITH INFERIOR PROGNOSIS IN LYMPHOMA PATIENTS

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Background: Previous epidemiological studies have shown that autoimmune diseases increase the risk of lymphoma development. Immune dysregulation could be the possible underlying pathogenesis. Whether autoimmune diseases deteriorate outcome of lymphoma patients, however, remains unclear.
Aims: The objective of this study is to compare the clinical outcome among lymphoma patients with and without autoimmune diseases.
Methods: From January 2008 to November 2016, we retrospectively reviewed medical records of 913 newly diagnosed lymphoma patients. From these 913 lymphoma patients, 34 (3.71%) patients were diagnosed to have autoimmune diseases before their lymphoma identification. Among these 34 patients, six patients lost their follow-up. A total of 28 lymphoma patients with pre-existing autoimmune diseases were finally analyzed. For the further comparison, 56 lymphoma patients without pre-existing autoimmune diseases who were adjusted for age and gender were considered to be the control group. Response rate, progression-free survival (PFS), and overall survival (OS) were compared between these two groups of patients.
Results: Rheumatoid arthritis was the most common autoimmune disease in lymphoma patients (11/34; 32.3%). The complete remission rate for lymphoma patients with and without autoimmune diseases were 72.0% and 83.3%, respectively (p=0.178). The PFS for patients with and without autoimmune diseases were 44.3±32.1 months and 50.9±28.6 months, respectively (mean±standard deviation; p=0.334). These two groups of patients had similar OS time as well (46.4 ±31.5 months *vs* 52.9±28.0; mean±standard deviation; p=0.337). Univariate analysis did not show autoimmune diseases were associated with inferior OS in lymphoma patients (crude hazard ratio: 1.32; 95% confidence interval: 0.43 - 4.07; P=0.627).
Summary/Conclusions: The results of this case-control study showed the autoimmune disease was not a poor prognostic factor for lymphoma patients.

PB1718

THE DIAGNOSTIC AND PROGNOSTIC IMPLICATIONS OF CIRCULATING MIRNA-21 IN A SAMPLE OF HEPATITIS C/NONE HEPATITIS DIFFUSE LARGE B-CELL LYMPHOMA EGYPTIAN PATIENTS

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Background: MicroRNAs (miRNAs) are small RNA molecules which control the expression of many target messenger RNAs involved in cell differentiation, proliferation and apoptosis. Circulating microRNAs are potential biomarkers of diagnostic and prognostic impact in various inflammatory and malignant diseases. Unlike all other malignancies, studies of the prognostic implication of miRNA-21 expression in diffuse large B-cell lymphoma (DLBCL) patients have been a matter of debate. To our knowledge, there are no existing data up to date on the expression of miRNA-21 in hepatitis C virus (HCV) associated DLBCL.
Aims: Linking inflammation with malignancy, we studied the expression of miRNA-21 in sera of hepatitis-C-virus and none hepatitis DLBCL patients, aiming to identify its differential expression and prognosis in DLBCL with its subtypes; germinal center B-cell (GCB) and activated B-cell-like (ABC) and to evaluate its relation with HCV.
Methods: MiRNA-21 expression was measured using Taq-Man quantitative RT-PCR in sera of 30 newly diagnosed DLBCL patients (HCV positive (n=10), HCV negative (n=20)) and 20 controls (HCV positive (n=10), HCV negative (n=10)). The diagnosis of DLBCL and its sub-classification in GCB and ABC subtypes were done by applying the criteria of the WHO classification of tumors of the hematopoietic and lymphoid tissues 2008 and revised in 2016 and were confirmed by Immunohistochemistry using antibodies to CD10, BCL-6, MUM-1 and BCL-2. HCV was diagnosed by detection of anti-HCV antibodies in sera of patients and controls by Enzyme-Linked Immunosorbent Assay (ELISA) technique and HCV genetic detection and quantification by polymerase chain reaction (PCR). All the patients received CHOP chemotherapy and were followed up for an average of 24 months.
Results: MiRNA-21 expression was significantly higher in DLBCL patients than in controls (p=0.00). Significant positive correlations between miRNA-21 and LDH, IPI and disease stage were detected (p <0.05). Significantly higher miRNA-21 levels were detected in ABC subtype compared to GCB subtype (p=0.00). Significantly higher miRNA-21 expression levels were detected in BCL6 negative, CD10 negative, MUM1 positive DLBCL cases compared to its levels in BCL6 positive, CD10 positive and MUM1 negative cases, (p=0.018, 0.002 and 0.001 respectively). Higher miRNA-21 was associated with worse response (p=0.016), 2-year overall (p=0.017) and 2-year progression free survival with statistical significance (p= 0.003). Significantly higher miRNA-21 levels were detected in HCV positive DLBCL patients compared to HCV-negative patients (p=0.00). Higher miRNA-21 levels were detected in HCV positive ABC subtype than GCB subtype (p=0.05). Significantly higher levels were also detected in HCV positive controls compared to HCV-negative controls.
Summary/Conclusions: Our study showed that miRNA-21 was overexpressed in DLBCL patients, displaying higher levels in ABC than in GCB subtypes. MiRNA-21 was associated with poor response to treatment and survival in DLBCL. According to our results, miRNA-21 is a potential marker of necro-inflammation independent of its role in tumorigenesis, showing higher expression in HCV positive DLBCL patients compared to none hepatitis patients.

PB1719

A NEW SCORING SYSTEM FOR PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA – A RETROSPECTIVE MULTI-CENTER ANALYSIS IN TAIWAN

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Background: Primary central nervous system lymphoma (PCNSL) is a rare type of non-Hodgkin's lymphoma. Two independent prognostic scoring systems have been developed at the Memorial Sloan-Kettering Cancer Center (MSKCC) and the International Extranodal Lymphoma Study Group (IELSG). The former considers age and Karnofski's performance status (PS) as prognostic parameters(*JCO.* 2006;24:5711). The latter includes age, Eastern Cooperative Oncology Group (ECOG) PS, the presence of deep lesions, serum lactate dehydrogenase (LDH) and total protein levels in the cerebrospinal fluid (CSF)(*JCO* 2003;21:266). Neither of the two systems has been verified in the Asian population, leading to concerns regarding applicability in this region.
Aims: This study was conducted to test the prognostic power of the 2 systems in PCNSL patients in Taiwan. In addition, we analyzed the parameters of the IELSG system to figure out the most powerful prognostic factors and then established a new scoring system.
Methods: The medical records of patients with tissue-proven PCNSL were retrieved from 15 academic hospitals in Taiwan through January 2002 to December 2011. They were stratified into different groups according to the MSKCC or the IELSG system and the overall survivals (OS) were evaluated. All parameters in the IELSG system were checked by multi-variable analysis to establish a new scoring system.
Results: When the IELSG scoring system was applied, the 2-year OS in low, intermediate and high-risk groups were 78.3%, 43.9% and 37.5% respectively with a crossover in the latter 2 groups (Figure 1A). When the patients were stratified by the MSKCC scoring system, the 2-year OS of class I, II and III were 65%, 68% and 20% (Figure 1B), respectively. We conducted single-variable analysis of the 5 parameters included in the IELSG scoring system and only age and ECOG PS were statistically significant. In the multi-variable analysis, these 2 factors were almost equally weighted. Based on these findings, we re-stratified the patients into 3 groups. Group 1 comprised patients with both age <60 and ECOG PS <2 and Group 3 with both age ≥60 and ECOG PS ≥2. The patients not fulfilling criteria of either Group1 or Group 3 were categorized as Group 2. According to this new scoring system, the median OS of Groups 1, 2 and 3 were 1,573, 548 and 304 days (Figure 1C), respectively, and their OS curves could be nicely distinguished.

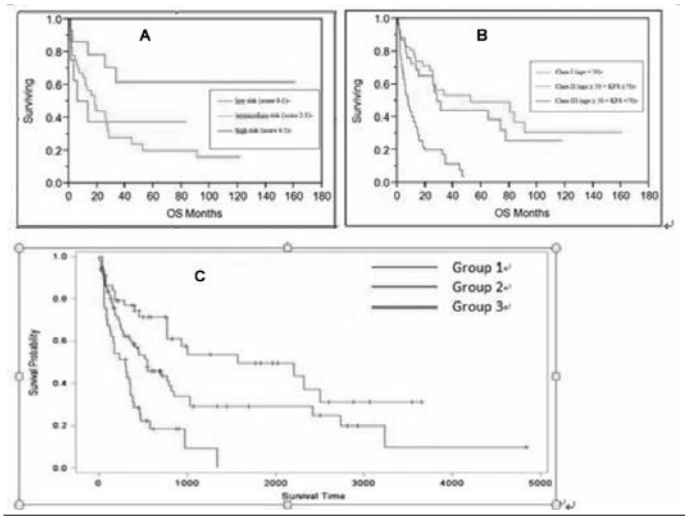

Figure 1.

Summary/Conclusions: Neither the IELSG nor MSKCC scoring system is ideal to distinguish the 2-year OS of the PCNSL patients in Taiwan. The new scoring system comprising age □ 60 years and ECOG PS □ 2 seemed to provide a better prognostic power for Taiwanese patients.

PB1720

RELEVANCE OF CIRS SCALE IN THE PROGNOSIS OF DIFFUSE LARGE B CELL LYMPHOMA IN ELDERLY PATIENTS

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Background: The incidence of lymphomas is increasing with age. Many aggressive lymphomas are now considered to be curable. All fit patients, even elders, are candidates for optimal treatment with a curative intent. Diffuse Large B Cell Lymphoma (DLBCL) is the most common non-Hodgkin Lymphoma, with 60% of curative rates after standard R-CHOP regimen. Patients that relapse can be rescued with salvage treatment in 20-30%. The elders are not considered for full standard treatment in many centers. Geriatric scales are starting to being used to stratify patients and offer them individualized treatments. The use of GSCF for neutropenia prophylaxis is not a standard of care in this population.
Aims: The objectives of this study were**:** 1) Validate CIRS score in a DLBCL cohort; 2) Analyse the impact of CIRS score in OS; 3) Analyse the impact of GSCF prophylaxis for neutropenic fever.
Methods: Between November 2008 and November 2015, 41 DLBCL patients with ≥60 years at diagnosis from a single institution and homogeneously treated with R-CHOP were analyzed. Patients were evaluated for comorbidities with Cumulative Illness Rating Scale (CIRS). CIRS score was used to detect the more unfit population and evaluate the average of admissions stay and the impact on OS. The CIRS scale was adjusted by removing the hematological question since all our patients were diagnosed with a hematologic malignancy. The cut-off point for CIRS score was selected using a ROC analysis. Neutropenic fever (NF) events were recorded and the use of GSCF in prophylaxis were analyzed, as well as the admission days for adverse events.
Results: In our series, 20 patients (48%) were males. Median age at diagnosis was 73 years old (range 60-90) With a median follow-up of 32 mo. (range 0-96), the median PFS was 51 months and the OS was 61 mo. The patients were stratified by the R-IPI and the NCCN-IPI. The ROC analysis showed a scoring of 5,5 in CIRS to identify two different risk groups, with an AUC of 70,5%, a sensibility of 87% and a specificity of 48% (p=0,02). In the low risk group, with CIRS <6 (n=17), 7 (41%) patients were admitted with a mean of stay of 6,2 days (range 1-16) *vs* the high-risk group with CIRS >6 (n=24). Of this group, 11(45%) patients were admitted with a mean of stay of 10,6 days (range 1-62), p=0,035. The CIRS scale was also used to discriminate two OS groups; the low risk showed a median OS not reached *vs* 29 mo. the high-risk group, with a Hazard ratio of 2,68 (CI95%: 1,031-5,882, p= 0,042). NF was the most common ER visit, n=18 (36%). Of the 18 patients with NF, 10 (55%) were prescribed with GCSF prophylaxis mid cycles. Of all patients with GCSF (n=43) only 10 (24%) NF were reported. 11/17 patients (65%) who didn't use GCSF prophylaxis had an NF episode. The Odds ratio (OR) for the patients under prophylaxis was 0,232 (CI 95%: 0,085-0,634, p=0,004) (Figure 1).

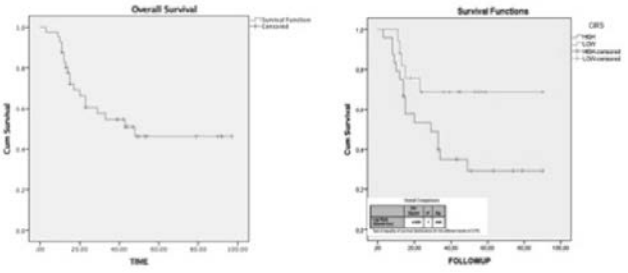

Figure 1.

Summary/Conclusions: The OS and the PFS in our sample is similar as described in larger studies. The days of admissions adjusted to the CIRS scale give us a tool to help physicians to discriminate patients with DLBCL that will have prolonged admissions when treated with the standard of care. The CIRS scale also help separate two distinct OS curves, giving physicians a new tool to help discriminate worse prognostic patients, making them good candidates for adapted therapies. The use of GSCF prophylactic can protect the elderly patients from NF, and should be used in all patients in this category.

PB1721

PRIMARY ADRENAL LYMPHOMA: A SINGLE-CENTER EXPERIENCE

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Background: Primary adrenal lymphoma (PAL) is rare, with slightly more than 250 cases currently described in the English-language literature. In current classifications, there is not yet a consensual definition of PAL.
Aims: The aim of this study was to report a large single-center clinical case series of primary adrenal lymphoma (PAL) in terms of clinical presentation, pathological and imaging features, and treatment outcome.
Methods: We performed a retrospective analysis of 21 patients diagnosed with PAL who presented to our center between January 2005 and January 2014.
Results: Median age at presentation was 48 years (range: 27–73) with a male-to-female ratio of 5:2. Bilateral and right-sided adrenal involvement were seen in 12/21 and 7/21 patients, respectively. Adrenal insufficiency (AI) was seen in

6/10 evaluated patients. Computed tomography scans showed slight to moderate contrast enhancement of adrenal masses in 4/5 patients (80%), and magnetic resonance imaging identified a normal T1 and longer T2 phase. Diffuse large B cell lymphoma (DLBCL) was the most common immunophenotype (85.6%). Two patients died due to rapid disease progression before treatment. Three patients were treated with chemotherapy±external beam radiotherapy. Two patients received autologous stem cell transplantation as consolidation therapy. Five-year overall survival and progression-free survival were 54.2% and 51.0%, respectively.
Summary/Conclusions: These findings suggest that PAL should always be considered in differential diagnosis of adrenal mass with AI. Moreover, DLBCL was observed as the most common histological subtype of PAL. Despite the contrasting previous reports, long-term prognosis of PAL is not necessarily inferior to that of non-Hodgkin lymphoma in general.

PB1722

EFFICACY AND SAFETY OF IBRUTINIB THERAPY IN RELAPSED/REFRACTORY MANTLE CELL LYMPHOMA IN REAL-LIFE – A MULTICENTRIC STUDY (R.E.P. - APULIAN HEMATOLOGY NETWORK)

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Background: Mantle cell lymphoma (MCL) is a rare subtype of non-Hodgkin lymphoma that has an aggressive clinical course and poor prognosis. Although current front-line combination chemo-immunotherapies followed by autologous stem-cell transplantation (ASCT) have improved the outcomes of affected patients (pts), this disease is still incurable and relapse is common. Ibrutinib is an oral covalent inhibitor of Bruton tyrosine kinase that showed significant activity in relapsed/refractory MCL in clinical trials, but in real-life routine, the efficacy and safety may not always mirror those seen in clinical trials.
Aims: We investigated the clinical use of ibrutinib as a single-agent in 31 pts with relapsed or refractory MCL to obtain additional information about predictive factors, outcomes and toxicity in a real-life context.
Methods: We studied a group of 31 pts treated (or still in treatment) with ibrutinib to assess effectiveness in terms of overall response rate, complete response rate, progression free survival and adverse events (AEs) in a real-life context. Data were collected also with reference to clinical and biological characteristics of the disease (MIPI, MIPIb, bone marrow involvement, stage, histology, presence of bulky mass and/or extranodal disease) both at the time of diagnosis and at the time of the start of ibrutinib therapy, and to the type and number of previous therapies.
Results: At the start of ibrutinib therapy, the median age was 70 years (range, 45-82), 100% of pts had high risk MCL according to the MIPI score, 83.9% of pts had disease stage III or higher, 41.9% of pts had bone marrow involvement, and 45.2% of pts presented extranodal involvement of MCL. 26 pts were treated for relapsed MCL, 5 for refractory disease. They had received a median of 2 (range, 1-5) prior regimens including different chemo-immunotherapy schemes, ASCT and newer agents such us bortezomib, lenalidomide, temsirolimus. We observed 6 complete responses, 1 after only 2 months of therapy, the others within 6 months of therapy. After 15 months, we observed 4 relapses, characterized by leukemic disease and one of them also presented central nervous system involvement, and 8 progression. 80% of pts treated for refractory disease presented progression within 6 months. The most common AEs were fatigue (13% of pts) and weight increase (13% of pts), followed by diarrhea and bleeding (grade ≤ 2) (6.4% of pts). The most common hematologic event observed was neutropenia (9.7% of pts, grade ≤ 2). With an estimated median follow-up of 6 months (range, 4-29), 19 pts are still receiving treatment, 12 have discontinued therapy for relapse or progression of disease. Follow-up is still ongoing.
Summary/Conclusions: Single-agent oral ibrutinib shows a high response rate and produces rapid responses regardless of the number and quality of prior regimens. However, the quality and time of response does not seem to be predictive of a better PFS or longer duration of response. Furthermore, resistance to ibrutinib in pts with MCL is associated with fulminant, severe progression. Ibrutinib is well tolerated also in real-life experience. The weight increase in 13% of pts suggests that ibrutinib may have an anabolic effect, including alterations in blood pressure and lipid profile. Larger cohorts of pts and longer follow-up are warranted to confirm these preliminary data.

PB1723

HEMATOLOGICAL MALIGNANCIES IN SOLID ORGAN TRANSPLANT RECIPIENTS: RETROSPECTIVE SINGLE-CENTER ANALYSIS IN JAPAN

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Background: Solid organ transplant recipients have elevated onset risks of hematological malignancies (HMs) due to long-term administration of immunosuppressant. However, few studies about the incidence and impact on survival of HMs following solid organ transplantation have been conducted in Asian countries.
Aims: The aim of this study was to identify the incidence, characteristics, risk factors and prognosis of HMs in solid organ transplant recipients at our institution.
Methods: Clinical data of patients undergoing kidney, liver and heart transplantation in Hokkaido University hospital between 1965 and 2015 were reviewed retrospectively. Kaplan-Meier analysis was performed for the cumulative incidence rates (CI) of HMs, graft survival and patient survival. Patient's characteristics were compared between groups by the student t-test or Kai-square test.
Results: A total of 16 cases of HMs were identified, 9 post-transplant lymphoproliferative disorder (PTLD), 5 acute myeloid leukemia (AML)/myelodysplastic syndrome (MDS), 1 myeloproliferative neoplasm (MPN)) and 1 recurrent non-Hodgkin lymphoma. The CI of PTLD were 1.1%, 1.5% at 5, 10 years in kidney transplant recipients (n=352), 0.92%, 2.6% at 5, 10 years in liver transplant recipients (n=287) and 20% at 1 year heart transplant recipients (n=5), respectively (P<0.0001). AML/MDS and MPN developed only in liver transplant recipients, and CI were 2.3% at 5 and 10 years (P<0.01). There was no difference in background factors other than transplanted organ type between recipients with HMs and without HMs. Patients with EBV-positive PTLD (n=5) were younger (P<0.05) and had less extranodal diseases (P<0.05) compared with EBV-negative PTLD (n=4). All patients with monomorphic PTLD (n= 4) were treated with chemotherapy combined with rituximab and had been in remission. In patients with other PTLD, reduction or withdrawal of immunosuppressant or rituximab alone resulted in stable disease or remission. All AML/MDS but 2 acute promyelocytic leukemia in pediatric patients were chemo-refractory and lethal. 10-year OS were 92% and 100% in kidney and heart transplant recipients. In liver transplant recipients, 10-year OS were 74%, 100% and 50% in patients without disease, with PTLD and with myeloid neoplasm, respectively. Survival in adult liver transplant recipients with myeloid neoplasms was inferior to that without disease (P<0.05). 10-year graft survival rates were 72% and 75% in kidney transplant recipients without disease and with PTLD.
Summary/Conclusions: The incidence of PTLD in solid organ transplant recipients in Japan is comparable to that in Western countries, whereas the incidence of myeloid neoplasms is higher in liver transplant recipients. PTLD dose not have a negative impact on the prognosis of solid organ transplant recipients under appropriate management, while heightened awareness and better clinical approach for myeloid neoplasms following solid organ transplantation are needed.

PB1724

MYC REARRANGEMENT HAS A STRONG PROGNOSTIC IMPACT IN THE FEMALE PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Cytogenetic abnormalities of *MYC* are associated with poor prognosis in patients with diffuse large B-cell lymphoma (DLBCL). Rearrangement of *MYC* reportedly occurs in approximately 10% of DLBCL cases. In addition, in various clinical trials of rituximab with standard dosing, female receiving rituximab have had better outcomes than male. However, gender-segregated outcomes of patients with *MYC* rearrangement have not been reported. In addition, the gender segregation of known prognostic factors, such as high international prognostic index (IPI) score, elevated lactate dehydrogenase (LDH) level, poor Eastern Cooperative Oncology Group performance status (PS), advanced stage, and ≥2 extranodal sites, not as yet been fully elucidated.
Aims: The aim of this study was to determine the gender segregation of clinicopathological and genetic prognostic factors, including *MYC* (fluorescence in-situ hybridization: FISH) in patients with DLBCL by analyzing data from consecutive DLBCL patients.
Methods: In order to identify prognostic factors, we analyzed gender segregation from the medical records of patients with DLBCL, including newly diagnosed and transformed, at Juntendo University Hospital from December 2009 to December 2016. We retrospectively analyzed the data of 161 consecutive DLBCL patients (male: 91 patients, female: 70 patients). Patients in this study were treated with R-CHOP or R-CHOP-based regimens with minor modifications. The relationships between overall survival (OS), progression free survival (PFS) and age, LDH level, PS, stage, ≥2 extranodal sites, IPI, cell of origin (COO), BCL2 (immunohistochemistry: IHC), BCL6 (IHC), MYC (IHC), double expressor (MYC and BCL2 expression on IHC), and *MYC* (FISH) were investigated. Univariate and multivariate analyses of estimated risk factors for OS and PFS were performed using the log-rank test and Cox proportional hazard regression analysis.
Results: The median age was 70 years (range: 27–92 years). The median follow-up was 17 months (range: 1–81 months). To adjust the impact of age, LDH level, PS, stage, ≥2 extranodal sites, IPI, COO, BCL2 (IHC), BCL6 (IHC), MYC (IHC), double expressor (IHC), *MYC* (FISH), and other significant factors, uni-

variate analysis was performed for the OS. Elevated LDH level, stage ≥3, PS ≥2, ≥2 extranodal sites, IPI≥3, BCL6 negative (IHC), and *MYC* rearrangement (FISH) were significant factors in the female patients; however, PS ≥2 and IPI≥3 were significant factors in the male patients. Univariate analysis was also performed for PFS. Elevated LDH level, PS ≥2, IPI≥3, BCL6 negative (IHC), and *MYC* rearrangement (FISH) were significant factors in the female patients; however, PS ≥2 was the only significant factor in the male patients. Multivariate analyses were then performed using these factors in the Cox proportional hazard model. *MYC* rearrangement (FISH) [hazard ratio (HR): 9.13, 95% confidence interval (CI): 2.33–35.77, *P*=0.0015], and IPI ≥3 were identified as independent significant prognostic factor for OS in the female patients with DLBCL. Furthermore, *MYC* rearrangement (FISH) (HR: 2.47, 95% CI: 1.87–327.8, *P*=0.01494], and elevated LDH level were identified as independent significant prognostic factor for PFS in the female patients with DLBCL. On the other hand, PS ≥2 was identified as the only significant prognostic factor for OS (HR: 44.27, 95% CI: 6.71–292.2, *P* <0.001), but not for PFS in the male patients with DLBCL. Five out of seven female patients with DLBCL and *MYC* rearrangement died from lymphoma progression. The median OS in the female patients with DLBCL and *MYC* rearrangement was 8.0 months (range: 1–35 months) compared to 21.5 months in those without *MYC* rearrangement (range: 1–79 months, *P*=0.003). On the other hand, in the male patients (n=13) with DLBCL, *MYC* rearrangement was not significantly associated with poor OS (Figure 1).

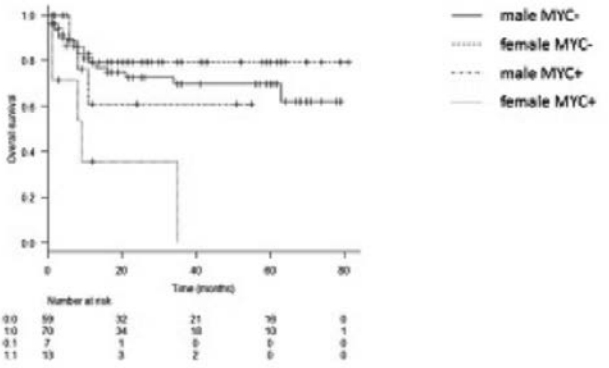

Figure 1. Overall survival.

Summary/Conclusions: These results suggest that *MYC* rearrangement by FISH is significantly associated with very poor OS and PFS in the female patients with DLBCL but not the male patients with DLBCL. On the other hand, PS ≥2 is significantly associated with poor OS in the male patients with DLBCL.

PB1725

ASSESSING THE RISK FOR PERFORATION IN DIFFUSE LARGE B-CELL LYMPHOMA INVOLVING THE INTESTINES USING COMPUTED TOMOGRAPHY CHARACTERISTICS.

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Background: Around 40% of all Diffuse Large B-Cell Lymphoma (DLBCL) cases involve extra-nodal sites, the most common being the gastro-intestinal (GI) tract. DLBCL patients with intestinal involvement are particularly prone to develop GI perforation, which might be life threatening and entail significant morbidity. Identification of patients at risk for perforation may promote the performance of pre-emptive surgical resection of the involved segment. Although computed tomography (CT) scan is widely used at diagnosis, incorporation of CT results into the risk stratification of perforation has not yet been performed.
Aims: To determine risk factors for perforation in patients with DLBCL and intestinal involvement, with an emphasis on CT findings.
Methods: A retrospective single center study, including all consecutive DLBCL patients that presented with intestinal involvement between 2005 and 2016. The analysis included clinical, laboratory, pathological and radiological parameters. Cases with DLBCL of the stomach were excluded.
Results: Forty-nine cases (30 men, 19 women) were included. Median age of the entire cohort was 64 years (54.5-77 IQR). Early stage (1, 2) according to the Lugano system was reported in 35% of cases. Small intestine involvement was most frequent (61%), followed by large intestine and ilio-cecum (23 and 16%, respectively). Forty-three (88%) patients underwent CT scan at diagnosis. Most lesions were defined radiology as concentric (n=27, 63%) (as opposed to eccentric), and transmural (n=31, 74%) (as opposed to non-transmural). Of note, 96.3% of the 27 concentric lesions were also transmural, compared with 31% (5/16) of the eccentric lesions. The median length and wall thickness of the involved site were 9.3 cm (5.8-13.5) and 15 mm (10-20), respectively. Ten (20%) patients developed an intestinal perforation. Six of the perforations (60%) involved the small intestine, 3 (33%) occurred at diagnosis prior chemotherapy, and 4 (40%) occurred within the first 21 days post therapy. All perforated lesions were concentric and transmural, with a median length of 11.2 cm. Eight (80%) patients underwent an urgent operation due to GI perforation, including 3 that resulted in an ostomy. Perforation led directly to 2 (20%) deaths. Perforation resulted in delayed administration of chemotherapy in 50% of cases (n=5). A univariate regression analysis found a higher risk of perforation in patients presenting with a concentric lesion (p=0.001. HR=46, CI 31.5-78.5), a transmural lesion (p=0.001. HR=34.6, CI 25.9-53.3) and a longer involved GI segment (p=0.008. HR=1.06, CI 1.017-1.116). Each extra centimeter to the length of the GI segment involved was associated with a 6% increase in the risk for perforation. There was no association between sex, age, performance status, hemoglobin, LDH, albumin, iron, ferritin, KI67, disease stage, anatomical location nor the involved site wall thickness and risk of perforation.
Summary/Conclusions: DLBCL patients presenting with an involvement of a long intestinal segment, especially with a concentric, transmural lesion, are at higher risk for perforation. These patients should be considered for a preemptive surgical resection, dependent on lesion site and operative risk.

PB1726

DOUBLE-HIT AND TRIPLE-HIT LYMPHOMAS: TREATMENT AND CLINICAL OUTCOME IN A SINGLE INSTITUTION

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Background: Five to 15% of patients with diffuse large B cell lymphoma (DLBCL) present *MYC* and *BCL2* and/or *BCL6* rearrangements which are detected by fluorescence *in situ* hybridization (FISH) or standard cytogenetic. This rearrangement defines a subgroup of DLBCL so-called double hit or triple hit lymphomas (DHL/THL) which are included in the 2016 WHO classification revision of lymphoid neoplasm in a new category "High-grade B-cell lymphoma with rearrangements of *MYC* and *BCL2* and/or *BCL6".* DHL/THL have an aggressive clinical course and poor response to standard chemotherapy and a median overall survival of 0.2-1.5 years. The best therapeutic option in these patients is not yet well established.
Aims: To evaluate retrospectively the incidence, clinical-biological characteristics, type of treatment, overall survival (OS) and progression-free survival (PFS) of patients diagnosed with DHL/THL and to compare them with patients with DLBCL without double/triple-hit genotype (DLBCL-noDH/TH) in a single institution.
Methods: From January 2000 to April 2016, we analized 18 patients with DHL/THL and 312 patients with DLBCL-noDH/TH. DHL/THL cases were identified using FISH for *MYC, BCL2* and *BCL6* in the tumor tissue (11 lymph node biopsy, 2 gastrointestinal biopsy, 1 bone marrow biopsy, 3 skin biopsy and 1 cerebrospinal fluid).
Results: The incidence of DHL/THL was 5.5%. The median age was 70 years [range 53-93]. The patients included in DHL/THL group had a higher prevalence of advanced disease and higher IPI (p=0.002). Thirteen patients received anthracyclines containing chemotherapy, 3 citoreductive treatment and 2 palliative care. No stem cell transplantation was performed in any patient as a consolidation therapy. Four out of 13 patients achieved complete remission, 3 patients partial response and 6 patients were refractory. At last follow up, 13/18 patients were dead (11 lymphoma progression; 2 infectious complications). Median follow-up 63 months. OS in DHL/THL was 9 months and in DLBCL-noDH/TH was not reached (p=0.001). The PFS in DHL/THL and in DLBCL-noDH/TH was 5.4 and 63 months, respectively (p<0.001) (Figure 1).

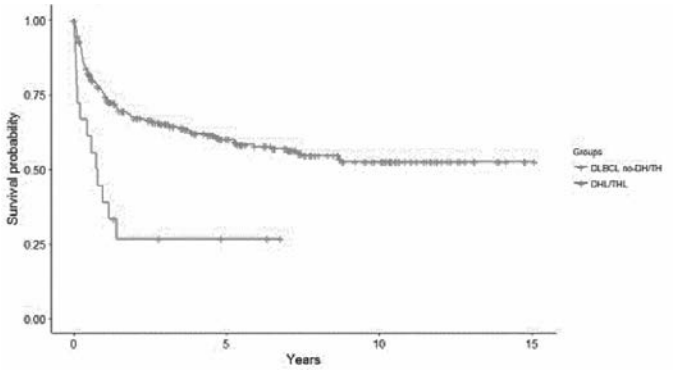

Figure 1. Overall survival.

Summary/Conclusions: 1)The incidence of double or triple hit lymphomas in our institution is consistent with the literature. 2)The most common regimen used in double or triple hit patients was anthracycline-cointaining chemotherapy achieving more than 50% of overall responses in our series. Nevertheless, the majority of patients relapse, showing a short PFS and worse outcome than DLBCL without double or triple hit, as reported previously.

PB1727

EFFECTIVE TREATMENTS ARE REQUIRED FOR PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA WITH PRIMARY REFRACTORY DISEASE

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Background: DLBCL is a heterogeneous disease; it has been described that around 30% of patients present a refractory/relapsing disease following R-CHOP treatment. Rituximab-containing salvage chemotherapy followed by high-dose therapy and autologous stem cell transplant (ASCT) in chemosensitive patients remains the standard of care for these patients.
Aims: We aimed to study the clinical features and outcome of patients diagnosed of DLBCL, homogeneously treated with R-CHOP/R-CHOP-like first line regimen, who have primary refractory disease (PRD).
Methods: Three hundred and sixty-seven patients were diagnosed of DLBCL between January 2004 to August 2016 in our center, 317/367 (86,3%) were treated with R-CHOP or R-CHOP-like in first line. Forty-four (13.9%) patients had PRD and 39 (12.3%) progressed during the follow up. Survival curves were estimated using the Kaplan-Meier method and compared using the Log-Rank test. Risk factors at diagnosis for PRD to R-CHOP were assessed. Univariate analyses were performed by Chi square test and multivariate analyses by Cox proportional hazard regression model.
Results: Among the 44 primary refractory patients, 15 (34%), with a median age of 76 years (range 63-91), were considered unfit, 11 received supportive care and 4 were treated with palliative chemotherapy (cyclophosphamide and prednisone). Twenty nine (66%) were eligible for salvage therapy and consolidation with ASCT. Characteristics of those 29 patients at the time of salvage therapy were: median age 50 years (range 21-71), males 19 (65.5%), ECOG 2-4 16 (55.2%), Ann Arbor stage III-IV 23 (79.3%), B-symptoms 9 (31%), bulky disease (20.7%), extranodal involvement 20 (69%), leptomeningeal infiltration 4 (13.8%), high LDH 19 (65.5%), IPI 3-5 21 (72.4%). Salvage therapies used were: R-ESHAP 23 (79.4%), R-ICE 1 (3.5%), MTX-ARAC 4 (13.8%) in patients with leptomeningeal infiltration andintensive burkitt-like therapy 1 (3.4%) in a double hit patient. Twelve (41.4%) did not complete the treatment: 2 (6.8%) for toxicity (1 cardiac event and 1 septic shock) and 10 (34.4%) for progression. The intention-to-treat response rate was: CR 1 (3.5%), PR 4 (13.8%), refractory disease/progression 22 (75.8%) and not evaluable 2 (6.9%). Five patients underwent an ASTC (BEAM as conditioning regimen). One died during transplant due to septic shock and 4 progressed with a median follow-up of 5 months. One patient was rescued with a third line of treatment (R-ICE) and allogeneic transplant, and he is currently in CR at 7 months. Median PFS was 2 months (CI 95% 1.2-2.7) and median OS was 5 months (CI 95% 3.4-6.6). Among the 15 primary refractory patients who were treated with palliative intention, median PFS was 1 month (CI 95% 0.19-1.80) and median OS 1 month (CI 95% 0.19-2.42). Among the 317 patients treated with R-CHOP, risk factors at diagnosis for having PRD to R-CHOP were: B-symptoms (HR 1.94, 95% CI: 1.05-3.61, p=0.034) and elevated LDH (HR 3.92, 95% CI: 1.61-9.51, p=0.003) (Table 1).

Table 1.

Risk factors at diagnosis for primary refractory DLBCL to R-CHOP

	Univariate (χ^2 test)	Multivariate (Cox regression model)		
	P value	HR	CI 95%	*P value*
Gender	0.226	0.55	0.299-1.04	0.067
Aged ≥ 60 years	0.757	1.05	0.567-1.962	0.865
ECOG ≥ 2	0.089	1.45	0.76-2.78	0.256
Ann Arbor stage III-IV	**0.002**	2.04	0.85-4.88	0.107
B-Symptoms	**0.005**	**1.94**	**1.05-3.61**	**0.034**
Bulky mass (>5cm)	0.053	1.67	0.88-3.18	0.116
Extra nodal involvement	0.453	0.71	0.36-1.40	0.326
High LDH	**0.000**	**3.92**	**1.61-9.51**	**0.003**

Summary/Conclusions: Patients with DLBCL refractory to first line R-CHOP are not rescued with current salvage therapies, and in this settingDLBCL must be considered an incurable disease with a very short survival, similar to that of patients treated with palliative care. Patients with B symptoms and elevated LDH at diagnosis have a significant higher risk to be refractory to R-CHOP. It is imperative to identify early these patients and to design new therapies for them.

PB1728

RITUXIMAB BENDAMUSTINE CYTARABINE IS A FEASIBLE AND SAFE INDUCTION REGIMEN PRIOR TO ASCT IN FRONTLINE MCL: A SINGLE CENTER RETROSPECTIVE REAL LIFE EVALUATION

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Background: Mantle cell lymphoma (MCL) is an uncommon, still incurable subtype of non Hodgkin lymphoma. The routine use of high dose Cytarabine and high dose chemotherapy followed by autologous stem cell transplant (ASCT) markedly improved the outcome and has become the standard treatment for fit, young (<65 years) patients. Recently, two phase II studies demonstrated that Rituximab, Bendamustine and Cytarabine (RBAC) combination has a remarkable activity with a favorable safety profile both in untreated and relapsed/refractory elderly MCL patients (Visco *et al.,* 2013 and 2017). These studies suggested that RBAC combination (with Cytarabine 800 mg/mq) is safe and effective as a CD34+ stem-cell mobilizing regimen. No data are available on RBAC with Cytarabine 500 mg/mq as mobilizing regimen in transplant-eligible patients.
Aims: To assess the efficacy and safety of RBAC as induction therapy and as a peripheral blood progenitor cell mobilization therapy in combination with granulocyte colony stimulating factor (Lenograstim) in newly diagnosed transplant-eligible mantle cell lymphoma patients.
Methods: From November 2009 to March 2016, 10 newly diagnosed MCL patients (median age 65 years; range 55-72) were treated as induction immunochemotherapy according to RBAC schedule (Rituximab 375 mg/mq day 1, Bendamustine 70 mg/mq day 2-3, Cytarabine 500 mg/mq day 2-3-4) for 4 cycles. 90% had a stage IV disease; MIPI score was high in one patient (10%), intermediate in 5 patients (50%) and low in 4 patients (40%). Stem cell harvest was performed after 2 cycles. Successful mobilization was defined as achieving physician-determined target PBSC yield which was CD34+ cells >= 2 x 10^6/Kg. The G-CSF (Lenograstim) infusion started per protocol at day 6 at the dose of 5 umg/kg.
Results: All patients completed the scheduled treatment (4 cycles). The ORR was 90%: CR 90% and PD 10% (1 patient). Overall, the rates of successful mobilization and the proportion of patients achieving a total PBSC yield of ≥2x10^6/kg were 100%, and the median PBSC yield was 10 x 10^6/kg (range 3-20x10^6/kg). The median time to stem cell harvest was 17 days (range 14-19). The median number of apheresis to achieve the PBSC target was 1 and only 1 patient (10%) required a second collection procedure. Plerixafor was not used. 80% of patients underwent high dose chemotherapy according to FEAM protocol (Fotemustine 150 mg/mq on days -7, -6, Etoposide 200 mg/mq) and Cytarabine 400 mg/mq on days -5, -4, -3, -2 and Melphalan 140 mg/mq on day -1) with infusion of at least 5 x10^6 /Kg of PBSC. The median day for neutrophils and platelet recovery (ANC >500/mmc, Plts >50,000/mmc) was 11 and 26 (range 10-29 and 14-34), respectively. There was no engraftment failure. Most frequent adverse events (according to CTCAE grading) during therapy were hematological: neutropenia (100%, all 3-4), thrombocytopenia (100%, 60% G3-4), anemia (100%, 50% G3-4). Among non hematological toxicities, 20% of patients had febrile neutropenia (G3-4), 20% mucositis (G1-2), 20% lung infections (G3), 10% hyperglycemia (G3). After a median follow up of 43 months the OS and PFS were 90% and 80% respectively.
Summary/Conclusions: As in the relapsed/refractory setting and in MCL patients ineligible for high dose chemotherapy, RBAC has been proven to be an efficacious induction and mobilization regimen also in transplant eligible MCL patients with an encouraging safety profile. Further investigations are needed to assess the optimal role of RBAC within the standard first line treatments.

PB1729

THE SAFETY OF LIPOSOMAL CYTARABINE IN CENTRAL NERVOUS SYSTEM INFILTRATION BY HAEMATOLOGIC MALIGNANCIES

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Background: Central nervous system (CNS) involvement, both leptomeningeal and parenchimatous conveys a poor prognostic in haematological malignancies. As well as systemic chemotherapy that crosses hematoencephalic barrier, intrathecal (IT) chemotherapy has become an attractive approach because of direct action in the cerebrospinal fluid (CSF). Liposomal cytarabine (Depocyte®) is a convenient formulation that maintains cytotoxic concentrations of cytarabine in CSF for an extended period of time (>14 days). This permits to decrease the frequency of lumbar punctures, without losing efficacy and minimizing the patient´s discomfort.

Aims: The objective of this retrospective, observational study is to evaluate the efficacy and safety of liposomal cytarabine in patients with CNS infiltration by haematological malignancies.
Methods: 36 consecutive patients with haematological disease and risk of CNS infiltration underwent flow cytometry (FC) analysis of CSF in a single center from December 2014 to December 2016. CNS involvement was assessed by using standard CSF cytology, 8-color flow cytometry or MRI imaging. Along with systemic therapy, all patients considered positive were treated 50 mg of IT Liposomal cytarabine administered by lumbar puncture every 2 weeks for 4 doses and every 4 weeks thereafter. Concomitant dexamethasone for arachnoiditis prophylaxis was added both i.v. and IT. We analysed the rate of adverse events (AE) and the time for CSF clearance. Short follow up precluded assessment of cumulative incidence of CNS relapse/progression.
Results: Data from 36 patients were analysed. A total of nine patients were considered to have CSF involvement, all of them detected by FC. Of note, all of them were considered negative for CSF infiltration by standard cytology. Three additional patients received IT therapy as prophylaxis since MRI imaging showed brain involvement by the malignancy. The median age of this 12 patients was 52 years (range16-69), 58.3% were female. Diagnosis were B-cell lymphoproliferative disorder 41.7% (CLL, Burkitt, DLBCL), ALL 25%, AML 25% and multiple myeloma 8.3%. The median number of doses per patient was 6.5 (SD 1.7). CSF clearance was achieved after a median of 1 dose (range 1-3) or 20 days (range 16-86). Overall rate of CNS response was 100%. Two patients (16.7%) had leptomenigeal relapse during the IT treatment. The overall AE incidence was 66.7%. The most common AE include: headache, peripheral sensory neuropathy, back pain and nausea. Severe neurotoxicity has been encountered in four patients: cauda equina syndrome (2), encephalitis (1) and arachnoiditis (1). Treatment had to be discontinued in 3 patients because of side effects but this did not lead to relapse. The median time to AE occurrence was 6 cycles (range 4-7) or 110 days (range 33-227). The incidence and severity of AE seemed to increase with the cumulative number of cycles administered. In most patients neurological complications resolved or improved with time.
Summary/Conclusions: use of liposomal formulation of cytarabine for IT administration has become an effective option for the treatment of leptomeningeal involvement by haematological malignancies. Neurological AE are reversible; however, they accumulate and worsen with time, thus precluding long-term use.

PB1730

RETROSPECTIVE ANALYSIS OF OUTCOMES FOR ELDERLY PATIENTS WITH STAGE 3 AND 4 DISEASE HIGH-GRADE DLBCL WITH REDUCED CYCLES OF R-CHOP OR R-GCVP : A 7 YEARS SINGLE-INSTITUTE EXPERIENCE.

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Background: The most common high-grade lymphoid malignancy in adults is Diffuse Large B-Cell Lymphoma (DLBCL), which has an increasing incidence with age (1). Over 40% of patients with DLBCL are above the age of 70, and the co-morbidities in this age-group present significant challenges and complexities with regards to selecting and implementing treatment regimens (2).
Aims: We present a retrospective analysis of outcomes for patients with high-grade DLBCL (stage 3 or 4 disease) who have received fewer than 6 cycles of full dose R-CHOP or R-GCVP because of poor tolerability or disease progression with treatment.
Patients and Methods: Retrospective data were collected from the cancer registry for all newly-diagnosed DLBCL patients who received R-CHOP or R-GCVP chemotherapy, with data collected from Jan 2010 to Feb 2017 from Ipswich Hospital NHS trust, United Kingdom. Patients who completed 6 cycles of chemotherapy were excluded. Interim PET-CT scan/staging CT scan was done to assess the disease response to therapy after 2 cycles of chemotherapy. The main baseline characteristics collected were age, sex, ECOG Performance Status, Ann-Arbor Stage and IPI risk stratification. The primary end point was progression-free survival (PFS) from completion of treatment. Secondary end points were overall response rate (ORR), overall survival (OS), and the reasons for premature ceasing of treatment based on graded toxicity according to NCI-CTCAE 4.0.
Results: Out of 87 patients, 12 patients were identified that fulfilled the inclusion criteria. The median age of patients was 72 years (range: 64-88 years), sex distribution was 7 male: 5 female, ECOG PS was 0-2 in 10 (83%) and ≥3 in 2 (17%) of the patients, Ann-Arbor Stage was 3 in 6 patients (50%) and 4 in 6 patients (50%), and IPI score was 3 in all 12 patients. 11 patients received R-CHOP and 1 patient received R-GCVP. The median length of treatment was 3.5 cycles (range: 1-5 cycles). The overall response rate was 50% on interim assessment and 75% at end of treatment assessment scan. The complete and partial response rates at the end of the treatment were 58% and 17% respectively. Progression free survival was 73% at 2 years (8 out of 11 patients) and 50% at 3 years (4 out of 8 patients). The median overall survival of deceased patients (4 out of 12) was 9.5 months (range: 2-42 months) and the median overall survival of living patients (8 out of 12) is at 40.5 months (range: 27-84 months). The most common reasons for stopping the treatment were intolerance of side-effects (4 out of 12) or neutropenic sepsis (3 out of 12). 2 out of 12 patients received an incomplete course of chemotherapy due to non-response or progression of disease with treatment.
Conclusions: DLBCL treated with less than 6 cycles of full dose R-CHOP or R-GCVP chemotherapy may achieve sustained long-term remission in selected patients with high IPI and significant co-morbidity. Further research on disease characteristics including molecular profile is needed to elucidate selected populations who may achieve long-term remission with shorter cycles of chemotherapy. Further insights may derive, for example, from analysis of polymorphism of folate pathway genes and /or of NF-kB,which have been previously suggested as pharmaco-genomic targets in lymphoid neoplasm. A risk stratification model needs to be developed to reduce drug toxicity and other short and long term treatment related complications so as to improve patient experience, and pharmaco-economic benefits.

PB1731

MULTIPLE NEOPLASMS CONSIST OF SOLID CANCER AND NON-HODGKIN LYMPHOMA

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Background: Malignant lymphoma is a nineth cause of death in Japan. And non-Hodgkin lymphoma(NHL) occupied more than 90%. We experienced cases and will report that we reviewed multiple neoplasms consisting non-Hodgkin lymphoma. We experienced 176 cases.
Aims: We aimed for epidemiology and prognosis improvement of malignant neoplasms including NHL. We want to look for a hint of the early detection.
Methods: We intended for multiple neoplasms 340 cases including hematological malignancy. We reviewed 190 cases of multiple neoplasms including malignant lymphoma. In 190 cases, NHL case were 176 cases. The examination factors are type of the hematological malignancy, gender, the age at onset of the first cancer, interval with the second cancer, treatment strategy. The definition of multiple neoplasms followed Warren & Gates theory. And as for the definition of synchronous type, a diagnosis interval is less than 6 months, metachronous type interval is more than 6 months. About statistical examination, we used SPSS statistics ver21.
Results: All cases are 176 cases, consist of male 108 cases, female 68 cases, synchronous type 45 cases, metachronous type 131 cases. Double neoplasms 149 cases, triple neoplasms 25 cases, quadple neoplasms 2 cases. The median age was 71yrs (ranged51-93yrs), the synchronous type 70yrs(ranged 51-88years), the metachronous type was 73yrs(ranged 57-93yrs). The counterpart of malignancies, Hodgkin's lymphoma 1 case, myelodysplastic syndrome 3 cases, acute myeloid leukemia 8 cases, multiple myeloma 4 cases, gastric cancer 38 cases, colon cancer 32 cases, lung cancer 26 cases, renal cell carcinoma 6 cases, prostate cancer 12 cases, breast cancer 14 cases, urinal bladder cancer 5 cases, uterin cancer 7 cases, esophangeal cancer 9 cases, hepatocellar carcinoma 12 cases. In double neoplasms was 149 cases, metachronous type was 112 cases. The median age of first diagnosis, 68yrs (ranged43-85yrs), the second cancer were 74yrs (ranged57-89yrs), About interval between solid cancer and NHL, median interval time was 58M, solid cancer precedence case was 53 cases, interval was 81M (ranged 7-564M), hematological malignancy precedence case was 59 cases interval was 55M (ranged 8-364M). The cause of death was that 15 cases were solid cancer, 72 cases were hematological malignancy and 6 cases were accident. The median overall survival was 18M(ranged 1-211M), synchronous type 14M(ranged 2-132M), metachronous type 22M (ranged1-116M).
Summary/Conclusions: In the case of a double cancer including solid cancer and NHL, the first cancer occurs in elderly. Diagnosis of malignant neoplasms within 3 years were 48 cases out of 149 cases(32.2%). The important point is that 3 years are required for careful observation at the time of malignancy diagnosis. It is nessessary to discover at the early stage. So it could be a lot of treatment options formalignant neoplasms. We think that a prognosis is improved.

PB1732

RETROSPECTIVE EVALUATION ON EFFICACY AND FEASIBILITY OF R-CODOX-M/IVAC REGIMEN IN AGGRESSIVE DLBCL

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Background: Diffuse Large B Cell Lymphoma (DLBCL) is an heterogeneous group of diseases. The aggressive behavior can be predicted by clinical risk scores, immunohistochemistry and cytogenetic. Among DLBCL, double hit lym-

phomas (DHL) and double or triple-protein-expression lymphomas (DPLs, TPLs) display a worse outcome. R-CHOP, which is the frontline treatment for DLBCL, showed a poor outcome in high risk IPI patients and DHLs or DPLs. From January 2011 in our centre (IRCCS AOU San Martino Hospital–IST, Genoa, Italy) R-CODOX-M/IVAC regimen has been adopted as first line in patients with aggressive DLBCL, defined by at least one among these features: high tumour burden, DPLs, IPI score >3 or by the presence of at least 1 extranodal site.

Aims: Our aim was to define the efficacy and feasibility of this frontline strategy and eventually identify the subgroups of patients who may benefit from this approach.

Methods: We retrospectively analyzed 20 patients affected by aggressive DLBCL treated with R-CODOX-M/IVAC. R-CODOX-M consists of rituximab 375 mg/sqm day 1, cyclophosphamide 800 mg/sqm day 1, 200 mg day 2-5, doxorubicin 40 mg/sqm day 1, vincristine 1,4 mg/sqm, methotrexate 6700 mg/sqm. IVAC-R contains rituximab 375 mg/sqm, iphosphamide 1500 mg/sqm day 1-5, etoposide 60 mg/sqm day 1-5, cytarabine 2000 mg/sqm bid day 1-2. In both cycles CNS prophylaxis was administered. According to Ann Arbor classification, 11 patients were on stage IV, 1 on stage III, 3 in stage II and 5 in stage I. Twelve patients had B symptoms. Median IPI score was 3. Eleven patients had DPLs and 4 of them had TPLs. Overall survival (OS) was calculated from the time of diagnosis to the dime of death or last follow-up.

Results: After a median follow-up of 28 months, 5 patients died (25%), OS at six and twelve months was 89,4 and 70,4%, respectively, median not reached (NR). Complete remission was achieved in 11 patients (69%), partial remission in 2 patients (13%). The overall response rate was 82%. Three patients (18%) were primary refractory. Among DPLs, OS at six and twelve months was 88,9 and 64,8%, respectively, not significantly lower than non DPLs patients (p=n.s., median NR). In patients with Ann Arbour stage III or IV, OS at six and twelve months was 90,9 and 60,6% (median NR). In patients with IPI score >3, OS at six and twelve months was 78,8 and 45% (median 12 months). The main toxicity during CODOX-M was grade >2 mucositis, 63% of patients. Infections occurred in 71% of patients. Renal and liver toxicity was mainly of low grade and was observed respectively in 38% and 50% of patients. Median severe neutropenia was 4,5 days (range 0-16) and median severe thrombocytopenia was only 1 day (range 0-21). Most patients (56%) needed transfusion support. In IVAC regimen the main toxicity was the haematological one with 7 days of median duration of severe neutropenia (range 3-10), and 7 days (range 6-23) of thrombocytopenia. Seventy-five patients required transfusion support. Infections occurred in 42% of patients. We observed few case of grade >2 mucositis (17%), renal toxicity (8%) and liver toxicity (17%).

Summary/Conclusions: R-CODOX-M/IVAC is a generally well tolerated regimen, with acceptable toxicity profile in the setting of aggressive DLBCL. Results in our cohort suggest a potential benefit for DPLs, whereas higher IPI scores retains a negative prognostic impact. The next step of the study will be retrospective FISH evaluation of C-MYC, BCL2 and BCL6 translocations, for lacking patients in our cohort, in order to disclose a potential benefit for double or triple hit lymphomas.

PB1733

OLDER PATIENTS WITH DLCLB- BODY MASS INDEX AS A PREDICTOR OF SURVIVAL

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Background: There are contradictory results of earlier studies regarding the impact of body mass index (BMI) on overall survival (OS) or progression-free survival (PFS) of diffuse large B cell lymphoma (DLCLB). Many factors like drug distribution and drug metabolism might influence the outcome in patients with excess body weight. The best method to predict outcome and adjust therapeutic approach is not known and elderly DLCLB patients do not always receive the appropriate therapeutic regimen.

Aims: To evaluate if BMI at diagnosis can predict clinical outcome in older patients with DLCLB receiving the first– line chemotherapy.

Methods: Patients at the Institution for Oncology and radiology of Serbia between 2005 and 2015 who were diagnosed and received first-line chemotherapy for DLCLB, older than 65 years were enrolled. Clinical and treatment data were recorded including BMI at the diagnosis. Patients were stratified into BMI groups according to WHO guidelines: underweight (BMI<18.5kg/m^2), normal weight (BMI 18.5 to<25kg/m^2), overweight (BMI 25 to<30kg/m^2), obesity class I (BMI 30 to<35kg/m^2). Survival time was estimated using the Kaplan- Meier (KM) method, and Cox proportional hazard model was used to evaluate the risk factors significance for survival. A p-value <0.05 was considered significant.

Results: 87 patients were included in the study. 23 (26.44%) patients were older than 75 years, 52 (59.77%) were female, 38 (43.67%) were Ann Arbor stage 1 and 2, 28 (32.18%) were International prognostic index (IPI) score 0-1. The majority of patients were diagnosed as normal weigth (39.08%) and overweight (31.03%), less were in obesity class I group (14.94%) and only 2,3% underweight. 38 (43.68%) patients received CHOP, 27 (31.03%) mCHOP, 12 (13.79%) CVP and 10 (11.49%) CEOP regimen, with or without Rituximab. In the whole group, obese patients had shorter OS and PFS. After a median follow-up of 43 months (range, 1-128), median OS times were 19 months (4-not reached) for obese, 54 months (not reached) for underweight, 90 months (53-not reached) for normal weight and not reached for overweight. PFS was 18 months (4-not reached) for obese, 67 (51-not reached) and 91 months (53-not reached) for overweight and normal weight. In the treatment of normal weight and overweight patients, the same chronological age, frequently was used anthracyclin based regimen (CHOP, 27 patients; mCHOP, 22 patients; CVP, 7 patients; CEOP, 7 patients). There was no difference in the frequency of different regimens in obesity group. In the group of patients treated with anthracyclin based regimens, obese patients tended to have shorter survival, the median OS was 33 months (9-not reached), while normal weight patients tended to have a longer OS, the median OS-not reached. The worse survival among non-anthracyclin regimen treated patients, had obese patients, median OS 26 months (9-not reached). Overweight females and men with normal weight exhibited the best median OS and PFS (not reached). In obese patients, females tended to have a longer OS and PFS (median OS/PFS 19/18 months for women *versus* 9/9 months for men), although the difference was not statistically significant (p=0.77).

Summary/Conclusions: Obesity was associated with shorter survival among older patients with DLCLB treated with different chemotherapy regimens. The impact of gender on PFS and OS varied with BMI. The use of anthracyclin did not influence the outcome of obese patients. This study suggests that BMI may predict survival in older patients with newly diagnosed DLCLB.

PB1734

STOMACH DIFFUSE LARGE B-CELL LYMPHOMA: A SINGLE CENTER EXPERIENCE

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Background: Primary gastric diffuse large B cell lymphoma is a relative rare type of diffuse large B cell lymphoma. Immunochemotherapy followed by consolidation radiation is the mainstay of treatment. However, the cycles of chemotherapy and the role of consolidation radiation are still under debate.

Aims: To review and analyze the treatment experience of newly diagnosed primary gastric diffuse large B cell lymphoma. We presented the treatment outcome of our institution.

Methods: We retrospectively reviewed medical records from Jan 2005 to Dec 2014 from our institution. 30 patients with primary gastric diffuse large B cell lymphoma were included. Clinical characteristics, treatment regimens, treatment response, treatment modality, and survival were analyzed.

Results: From Jan 2005 to Dec 2014, there were 30 patients with primary gastric diffuse large B cell lymphoma. Median age was 65 years of age. 53%(n=16) of patients were male. All 30 patients (100%) have received chemotherapy. 13 of them (43%) have received involved field radiation therapy(IFRT). RCHOP or RCEOP was administered in 86%(n=26) of patients. Complete response(CR) rate was 80%(n=24). 5-year survival was 69%. In patients who achieved complete response(n=24), 5-year survival for 4 cycles of chemotherapy *vs* 6 cycles of chemotherapy were 88% *vs* 86%(p=0.42), respectively. For addition of IFRT in CR patients, 5-year survival for IFRT *vs* no IFRT were 83% *vs* 90%(p=0.93), respectively. Treatment-related mortality(TRM) was 10%(n=3) and primary refractory disease was 10%(n=3). All of them are non-CR patients. Gastrointestinal bleeding which required admission occurred in 10%(n=3) of patients. In patients who developed GI bleeding, 2 of them were non-CR patients and they all died. No patient died of disease relapse after complete response.

Summary/Conclusions: In our series, the 5-year survival was good. In patients who achieved CR, cycles of chemotherapy and consolidation radiation did not make significant difference to the survival. Prevention of early mortality may improve the outcome of this disease. Gastrointestinal bleeding in treatment is rare but with high mortality.

PB1735

IMMUNOHISTOCHEMISTRY BIOMARKERS IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA: A RETROSPECTIVE STUDY

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Background: Diffuse Large B-Cell Lymphoma (DLBCL) is a heterogeneous disease with variable clinical course. The International Prognostic Index (IPI) is the most important tool to identify subgroups with different survival, however, certain biological markers seem to have a prognostic value relevant and independent of IPI.

Aims: To analyze the evolution of patients diagnosed with DLBCL and the expression of BCL2, BCL6 and MYC.
Methods: We conducted a retrospective study that included hospitalized patients with *de novo* CD20+ DLBCL, with expression of BCL2+, BCL6+, BCL2/BCL6, MYC/BCL2, MYC/BCL6 treated with regimens containing rituximab, from February 2012 to November 2016. Samples were analyzed by immunohistochemistry. Statistical analysis with the SPSS V17.0 program.
Results: We included 43 patients with a median age of 65 years (22-87), 59.5% male, 45,2% had IPI 0-2, 54,8% had IPI 3-5, 26,2% stage I-II, 73,8% stage III-IV, 61,9% had extranodal disease and 23,8% bulky disease. Ki-67 was elevated in all patients who did this evaluation (n=28). In 13 patients was identified BCL2+, BCL6+ in 6, and 21 patients had co-expression of BCL2/BCL6, 1 patient had MYC/BCL2 and 1 had MYC/BCL6. The R-CHOP regimen was first line treatment in 92,8% of patients. The ORR was 82,5%, with 65% of CR, 15% PR and 17,5% PD. Of those patients who received second line treatment, 8 expressed BCL2/BCL6, 4 BCL2, 2 BCL6, 1 MYC/BCL2, and 1 MYC/BCL6. Of the 5 patients who had third line treatment 3 expressed BCL2/BCL6, 1 BCL2, and 1 MYC/BCL6. The average time to next treatment (TNT) was 5,2 months (0,5-19) for second line and 4,9 for third line. Mortality rate was 45,2%. With a median follow up of 18,6 months (3-58,6), the overall survival was 24,6 months (3-62).
Summary/Conclusions: The identification of biomarkers by immunohistochemistry is a relatively inexpensive process, which, when well elaborated and interpreted, allows to find in a safe way, subgroups of patients at high risk, who benefit from more aggressive 1st line therapy and, whenever possible, from the Inclusion in clinical trials with new drugs.

PB1736

INVESTIGATION ON TREATMENT STRATEGY, PROGNOSTIC FACTORS, AND RISK FACTORS FOR EARLY DEATH IN ELDERLY TAIWANESE PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Given that the population of elderly cancer patients, including those with diffuse large B-cell lymphoma(DLBCL), is increasing, the management of cancer in the elderly has emerged as an increasingly common problem.
Aims: This study aimed to investigate the treatment strategy, prognostic factors, and risk factors of early death in elderly patients (age ≥65 years) with DLBCL in the rituximab era.
Methods: Elderly patients diagnosed with DLBCL between 2008 and 2014 were enrolled for analysis.
Results: There were 145 elderly patients with DLBCL diagnosed between 2008 and 2014. After excluding patients with primary central nervous system DLBCL (n=9) and incomplete data (n=3), a total of 133 patients (64 male and 69 female) with a median age of 74 years (range 65 to 94 years) were enrolled in the present study. Patients at a younger age and with better performance status were more likely to receive intensive frontline treatment. The median progression-free survival (PFS) and overall survival were 15 and 21 months, respectively. Anthracycline-containing chemotherapy achieved a higher remission rate and showed a trend toward better overall survival at the expense of a higher risk of severe neutropenia. Multivariate analysis revealed that very old age (≥81 years), a high-risk age-adjusted international prognostic index (aaIPI) score, and bone marrow involvement were associated with poorer PFS and overall survival. Progression of lymphoma was the major cause of death in the study population. In addition, approximately 25% of patients died within 120 days of their diagnosis. The risk factors for early mortality included very old age, a high-risk aaIPI score, and bone marrow involvement. The appearance of symptoms or signs of tumor lysis syndrome at diagnosis was associated with a trend toward early death.
Summary/Conclusions: Treatment of elderly patients with DLBCL remains a challenge in clinical practice, and comprehensive evaluation to tailor therapeutic interventions and offer the best supportive care may reduce complications and improve the clinical outcome of these patients.

PB1737

TREATMENT OUTCOME OF MONOMORPHIC EPITHELIOTROPIC INTESTINAL T-CELL LYMPHOMA: EXPERIENCE FROM AN ASIAN CANCER CENTER

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Background: Monomorphic epitheliotropic intestinal T-cell lymphoma (MEITL), previously type II enteropathy-associated T-cell lymphoma(EATL), primarily occurred in Asian countries. It is refractory to chemotherapy and the prognosis is poor. Intensive chemotherapy has been proposed to improve treatment outcome.
Aims: We examined the treatment outcome of MEITL in our institution.
Methods: We retrospectively searched our institutional database from 1996 to 2014 for intestinal T-cell lymphoma. Medical records were reviewed and the patients were classified on the basis of WHO-2016 classification. Patient's characteristics, treatment modalities, response and survival were collected and analyzed.
Results: Ten patients with intestinal T-cell lymphoma were identified. One patient had enteropathy-associated T-cell lymphoma (EATL) presenting with celiac sprue. Five patients had intestinal T-cell lymphoma, NOS. Four patients were diagnosed with monomorphic epitheliotropic intestinal T-cell lymphoma (MEITL). For patients with MEITL, median overall survival was 7.9 months (4.2-15.0 months). Median age was 46 years of age. Bowel perforation was the initial presentation in 3 patients (3/4, 75%). One patient was treated with chemotherapy with CHOP regimen, while another patient underwent surgery alone. The remaining two patients (2/4, 50%) received surgery followed by chemotherapy (one with CHOP, the other with BFM-90 protocol). Only one patient (1/4, 25%) entered complete response. Of concern, the unique patient achieved complete response received surgery followed by chemotherapy with Berlin-Frankfurt-Munster(BFM)-90 protocol. Remission duration was 10.3 months. He passed away 15.0 months after remission because of relapsed lymphoma.
Summary/Conclusions: Though the prognosis of MEITL is poor, operation followed by high dose chemotherapy such as BFM-90 protocol may have better treatment response, response duration and survival. It deserves further investigation.

PB1738

OSTEOPONTIN AS PRONOSTIC FACTOR OF DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Diffuse large B-cell lymphoma (DLBCL) is the most common type of lymphoma. It is a heterogeneous disease whose prognosis depends on the histological subtype (centralgerminal, non-centrogerminal), as well as other factors such as age, clinical stage, extranodal disease, ECOG scale and levels of lactate dehydrogenase (LDH) identified by established scales (IPI, NCCN-IPI). Osteopontin (OPN) a protein that is secreted by various cells and fulfills physiological functions, when produced by neoplastic cells favors tumor growth and metastasis. This has been corroborated in different types of cancer and there are few reports of cases of patients with DLBCL in which the tumor cell expresses osteopontin, characteristically these cases have presented an aggressive clinical behavior with extranodal disease.
Aims: To evaluate the expression of osteopontin in neoplastic lymphocytes and their association with overall survival; the percentage of patients who expressed osteopontin at diagnosis; the association between the expression of osteopontin and the histological subtype (germinal center, not centrogerminal, unclassifiable); the association between osteopontin expression and age, elevation of DHL, ECOG scale, clinical stage, extranodal invasion and the application of the IPI and NCCN-IPI scales.
Methods: Tissue samples were obtained from DLBCL patients diagnosed at the Instituto Nacional de Cancerología between December 2014 and January 2016. Morphologic and immunochemistry features were studied on paraffin-embedded tissue microarray (TMA). Single antibody staining was performed for OPN. OPN expression was semiquantitatively assessed by three different pathologists scoring the proportion and intensity of stained cells. Positive cases were those that showed any degree of expression in the nucleus or cytoplasm of the tumor cell. Age, ECOG, clinical stage, LDH, extranodal invasion, histological subtype, IPI and NCCN-IPI score were independently documented. Overall survival (OS) analysis was performed by the Kaplan-Meier method, the comparison between different curves was performed using the log-rank test; for the analysis of the relationship between variables we used the X^2 test with a statistical significance of $p < 0.05$.
Results: 81 patients were evaluable. 43.2% of the cases were positive for OPN in neoplastic cells. The mean survival of patients with positive OPN was 14.8 months *versus* 16.5 months for patients with no OPN expression (p=0.628). OPN positivity was not significantly associated with increased age, impaired functional status (ECOG 2,3,4), advanced clinical stage (III, IV), increased LDH or extranodal invasion (including central nervous system); neither was it associated with a specific histological subtype. Survival significantly decreased in patients with increased LDH (p=0.000137), ECOG 2,3,4 (p=1,7374E-7). Survival decreased significantly as the risk measured by the IPI and NCCN-IPI scales increased (p=0.000001, p=0.000013 respectively) with an average survival of 18.6 months for the low-risk group, compared with 6.4 months for the high-risk group (Figure 1).

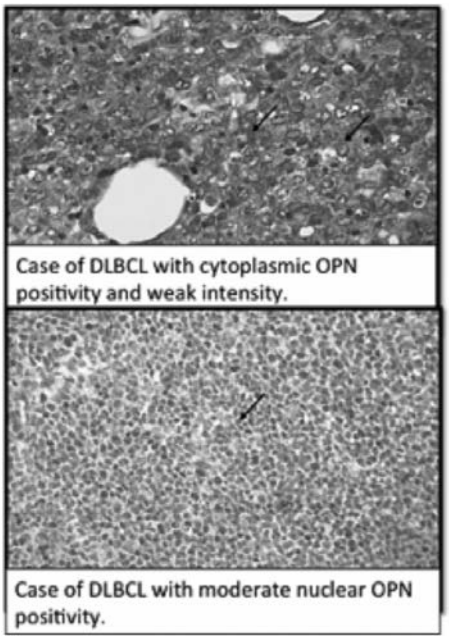

Figure 1.

Summary/Conclusions: Our findings demonstrate that approximately half of the cases evaluated express OPN at diagnosis and tend to have a lower survival rate, however, a longer follow-up time is needed, as well as other studies that discriminate between different isoforms or post-translational modifications of osteopontin to determine if this trend can reach significance. By demonstrating OPN expression by neoplastic cells we can devise new protocols that evaluate its usefulness as a surrogate marker of tumoral activity in DLBCL using non-invasive techniques (*e.g.*, quantification of serum levels), which would improve surveillance of these patients.

PB1739

TREATMENT OF NEWLY DIAGNOSED CENTRAL NERVOUS SYSTEM LYMPHOMA PATIENTS BASED ON COMORBIDITIES & PERFORMANCE STATUS: A SINGLE-CENTRE EXPERIENCE

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Background: Combination chemotherapy incorporating high dose methotrexate (HD-Mtx) and high dose cytarabine (Ara-C) is the standard chemotherapeutic approach for newly diagnosed primary CNS lymphoma (PCNSL). However, patients >60 years old account for 50% of cases and combining HD-Mtx with Ara-C can be associated with high toxicity and early mortality. The management of secondary CNS lymphoma (SCNSL) is less clear, but is often based upon a similar approach.
Aims: We present a tertiary centre experience in management of primary (PCNSL) and secondary CNS lymphoma (SCNSL), with therapy based on comorbidities and performance status.
Methods: We performed a retrospective analysis of patients with a diagnosis of CNS lymphoma seen at our centre between 2011 and 2016. These were categorized into 3 groups, Group 1: treatment of newly diagnosed PCNSL prior to September 2014 where majority of patients received HD-Mtx & Ara-C combination chemotherapy, Group 2: treatment of PCNSL after September 2014 where patients were selected based on co-morbidities to receive Mtx with or without Ara-C, Group 3: treatment of newly diagnosed SCNSL. The median survival for each group was estimated using the Kaplan-Meier method and log-ranked test. Overall response rates, 30 day and 90 day survival between groups 1 & 2 were compared using unpaired t test.
Results: 60 pts with a median age of 65 years old were recruited. 40 pts were diagnosed to have PCNSL at presentation, while 20 patients had SCNSL. 5 pts were excluded from this study as they did not receive any treatment. In group 1, 21 pts (84%) received combination chemotherapy incorporating HD-MTX and Ara-C, 3 pts (12%) received HD-MTX monotherapy and 1 pt (4%) received radiotherapy only. In group 2, 7 pts (53.8%) received HD-MTX and Ara-C as part of MATRix protocol or with single agent rituximab, 3 pts (23%) received HD-MTX as part of RMP protocol or with single agent rituximab, 1 pt (7.7%) received a single alkylating agent and 1 pt (7.7%) received radiotherapy only. In group 3 15 pts (88.3%) received chemotherapy incorporating HD-MTX and Ara-C, 2 pt (11.8%) received HD-MTX without Ara-C. 30 day mortality was 7 (28%) in group 1 and 0 in group 2 (0%) (p=0.03). 90 day mortality was 7 (28%) in group 1 and 2 in group 2 (15.4%) (p=0.39). Overall response rate was 9 (36%) in group 1 and 8 (61.5%) in group 2 (p= 0.13). A Kaplan Meier curve of all 3 groups is illustrated in Figure 1 below.

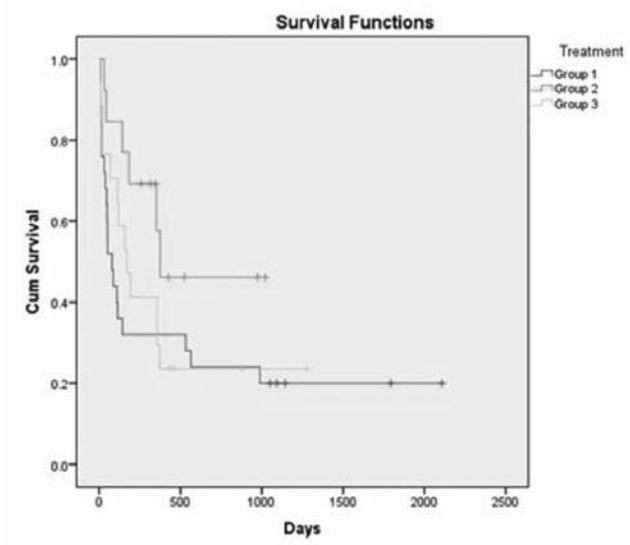

Figure 1.

Summary/Conclusions: This single centre study demonstrated that patient selection, based upon comorbidities and performance status, for high dose combination chemotherapy in the treatment of PCNSL improves 30 day mortality, often associated with death from myelosuppression due to chemotherapy. The overall response rate, with appropriate selection of combination chemotherapy regimens, was improved. This also applies to patients with SCNSL in subgroup analysis. Longer follow up of patients will be needed to further demonstrate an overall survival benefit.

PB1740

AN AUDIT OF THE USE OF RASBURICASE FOR THE PREVENTION AND TREATMENT OF TUMOUR LYSIS SYNDROME IN PATIENTS RECEIVING TREATMENT AT THE NORTHERN CENTRE FOR CANCER CARE, NEWCASTLE UPON TYNE, UK

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Background: Tumour Lysis Syndrome (TLS) is a known complication of haemato-oncological treatment. Although clinical TLS is rare, the consequences are significant, with one third of affected patients requiring dialysis and an overall mortality rate of around 15%[1,2]. A new British Society for Haematology (BSH) guideline was published in April 2015 to guide physicians on how to risk stratify patients (based upon the Cairo Risk Stratification 2010[3]), choice of prophylaxis, and treatment of established TLS[4]. We audited all patients who received rasburicase at the Northern Centre for Cancer Care from 16th April 2015 to 3rd February 2016, and compared their management with BSH guidelines[4].
Aims: To compare our practice with BSH guidelines.
Methods: Retrospective review of electronic patient presciption records, biochemistry results, and paper notes.
Results: 27 patients received rasburicase in the study period. 20 patients met Cairo criteria/BSH criteria as having High Risk Disease (HRD) or Intermediate Risk Disease(IRD)/Low Risk Disease (LRD)with renal impairment, and therefore should have received 3mg rasburicase prophylaxis if no evidence of TLS according to the guideline. Of those 20, 11 had laboratory TLS, and therefore BSH guidelines would recommend 0.2mg/kg/day [JG1] rasburicase, however only 3/11 were given the drug at treatment doses. 1/3 had clinical TLS at presentation and received treatment according to the guideline. The 2 other patients received larger doses of rasburicase but less than the BSH would recommend. A further 7 patients with IRD received rasburicase prophylaxis but on review did not meet the criteria for rasburicase as set out in the guidelines. 5 patients died during the study period. 2 patients died on ITU of multi-organ failure <7 days into chemotherapy. A third patient died of sepsis, and the other 2 deaths were in deteriorating patients where a decision was made to palliate.
Summary/Conclusions: When assessed against BSH standards, all patients in this cohort who should have received rasburicase prophylaxis, were given the drug. 2 patients with lab TLS developed clinical TLS. 8 others with lab TLS received lower doses than the BSH would recommend, but did not progress to clinical TLS. Although there were 5 deaths in our cohort, none were directly attributable to TLS. In order to comply with the guidelines, particular importance must placed on formally assessing the TLS risk score as per Cairo

criteria at the outset and analyzing the possible features of laboratory TLS. Although dosing did not always follow BSH guidelines, we did respond to biochemical deterioration. The majority of patients with HRD developed acute kidney injury despite rasburicase. Doses were increased in response to creatinine increases, albeit not as per guideline. It is notable that despite lower than the recommended doses of rasburicase, 6/8 patients with lab TLS did not progress to clinical TLS, and none required dialysis. The guideline is a good tool for the risk stratifcation and treatment of patients at risk of TLS. In clinical practice 100% compliance is hard to achieve. Responding to trends in creatinine may explain why, despite lower than recommended doses, our outcomes were still good. It would be interesting to see if further work with larger numbers of patients would support this. Since this audit was completed, the ePrescribing system has been altered to improve practice and a re-audit is planned.

PB1741

IMPLICATION OF BASIC VALUES OF VITAMIN D IN THE CLINICAL COMPLICATIONS OF PATIENTS WITH NO HODGKIN LYMPHOMA IN ACTIVE CHEMOTHERAPY TREATMENT

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Background: The incidence and prevalence of Non-Hodgkin's Lymphoma B (NHL-B) has increased in recent years, reaching approximately 3-7 cases / 100,000 habitants. For this reason, the number of patients who receive chemotherapy treatment is also considerably higher; this implies a greater presence of adverse events. In many of these patients, baseline vitamin D values at the time of diagnosis are decreased, and may be related to the development of the tumor pathology, also to the severity of the adverse events.
Aims: To assess the implication of vitamin D values in the development of relevant clinical complications in patients diagnosed with NHL-B receiving chemotherapy. To determine its clinical evolution after correcting the vitamin deficit.
Methods: Retrospective study (January2013/January2017),which includes patients diagnosed with NHL-B with histological confirmation. We analyze demographic parameters (age, sex), histological subtype of NHL-B according to WHO classification, laboratory values of vitamin D (cut-off values:optimal 25-66pg/mL; low 25-18pg/mL or very low<18pg/mL), adverse effects:hematological toxicity,infection,gastrointestinal toxicity,hospital admissions and exitus.A subanalysis of complications was performed in patients with vitamin D deficiency who received corrective treatment.
Results: 68patients were analyzed, and 57cases (84%) were valid because they had vitamin D determination in the 8weeks near the diagnosis. The distribution was:58% ♂(n=33)/42%♀(n=24),with median age 59years (range:29-91 years).The subtypes of LNH-B:Follicular n=23(40%),Diffuse large cell n=21(37%), Mantle n=6(11%), Marginal n=4(7%) and others n=3(5%).Patients were included in 3 groups according to serum vitamin D levels: patients with optimal levels (n=23;40%),low levels (n=27; 48%) and very low levels (n=7;12%). Hematological toxicities were higher for the subgroup with decreased vitamin D levels *vs* subgroup with level in range (28%*vs*72%) (p<0.01). Neutropenia was more severe (grade>2) in patients with very low levels of vitamin D(p<0.01).No patient with optimal vitamin D levels had severe anemia(Hb<8g/dl) or thrombopenia<70000/mm3, in relation to 28cases of severe toxicity diagnosed in vitamin D deficient groups. In this group were documented the two infections of the study (both pneumonias), a gastrointestinal toxicities(86%),hospitalizations for complications(69%) and only one exitus. After treatment, it was found that 74%(n=25patients) corrected levels, presenting a lower incidence of toxicity to the treatment *vs.*26%(n=9) who did not correct levels and presented more complications (especially hematological toxicity) more complex and durable.
Summary/Conclusions: Vitamin D deficiency in the diagnosis of patients with NHL-B has been correlated with a higher incidence of medical complications due to the treatment of chemotherapy. In our series, patients had greater hematological toxicity and greater severity (p <0.01), more infectious episodes and a higher hospital admission rate. These adverse effects are even more pronounced the lower the vitamin D levels (<18pg/mL). A study conducted by *Drake et al. (JCO, 2010)* on 980patients presents similar data, with a significantly higher incidence of complications in vitamin D deficient patients. After treatment with vitamin D, patients who corrected levels had a more favorable evolution with fewer hematological and infectious complications (p<0.01) in relation to those patients in whom the vitamin deficit persisted despite the treatment. At this time, the monitoring period is not completed, so the data related to OS and SLE still have to be updated and will be presented at the next congress. We believe that the determination of vitamin D levels should be routinely included in the diagnosis in patients with NHL-B because could be a modifiable risk factor in the complications of this patients.

PB1742

PROGNOSTIC IMPACT OF SYNCHRONOUS MULTIPLE PRIMARY MALIGNANT TUMORS ON NEWLY DIAGNOSED LYMPHOMA PATIENTS

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Background: Synchronous multiple primary malignant tumors (sMPMTs) are occasionally diagnosed during screening for a newly diagnosed malignant neoplasm. Lymphoma is one of the most common hematological malignancies, and number of lymphoma patients with sMPMTs seems to grow as the population ages. Since the standard chemotherapy for lymphoma takes a few months, treatment strategy sometimes comes to an issue.
Aims: To answer a clinical question of how to handle sMPMTs in the treatment of lymphoma, we investigated prognostic significance of sMPMTs and suitable treatment strategy for a newly diagnosed lymphoma with sMPMTs.
Methods: We retrospectively analyzed patients with malignant lymphoma newly diagnosed between 2009 and 2015. The definition of sMPMTs was patients who were also diagnosed as a solid tumor within 6 months of the diagnosis of lymphoma. Therapeutic strategy was according to physician's choice. Impact of sMPMTs on treatment outcome of lymphoma was analyzed. Also, relation between treatment of lymphoma and concomitant solid tumors was closely analyzed.
Results: Total of 505 lymphoma patients was included. Median age was 69 (range20-99). The most common diagnosis was diffuse large B-cell lymphoma (63%), and patients with aggressive lymphoma accounted for 77% (391/505). High risk disease, which was defined as international prognostic score 3 or higher, accounted for 36% (184/505). sMPMTs were identified in 16 patients (3.2%). There was no difference of distribution between patients with and without sMPMTs regarding age, grade of lymphoma, and disease risk. The overall survival (OS) and disease-free survival (DFS) were not significantly different between the two groups (with sMPMTs: 53% and 47% *vs* without sMPMTs: 77% and 61% at 3 years, P=0.20 and P=0.31). Cumulative incidence of lymphoma relapse was similar between the two groups (with sMPMTs 29% *vs* without sMPMTs 27% at 3 years, P=0.28). In multivariate analyses, age (75 years<) and disease risk (high) were identified significant risk factors for OS, and age was an only significant risk factor for DFS. Existence of sMPMTs was not a significant risk factor for either OS or DFS (OS: HR 1.29, 95%CI 0.52-3.20, P=0.58; DFS: HR 1.06, 95%CI 0.49-2.27, P=0.88). Among 16 patients with sMPMTs, half of the patients had high-risk lymphoma, and half of the solid tumors were gastric cancer. Treatment was initiated for the disease which was diagnosed earlier in all patients except one. Interval from diagnosis to the first treatment was significantly shorter in patients whose lymphoma was treated earlier (median 11 days *vs* 38.5 days, P=0.004). OS was not significantly different according to the sequencing of treatment (lymphoma earlier: 59% *vs* Solid tumor earlier: 40% at 3 years, P=0.84). In 8 of 10 patients whose lymphoma was treated earlier, treatment of lymphoma was interrupted for the treatment of comorbid solid tumor. Interruption of treatment had no significant effect on OS (interruption+: 60% *vs* interruption-: 50% at 3 years, P=0.13).
Summary/Conclusions: Existence of sMPMTs was not a significant risk factor for newly diagnosed lymphoma patients. It is important to provide adequate treatment for both lymphoma and solid tumor at physician's discretion.

Bleeding disorders (congenital and acquired)

PB1743

GLOBAL HEMOSTATIC ASSAY AT DIFFERENT TARGET ACTIVITY OF FACTOR VIII AND FACTOR IX

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Background: Based on reports addressing hemophilia B patients bleed less common and less intensively than hemophilia A, it has been expected that the hemostatic level of factor IX (FIX) activity can be lowered than that of factor VIII (FVIII) activity.
Aims: We compared the hemostatic efficacy of the different hemostatic level of FIX and FVIII activity using global hemostatic assay.
Methods: A total of 17 severe hemophilia patients without inhibitor, aged more than 15 years old were subjected; 12 hemophilia A patients and 7 hemophilia B patients. Factor concentrates were injected to reach the target activity of 60% in hemophilia A and 40% in hemophilia B which is given by Korean health insurance guideline. All patients were in non-bleeding state and kept the wash-out period of 3 days for hemophilia A and 5 days of hemophilia B. Before and on 15 minutes after injections, we conducted one-stage factor assay, thrombin generation assay (TGA), thromboelastography (TEG) and clot-wave form analysis (CWA).
Results: Median ages of hemophilia A and hemophilia B patients were 28 and 33 years old. Baseline FVIII:C and FIX:C were 0.6% and 1.8% and they rose after injection rose to 70.8% and 49.8%. The dosage of FVIII concentrates and recombinant FIX concentrates were 28.4 IU/kg and 50.7 IU/kg. In-vivo recovery (IVR) in hemophilia A and hemophilia B patients recorded 2.43%/U/kg and 0.91%/U/kg. Peak thrombin of FVIII and FIX were 451.3 nM and 376.6 nM (P=0.108, normal range, 458 nM±60). TEG index of FVIII and FIX were -1.60 and -3.77 (P=0.004, normal range, -2~+2). MIN2 of CWA of FVIII and FIX were 0.62 and 0.59 (P=1.000).
Summary/Conclusions: Global hemostatic assay indicates even though IVR of FVIII and FIX are normal, less amount of FIX is insufficient to normalize hemostatic parameters in comparison with FVIII.

PB1744

THE RATE OF SUCCESSFUL IMMUNOTOLERANCE INDUCTION IN HAEMOPHILIA A BOYS TREATED WITH OCTOCOG ALFA - THE EXPERIENCE OF POLISH PAEDIATRIC HAEMOPHILIA CARE CENTRES

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Background: Development of neutralizing anti-factor VIII alloantibodies (inhibitor; INH) is the most challenging complication of haemophilia replacement therapy (HRT). It occurs in up to 30% of severe haemophilia A (HA) patients. Data published recently indicate that immunotolerance induction (ITI) is effective in 62–87% of cases.
Aims: To assess the rate of successful ITI in boys with severe HA treated with full length recombinant FVIII (octocog α) in all Polish Paediatric Haemophilia Care Centres between 2011-2016.
Methods: From 2011 to 2016 in all Polish Paediatric Haemophilia Care Centres 14/88 (15.9%) boys with severe HA on prophylaxis or on demond treatment with octocog α developed INH after 3 - 489 (median 20) exposure days (EDs). Twelve of them (85.7%) were high responders with the peak inhibitor titre (PIT) 5,88 - 716.8 (median 20.1) BU/ml. Two patients were low responders (14,3%) and had PIT 2.8 and 3.02BU/ml. All except one boys were Caucasians and only one had a positive family history of INH formation. Characteristics of patients is given in Table 1.
Results: INH titres prior to ITI were 1.2 - 37 (median 6,75) BU/ml. One of low responders eliminated INH spontaneously, 1 patient is waiting for ITI initiation. ITI with octocog α was initiated in 12/14 boys after 0.2 to 8.2 (median 2.0) months from INH diagnosis and completed in 9 patients. Three patients are still on ITI. INH eradication was observed in 7/9 (77.8%) of those who completed ITI. Eradication of INH was not achieved in 2 patients; both have already started prophylaxis with activated prothrombin complex concentrate (APCC). The remaining 3 patients are still on ITI. All 7 patients after successful ITI were put back on prophylaxis with octocog α.
Summary/Conclusions: 1. Octocog α is effective in induction of immunotolerance in severe haemophilia A boys who developed inhibitor on prophylaxis with octocog α.

Table 1. Characteristic of patients.

Patient	Age at: 1st dose of FVIII / start of prophylaxis [mth]	Peak inhibitor titer BU/ml	Number of EDs at INH diagnosis	Type of treatment	Surgery	High doses of FVIII due to bleeding	Factor VIII given on: first day of infection / day of vaccination
1	0.1 / 22.8	2,8	21	P		subgaleal	Y / N
2	11.9 / 11.9	3.02	10	P			N / N
3	11.9 / 11.9	5,88	20	P			N / N
4	5 / -	6,4	3	OD		intramuscular	N / N
5	32.9 / -	7.6	20	OD		GI	N / N
6	0 / -	11.0	21	OD		CNS	N / N
7	11.6 / 11.6	14	489	P	CVA		N / N
8	11.9 / 12.6	15.84	6	OD + P		oral mucosa and fremulla of the mouth	N / N
9	4.2 / -	20,1	22	OD	CVA		Y / N
10	0.1 / 10.6	22	21	OD + P		subgaleal	N / N
11	12.2 / 12.2	37	15	P			N / N
12	12.1 / 12.1	88,96	14	P			N / N
13	3.4 / 7.1	131	10	P			N / N
14	11,2 / -	252,5	20	OD		massive to scrotum + after venopuncture	N / N

OD, on demand; P, prophylxis; CVA, central venous access; N, no; Y, yes; mth, mounth.

PB1745

APPROACH TO PREGNANCY IN NIEMANN PICK DISEASE TYPE B PATIENT

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Background: Niemann Pick Disease type A and B is a rare autosomal recessive disorder caused by sphingomyelinasa deficiency resulting in sphingomyelin accumulation in macrophages of varies organs. In type B usually patients survive in adulthood. Usually, they have hepatosplenomegaly, thrombocytopenia, and dyslipidemia. Lung and liver function are influenced, and they have bleeding risk.
Aims: Pregnancy in this situation is always risky and multidisciplinary approach is needed. Searching on Medline we found only two case reports of childbirth by women with this condition.
Methods: We presented a case of pregnancy in 34 year old women with Nieman Pick disease type B. She had marked splenomegaly, mild thrombocytopenia and partial respiratory insufficiency. Previously, she had two artificial abortions without more than expected bleeding. Also she had surgery of left side inguinal hernia and after that she was given platelet concentrates. Risk factors for pregnancy were presented to her.
Results: Laboratory controls were done periodically, ultrasound examination of abdomen and portal vein system, lung capacity and echocardiography were performed, too. Results of CBC were stable. Repeated tests of hemostasis were normal. Hyperlipoproteinaemia type IIb with hypoHDL cholesterolemia was present. We assumed that platelets dysfunction could exist, therefore before planned amniocentesis we performed platelets aggregation tests with ADP, TRAP and collagen. All of them were below lower limit: ADP 43 (55-117), TRAP 71 (92-151), col. 30 (61-108). Ultrasound examination of abdomen and portal vein sistem revieled liver diameter 17cm, craniocaudal diameter of spleen 22cm, portal vein had not been seen. There were no sign of trombosis in portal branches. Amniocentesis was done without complication and there was no need for platelet substitution. Normal male kariotype was found. We prepare her for planned caesarian section with platelet concentrates. She was given corticosteroids for lung maturation. In 35^{th}+5d gestational week she was operated. Before surgery platelets count was $87x10^9$/l, she was given seven concentrates of platelets (1 per 10 kg body weight) before and seven during procedure. She also received antibiotic prophylaxis. Newborn was 47cm, 2490g weight and Apgar score was 7/8. There was no major blood loss and no need for red blood cell transfusion or platelets transfusion in follow up period. We decided not to make splenectomy or partial resection because there were no significant differences in spleen measurements before and during the pregnancy, and there was no sign of spleen trauma. Also, in literature we found data about worsening lung function after this procedure caused by more shingomyelin accumulation in pulmonal tissue. Published data and findings of abnormal platelet function in our patient and experience with previous abdominal surgery led our decision to give her platelet concentrates before section and according to obstetrician's estimation during the operation. Pregnancy did not cause health state deterioration in our patient and there are no clinical findings of Niemann Pick disease in newborn.
Summary/Conclusions: We presented a case of pregnancy in 34 year old women with Nieman Pick disease type B. Marked splenomegaly, mild thrombocytopenia and partial respiratory insufficiency existed before this pregnancy. Decisions about diagnostic assesment, platelet transfusion, splenectomy, and

dyslipidemia treatment were made upon data from literature and patient's findings. Multidisciplinary approach in this setting is needed. Bleeding risk is not connected only with platelet count, but also with their function and degree of splenomegaly. Liver function can also be disturbed and can influence hemostasis. Pregnancy in our patient did not cause health state deterioration and there were no clinical findings of Niemann Pick disease in newborn.

PB1746

SINGLE CENTRE FX DEFICIENCY EXPERIENCE

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Background: Factor X is a vitamin K–dependent serine protease that works at the crossroads of the extrinsic and intrinsic pathways to cleave prothrombin into thrombin. Inheritance pattern of factor X deficiency is autosomal recessive, with heterozygote patients most often remaining asymptomatic or having only a mild bleeding phenotype. (1) Homozygous individuals may experience haemorrhagic symptoms, including easy bruising, haematuria, soft-tissue haemorrhages, haemarthroses, recurrent epistaxis, and menorrhagia (2) Congenital factor X deficiency is among the most rare factor disorders. We present here our experience with patients having congenital factor X deficiency.
Aims: We aimed to present our experience with rare FX deficiency in our centre.
Methods: There are currently 4 patients with factor X deficiency (F/M: 3/1) that are followed at our centre.
Results: First patient is 40 years old man who got his diagnosis at the age of 31 years following a gastrointestinal bleeding. He was treated with fresh frozen plasma (FFP) at that time. His FX was found:%0. Two years later underwent a planned tooth operation under the coverage of prothrombin complex concentrate (PCC) (Table 1). Three years after the tooth extraction he underwent an intraocular lens operation under PCC prophylaxis. No complication was observed while on PCC treatmet.

Table 1.

weight : 70 kg	PCC
Operation day	750 unit
2nd day	500 unit
4th day	500 unit
6th day	250 unit

Our second patient is a woman who was diagnosed at the age of 3 because of recurring gum bleeding. She has been treated with FFP replacement throughout her childhood and adolescence due to recurring nose and soft tissue bleeds as well as menorrhagia. She was first referred to our hospital at the age of 42 due to soft tissue bleeding. Given the lack of health insurance she mainly received FFP and tranexamic acid tablets during most of her bleeding attacks. However, PCC of 1000 unit for two days had to be used for her excessive vaginal bleeding irresponsive to FFP. Her number of annual bleeding is 15-20 times in a year and most of them are gum bleeding and rarely vaginal bleeding. Third and 4th patients were referred to our centre because of prolonged the prothrombin time (PT) and the activated partial thromboplastin time (aPTT) and received the diagnosis of FX deficiency.
Summary/Conclusions: Bleeding phenotype differs in a wide range in patients with congenital FX deficiency. Secondary causes including amyloidosis should be excluded especially in patients receving diagnosis at advanced ages. Usually the factor level does not correspond to the severitiy of the bleeding phenotype. Therefore bleeding pattern of the patients with FX deficiency should be carefully observed and considered while planning a prophylactic treatment with PCCs to prevent the risk for thrombosis and unnecessary utilisation of PCCs. FFP and PCCs replacement continue to be the source for FX in bleeding patients or in individuals requiring prophylaxis. Recently, a FX concentrate has entered the market in the USA and the Europaen Community.

PB1747

IMPROVEMENT OF THE SURVIVAL FOR LIFE-THREATENING HEMORRHAGE WITH HEMOPHILIA PATIENT

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Background: In life threatening hemorrhage such as brain and abdomen, several important factors are affect for improving the survival. One tenth (223) of hemophilia patients in Korea lived in Daegu city and Kyungpook province and have treated in our regional treatment center.
Aims: We reviewed the result of life threatening hemorrhage and our unique care of hemophilia patients for 34 years.
Methods: Korea Hemophilia Foundation was established in 1991. After that all factor concentrates were free to all hemophilia patients. Home treatment are available for rapid administration of factor concentrate of full required amount. Rapid transportation to emergency room are available for immediate operation. Hot line of mobile phone between patient and doctor for 24 hours are available for emergency care. Monthly group education has done. Prophylactic treatment was started to all who had a life threatening hemorrhage history in our hospital since 1996. But HIRA permitted officially since 2011.
And then recovery rate test was done for the optimal blood level for life threatening hemorrhage patient. Continuous infusion with every 2 to 4 hours reconstitution dilution fluid has been done for preserve *in vitro* factor activity to all surgery cases.
Results: Thirty five events were intracranial hemorrhage in 17, general surgery in 9 and orthopedic surgery in 9. Age distribution was 0-32 yr (mean; 24.8 yr). Severity was severe (16), moderate (7) and mild (5). Time interval between first symptom and arrival at ER were 15 min to 10 days (mean; 1.7days). We confirmed *in vivo* factor activity within permissible level in all patients. All recovered from hemorrhage or surgery and are healthy, but one had limping gate and one had mild neurologic sequela for more than 10 years follow-up period.
Summary/Conclusions: Education, financial support, home and prophylactic treatment, hot-line, individual pharmacokinetics with effective blood level and fresh concentrate during continuous infusion are important factors to improve the survival of surgery case.

PB1748

CAN BLEEDING SCORE AND FACTOR LEVELS DETERMINE HEMOPHILIA CARRIERS?

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Background: Hemophilia A and B are X-linked recessive hemorrhagic disease. Due to this type of inheritance, males are usually affected, but girls are carriers. Factor levels are usually detected around 50% because only one chromosome is affected in carriers. Inconsistently, it has been reported that factor activity can be detected in a wide range of 22% -116% as a result of random inactivation (lyonization) of one of two X chromosomes. It is specified that factor levels may be very low due to excessive inactivation in a significant part of the hemophilia carriers, which creates a risk of bleeding in carriers.
Aims: In this study, we aimed to investigate the role of bleeding score and factor levels in detecting hemophilia carriers.
Methods: Bleeding Assessment Tool (BAT) for hereditary factor deficiencies of the International Society on Thrombosis and Haemostasis (ISTH/SSC) were applied to the mother and sisters of 32 hemophilia patients who were followed-up in Dr Behçet Uz Children's Diseases and Surgery Training and Research Hospital. Mothers whose at least one of the other members of the family and their sons had hemophilia, mothers with more than one hemophilic son and girls whose father had hemophilia were evaluated as an obligate carrier. Sisters or mothers who do not meet the obligatory carrier criteria but whose siblings or sons are hemophilic were identified as possible carriers. Factor activity of obligate or probable carriers was studied after their informed consent was obtained.
Results: Thirty-two mothers and 13 sisters of hemophilia patients were included in this study. The mean age was 31.6 (4-57) years. Three of the patients were mild, 3 were moderate, 23 were severe hemophilia A; 2 were severe and 1 had moderate hemophilia B. Twelve were obligate and 33 were probable carriers. Only seven in 45 (15.5%) probable and obligate hemophilia carriers had high bleeding scores (≥4). Those with high bleeding scores, three were obligate carriers and four were probable carriers. The mean factor activity of 12 obligate and 18 probable carriers were 78.9% (20.8%>189%). Factor activities of the three obligate carriers with high bleeding scores were 77%, 80% and 98%, respectively. Factor activities of the three probable carriers with high bleeding scores were 58.8%, 69.3% and 112%, respectively. The median bleeding scores of four probable and one obligate carriers with low factor activity (<60%) were 2.8 (1-4).
Summary/Conclusions: Measurement of factor activity seems to be insufficient to detect hemophilia carriers**.** ISTH/SSC-BAT may help to determine the carriers. However, a larger study is needed to understand the diagnostic value of the BAT.

PB1749

FETAL INTRACRANIAL HEMORRHAGE AS A PRESENTING FEATURE OF SEVERE CONGENITAL FACTOR VII DEFICIENCY: THE NEED FOR EARLY PROPHYLAXIS

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Background: Congenital factor VII (FVII) deficiency is a rare autosomal reces-

sive bleeding disorder, with an estimated prevalence of 1:300,000. Compared to western countries, rare bleeding disorders (RBDs) are relatively commoner in Oman, owing to high rate of consanguineous marriage.
Aims: To discuss an interesting case fo severe congenital factor VII deficiency and to explore the need for early prophylaxis.
Methods: Case report and retrospective data analysis of all children diagnosed with inherited coagulation factor deficiencies in Pediatric Hematology Unit, Sultan Qaboos University Hospital, Muscat, Oman from January 2009 till December 2016.
Results: We report a male full term baby, delivered by cesarean section. His older sister is a known case of severe congenital factor VII deficiency. Antenatal scans of this baby revealed two intracerebral hematomas and dilated cerebral ventricles. Postnatally, the diagnosis of severe congenital FVII deficiency was confirmed. CT scan revealed obstructive hydrocephalus at the level of aqueduct of Sylvius (Figure 1). At day 10 of life, ventriculo-peritoneal shunt has been done successfully under cover of recombinant activated factor VII replacement therapy. Afterwards, the patient has been initiated on rFVIIa prophylaxis at a dose of 30 ug/kg three times weekly. In our center, deficiencies of fibrinogen, FV, FVII, FX and FXIII were diagnosed in 22 pediatric patients (10 males and 12 females), accounting for 11.1% (22/198) of all children with inherited coagulation factor deficiencies. The age ranges from 1 day to 6 years and consanguinity is found in 19/22 cases (86.4%). Hypofibrinogenemia, FV and FVII deficiency are the commonest RBDs, diagnosed in 8, 6 and 5 patients respectively. As an initial presentation, intracranial hemorrhage occurred in 7/22 cases (31.8%). Three patients with FV, FVII and FXIII deficiencies suffered from global developmental delay due to severe intracranial hemorrhage. As regards management, 4 patients with severe FV deficiency and one with severe FXIII deficiency are on fresh frozen plasma (FFP) and recombinant FXIII prophylaxis respectively. Other patients receive on-demand therapy.

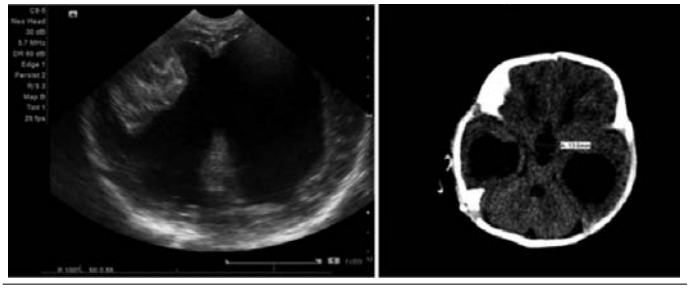

Figure 1.

Summary/Conclusions: Children with RBDs constitute more than one tenth of cases of hereditary coagulation factor deficiencies in our center. They have some unique features in terms of severity, clinical profile and the need for prophylaxis early in life. We recommend establishing a national/regional registry of RBDs to identify the magnitude and the peculiar genotype-phenotype correlations of such rare, yet significant disorders.

PB1750

THE ASSOCIATION OF BLOOD TYPE WITH THE NEED FOR TRANSFUSIONS IN PATIENTS WITH VENTRICULAR ASSIST DEVICES

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Background: Patients who have implantation of continuous flow ventricular assist devices (VAD) as a bridge to heart transplantation are subjected to complications secondary to pump support. The use of antiplatelets either alone or in combination with acticoagulation is necessary to avoid clot formation and pump thrombosis. However, a proportion of patients reveal an increasing risk of bleeding episodes. A possible reason of this situation could be that high shear forces lead to devastation of high molecular weight von Willebrand factor (vWF) making it functionally inactive and resulting in acquired von Willebrand disease (vWD). People with blood type O have lower baseline vWF levels and this abnormality could exacerbate the bleeding risk of patients with blood type O with VAD, resulting in more frequent bleeding episodes and need for transfusions.
Aims: The aim of current study was to investigate the possible association of blood type with acquired vWD induced by VAD, with the need for transfusions.
Methods: In this retrospective study, 17 patients who had a VAD implant in our hospital in a six-month period were included for analysis. The investigation of underlying vWD was estimated by ristocetin-induced platelet aggregation (RIPA) using classical light transmission aggregometer.
Results: Six patients (35.3%) had left-VAD (L-VAD) implantation while the others had biventricular VAD implantation (BiVAD). The mean age was 42.41 years (SD±15.33) and 9 patients (52.9%) were male. Female patients had VAD implantation at younger age than male ($p<0.001$). The mean follow-up after VAD implantation was 15 months (SD±11.88). At the time of analysis, 13 patients (76.5%) were alive, 2 patients (11.8%) had died while 2 patients (11.8%) had been heart-transplanted. Eight patients (47.1%) had blood type O, 8 patients (47.1%) had blood type A and a patient (5.9%) had AB. Mean RIPA before VAD implantation was 59.3% (SD±14.76) while after VAD implantation was 47.29% (SD±15.47), whereas the decrease was no statistically related. No statistical correlation was found between RIPA among different blood types. Among patients with blood type O, the need for blood transfusions was associated with the duration of having the VAD implantation in months ($p<0.001$) while the need for fresh frozen plasma (FFP) transfusions was associated with RIPA before VAD implantation ($p=0.016$). In non-blood O type patients no statistical correlation was found with the need for transfusions with RIPA percentage or median follow-up of patients.
Summary/Conclusions: It has been shown by several studies that patients with VAD show a decrease in vWF increasing the bleeding risk. Thus the best antiplatelet treatment and/or anticoagulation that those patient needs, remains challenging. In our study, there was a decrease in mean RIPA percentage after VAD implantation and patients with blood type O had lower RIPA before implantation. However, none of these measurements was statistically significant. The blood type O patients showed an increased need for transfusions in correlation with the duration of VAD implants and an increased need for FFP in correlation with RIPA baseline. Our study has limitations due to the small population and the fact that vWF was not estimated within the different blood groups at baseline and after VAD implantation.

Bone marrow failure syndromes incl. PNH - Clinical

PB1751

ACQUIRED PURE RED CELL APLASIA ASSOCIATED WITH LYMPHOPROLIFERATIVE DISEASES IN ERYTHROPOIETIN-REFRACTORY ANEMIA PATIENTS ON DIALYSIS

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Background: Erythropoietin-refractory anemia is a serious problem and complicated causes should be ruled out in patients on dialysis. Acquired pure red cell aplasia (PRCA) may be hidden behind anemia of chronic kidney disease. Recently it was reported that PRCA patients with large granular lymphocyte frequently had *STAT3* mutations (Oie ZY *et al.* J Hematol & Oncol 2013, Ishida F *et al.* Cancer sci 2014). Molecular or flow-cytometric analysis is useful for detecting a small amount of abnormal lymphocytes.
Aims: We conducted this study to determine the clinical characteristics and *STAT3* mutations of patients with acquired PRCA on dialysis with lymphoproliferative diseases.
Methods: In our hospital, 4 patients were diagnosed as having acquired PRCA on dialysis with lymphoproliferative diseases after 2005. Patients were retrospectively studied for presenting feature, laboratory data, and clinical course. Surface markers of lymphocytes were examined by flow cytometric analysis, and T-cell receptor (TCR) rearrangements were examined by Southern blot analysis. Mononuclear cells were separated after obtaining written informed consent. *STAT3* (Y640F and D661Y) mutations were examined by allele-specific PCR. Current study was conducted within the guidelines and with the approval of the institutional ethical committee.
Results: In spite of adequate administration of erythroid colony-stimulating factor, all 4 patients required blood transfusion due to erythropoietin-refractory anemia. Median leukocyte and lymphocyte counts at diagnosis were 4650/mL (range, 3180-4850) and 1794 mL (range, 1183-2859), respectively. Two patients (Cases 1 and 2) had low percentage of CD4+ CD8+ by flow-cytometry and TCR C beta1 and gamma rearrangements by Southern blot analysis. Another patient (Case 3) had high percentage of gamma-delta T cell component (66.2%) with TCR delta rearrangement. The other patient (Case 4) had high CD16+CD56+ NK cell percentage without TCR receptor rearrangement. The median duration from the start of dialysis to onset of PRCA was 13 years (range, 5-19 years). Of the 4 patients, only one patient (Case 3) had the mutations of the *STAT3* gene (Y640F). This patient first received cyclophosphamide but he did not respond to the therapy. He subsequently received cyclosporine (CyA). The other three patients received CyA as an initial therapy, and it was effective in all 4 patients. Median follow-up were 7 years from diagnosis, and two patients died during follow-up period. One patient (Case 4) died of cardiac failure 7 years from the diagnosis. Another patient (Case 2) developed diffuse large B-cell lymphoma 5 years after the administration of CyA. He was treated with R-CHOP chemotherapy and complete remission (CR) was achieved. Although he had been in CR, he died of refractory pancytopenia with infection, 2 years after the lymphoma onset. The other two patients are still alive without blood transfusion for 6 and 7 years.
Summary/Conclusions: A proportion of erythropoietin-refractory anemia patients on dialysis have acquired PRCA associated with lymphoproliferative diseases. The combined analyses of flow-cytometry and TCR rearrangement of lymphocytes were useful for diagnosis of acquired PRCA associated with lymphoproliferative diseases. Further accumulations of patients were required for understanding the pathogenesis of lymphoproliferative diseases causing acquired PRCA on dialysis.

PB1752

ADULT PATIENTS WITH ACQUIRED PURE RED CELL APLASIA: TREATED BY CYCLOSPORINE A OR CORTICOSTEROIDS□SIMILAR EFFICIENCY

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Background: Adult pure red cell aplasia (PRCA) is a syndrome characterized by a severe normocytic anemia, reticulocytopenia, and absence of erythroblasts from an otherwise normal bone marrow. Immunosuppressive therapy has been used as the initial treatment foracquired chronic PRCA.
Aims: This article aims to evaluate the efficacy of cyclosporine A, and/or corticosteroids, and possible factors influencing it.
Methods: 34 cases of PRCA were retrospectively analyzed at our institution. Clinical data of 23 inpatient cases and 11 outpatient cases since 2009 October were collected. These patients were treated by cyclosporine A (CsA), and/or corticosteroids (CS), or other immunosuppressive agents if become refractory and relapsed.
Results: 31 patients were evaluated in our institution (one patient lost to follow-up and two patients with short observation period). The remission induction therapy included CsA (n=13), CS (n=13), or a simultaneous combination of CsA and CS (n=5). The initial response rate of CsA alone, CS alone, combination of CS and CsA were 69.2%, 46.2%, 80%, respectively (*P=0.422*). There was no statistical difference in response rate and CR rate between CsA-containing group and CS group, although the patients treated with CsA had a better response than those treated with CS (response rate 72.2%*vs* 46.2%, *P=0.262*; CR rate 33.3% *vs* 23.1%, *P=0.696)*. Including patients who had crossed over from other treatment groups, the cumulative response rate of CsA, CS, combination of CS and CsA, was 73.7% (14/19), 46.7% (7/15), 83.3% (5/6), respectively (*P=0.193*); the cumulative rate of CR was 26.3% (5/19), 26.67% (4/15), 66.7% (4/6), respectively (*P=0.202*). In 23 refractory and relapsed PRCA patients, 8 out of 12 (66.7%) refractory patients and 4 out of 11 (36.4%) relapsed patients achieved remission. The response rate of treatment with traditional immunosuppressive agents (CS and/or CsA) was higher than other immunosuppressive agents (65.0% *vs* 20%, *P=0.014)*.
Summary/Conclusions: CsA and/or CS are effective similarly in treating PRCA. For patients with relapse or refractory PRCA, there were no satisfactory treatment measures if CsA and/or CS were not be administered or un-effective. It was still needed to explore a more effective therapy for them.

PB1753

REACTIVATION OF HEPATITIS B VIRUS INFECTION IN APLASTIC ANEMIA PATIENTS

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Background: There is litter data about the influence of infection of HBV impact on the therapy of aplastic anemia.
Aims: This article is aimed at assessment the HBV reactivation risk in HBsAg-positive or HBsAg-negative, antihepatitis B core antigen antibody (anti-HBc) - positive patients with AA receiving CsA and/or ATG.
Methods: We analysis the clinical data of 60 AA patients with HBV infection out of 201 cases of AA from our center at AA diagnosis during the recent 3 years, and laboratory test data such as levels of liver enzyme, HBV DNA in serum, HBsAg anti-HBs and anti-HBc were monitored. Entecavir (ETV) or lamivudine □LAM□ was started when HBV reactivation (defined as detectable HBV DNA) was encountered or as a antiviral prophylaxis regimen for some HBVsAg-positive patients.
Results: Among 60(29.8%) AA patients, 12 were chronically infected (HBsAg positive) and 48 were previously exposed (HBsAg negative/anti-HBc positive). 5 patients (8.33%) who were HBsAg positive and not given any rophylactic antiviral therapy suffered HBV reactivation. 7 patientswho were HBsAg positive but given were found no HBV reactivation. All the 48 patients with negative HBsAg and positive anti-HBc were found no HBV reactivation during the follow-up.
Summary/Conclusions: Antiviral prophylaxis should be recommended for HBsAg-positive patients who will receive IST with AA as they had high rate (41.6%) of HBV reactivation. HBV infection were found no influence to the clinic course in AA and antiviral therapy had no influence to the effect of IST.

PB1754

MULTICENTER RESULTS OF SCHWACHMAN-DIAMOND SYNDROME PATIENTS

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Background: Shwachman-Diamond syndrome (SDS) is an autosomal recessively inherited disease characterized with neutropenia, exocrine pancreas insufficiency, failure to thrive and skeletal abnormalities. In approximately 90% of the patients, the molecular defect is related to SBDS gene mutations. The classical triad is present in one-forth of the patients and a high degree of suspicion is required in order to make the diagnosis. In this study, molecular work-up to patients with suspected SDS were made and the clinical and laboratory findings that predict the SDS diagnosis were investigated.
Aims: Aim of the study was to find out the predictive clinical and laboratory characeteristics of SDS patients.
Methods: The patients who were sent to Hacettepe Inherited Bone Marrow Failure Center for molecular work-up between June 2015 and August 2016 were evaluated with clinical and laboratory data obtained from a standardized patient registry form.

Results: Molecular work-up was performed in 20 patients referred to our center with a suspected diagnosis of SDS. Of these 20 patients (12 girls), 4 (20%) (3 boys) were found to have mutation in SBDS gene. The median age of these patients was 3.2 years (1-18). Of the 4 patients with genetically verified SDS, 1 (25%) had history of chronic diarrhea and pancreas atrophy was detected in ultrasonography of that patient Another patient (25%) with SDS had skeletal abnormality, and 3 (75%) of the patientshad failure to thrive. Three patients (75%) had anemia associated to neutropenia, and 1 patient (25%) had pancytopenia at presentation. On the other hand of the patients who were referred with a suspicion of SDS but was found to have no mutation, 43% had neutropenia, 25% had bicytopenia, 10% had pancytopenia. The patientsin the latter group had failure to thrive in 25% of the patients and chronic or persistent diarrhea was present in 25% of this group. There was no statistically significant difference in the clinical and laboratory parameters of the genetically verified SDS patients and patients without SDS but was referred with SDS suspicion.
Summary/Conclusions: Although, there was no statistically significant difference in the clinical and laboratory parameters of the genetically verified SDS patients and patients without SDS but was referred with SDS suspicion, this might be attributed to the small sample sizes. Compatible with the previous literature data, SDS is a cryptic disorder and the classical triiad is not commonly fulfilled in most of the patients. On the other hand, failure to thrive/growth retardation was three times more common in patients with SDS. Thus, in patients neutropenia, accompanying failure to thrive/growth retardation might be an indicative to make molecular work-up for SDS. Additionally, not only neutropenia, but bicytopenia or pancytopenia might be the hematological presentational findings of SDS.

PB1755

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA AND APLASTIC ANAEMIA – DATA FROM THE SPANISH PNH REGISTRY

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Background: Aplastic anaemia (AA) and Paroxysmal Nocturnal Hemoglobinuria (PNH) are included, together with other pathologies, within the bone marrow failure syndromes (BMFS). In the present time, these clinical entities cannot be understood as independent pathologies, due to the extremely frequent evolution among them and with other BMFS, along with the development of new clones in the context of haematopoietic stem cell's kinetics.
Aims: The aims of this study were analyzing and comparing the behaviour of patients who suffered from PNH with pancytopenia with respect to that of patients who were initially diagnosed of AA and who later developed a PNH clone.
Methods: A clinical form was elaborated and distributed among the investigators of the PNH Spanish Registry. Clicnical, laboratory and treatment data of the patient were asked. Soon after, a descriptive analysis of the data was performed.
Results: 34 patients were recruited and analyzed (12 women and 22 men). Their age interval ranged from 2 to 87 years, and all of the patients suffered from either PNH with pancytopenia and/or AA with a developing PNH clone. The average age at the time of initial diagnosis was 28,5 years old (4m-72y). The initial diagnosis was: PNH with pancytopenia (1), moderate AA (16), severe AA (10), very severe AA (7). 15 patients presented a PNH clone in their granulocytes and/or monocytes at the time of diagnosis, being 24% the average of such clone (0-08-95%) and less than 2% in 7 patients. All of the cases that showed hemolitic signs at diagnosis presented clones >20%. The time of the clone's development in the remaining cases was 8,7 years (5m-28y), appearing in 2 patients just after resolution. Patients with AA received an average of 3 treatments, mostly cyclosporine with/without ATG (anti-thymocyte globulin). 10 patients underwent HCT (7 allogeneic HCT and 3 matched unrelated-donor HCT). 14 patients received eculizumab, being 88.2% the average size of the PNH clone at diagnosis (65-99%). Treatment response with eculizumab was total in 11 cases and partial in 3. The following complications were observed: cholelithiasis (3), renal failure (6; 50% secondary to treatment), iron overload that required chelation therapy (3), transient aplastic crisis due to parvovirus B19 (1), HCV infection (1), thrombosis (6). 4 patients started anticoagulant treatment prior to eculizumab with no evidence of further thrombosis once the treatment was initiated. 28 patients remain alive (26 of them with very good quality of life). 3 of them died due to HCT-complications and follow-up was lost in the 3 remaining cases.
Summary/Conclusions: Clonal evolution in AA is frequently associated with the development of a PNH clone at the time of diagnosis, throughout the pathology' natural course or even after disease's resolution. The development of such clone has been related to better prognosis in AA right after the immunosuppressive therapy (IST). Our experience demonstrated the presence of hemolisis in at least half of the cases, making it necessary in these patients treatment with eculizumab, generally obtaining a very good response.

PB1756

AUTOIMMUNE CYTOPENIAS IN PRIMARY IMMUNODEFICIENCY DISEASES: SINGLE CENTER EXPERIENCE

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Aims: Primary immunodeficiency diseases (PID) are associated with hematologic complications such autoimmune hemolytic anemia (AIHA) and thrombocytopenia (ITP). The most common autoimmune cytopenia is ITP. Although ITP is observed in 7.6% of patients with PID, AIHA is seen at 4.8%. Also, we aimed to present the patients who had autoimmune cytopenias and PID.
Methods: Fifty six PID patients who were followed at the Pediatric Immunology Department of Erciyes University Medical Faculty (they were analyzed genetically) were evaluated retrospectively. Autoimmune cytopenias such as ITP and AIHA were detected in 9 (%16. 07) of the patients (combined immunodeficiency:4 patients, common variable immunodeficiency: 2 patients, hyper immunoglobulin E syndrome:1 patient, X-linked lymphoproliferative :1 patient, chronic granulomatous disease:1 patient). ITP was detected in 8 of 9 patients and AIHA was also detected in 6 patients. In four patients (LRBA deficiency: 2 patients, hyper IgE syndrome:1 patient and CGD:1patient), both ITP and AIHA were observed. Immunosuppressive therapy with steroid, cyclosporine, mycophenolate mofetyl and intravenous immunoglobulin were given to all patients. Bone marrow transplantation was performed to the four patients. However, five patients died because of immunodeficiency.
Results: There is a paradoxal situation between PID and autoimmunity. The reduction of central and peripheral tolerance is held responsible for autoimmunity in PID.
Summary/Conclusions: As a conclusion, we wanted to point out autoimmune cytopenias in patients with PID and the requirement of multidisciplinary approach for treatment.

PB1757

HEAVY METAL LEVELS IN PATIENTS WITH FANCONI APLASTIC ANEMIA

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Background: Fanconi aplastic anemia (FAA) is a rare, autosomal recessively inherited bone marrow failure disorder. Various congenital anomalies may accompany disease and various complications including malignancy and endocrinopathies may develop during the course.
Aims: Heavy metal levels in patients with different chronic disorders have been investigated, however, up to our knowledge there is no literature data on the heavy metal levels in patients with FAA. Heavy metal levels in patients with different chronic disorders have been investigated, however, up to our knowledge there is no literature data on the heavy metal levels in patients with FAA.
Methods: Study was performed between July 2015 and April 2016 among patients with FAA and the results were compared with age and gender matched control group**.** Plasma copper (Cu), iron (Fe), zinc (Zn), and whole blood chromium (Cr), cobalt (Co), selenium (Se) levels were measured in patients with FAA.
Results: Total of 17 patients with FAA were included in the study. Median age was 9 years (1 – 30), female to male ratio was 8/9. One patient had undergone stem cell transplantation, four patients were transfusion dependent. When we compared patients with FAA and age/sex matched healthy group (16 volunteers) Cr and Cu levels were higher and Se level was lower in FAA group significantly (Table 1). However, all patients had chromium level within normal range, two patients with FAA and two volunteers had copper levels higher than the normal ranges (Table 2).

Table 1. Heavy metal levels in patients and control group.

	FAA	Control	*p*
Chromium (mcg/L) (2.8-45)	8.5 (6.3 – 26.2)	6.5 (4.2 – 13.5)	**0.09**
Cobalt (mcg/L) (0.5-3.9)	1.6 (0.09 – 3.2)	1.6 (1.1 – 3.3)	0.84
Copper (mcg/dL) (70-150)	111.2 (65.3 – 181.3)	80.7 (49.5 – 204.9)	**0.01**
Iron (mcg/dL) (50-150)	187.2 (119.7 – 391.2)	254.2 (91.7 – 353)	0.62
Selenium (mcg/L) (0-150)	46.4 (30.6 – 68.5)	65 (44 – 112)	**0.00 1**
Zinc (mcg/dL) (70-120)	86 (53 – 124)	77.1 (47.3 – 131)	0.34

FAA; Fanconi aplastic anemia.

Table 2. Classified heavy metal level in patients and controls.

	FAA	Control
Chromium:		
Low	0	0
Normal	17	16
High	0	0
Cobalt:		
Low	1	0
Normal	16	16
High	0	0
Copper:		
Low	1	3
Normal	14	11
High	2	2
Iron:		
Low	0	0
Normal	6	5
High	11	11
Selenium:		
Normal	17	16
High	0	0
Zinc:		
Low	3	6
Normal	13	7
High	1	3

FAA; Fanconi aplastic anemia.

Summary/Conclusions: In our study we found chromium and cobalt levels higher in patients with FAA than control group. In-vitro studies have revealed that FAA cells are more sensitive to chromium toxicity. With larger number of patients chromium level and clinical association should be investigated in further studies. Lower Se level in patients with FAA may be related with oxidative stress in these patients.

PB1758

CLINICAL IMPACT OF AGE AND COMORBIDITY IN PNH PATIENTS

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Background: PNH is an ultra-rare disorder affecting mainly young adults, but can be diagnosed in geriatric population. Comorbitidy is more prevalent in general geriatric population and can either hamper diagnostic evaluation or increase the complexity of PNH patient care.
Aims: To identify geriatric-age PNH in Spanish PNH registry. To study the clinical characteristics at diagnosis and evolution of geriatric-age PNH and compare them to non-geriatric PNH population. To analyse the impact of both age and prognosis in the PNH setting. To evaluate the use of eculizumab in geriatric age patients.
Methods: In a multicentric retrospective study, Cumulative Illness Rating Scale for Geriatrics (CIRS-G) and clinical and biological variables have been collected from a Spanish PNH Group patient cohort. Statistical analysis was performed using GraphPad Prism v5 (La Jolla, CA).
Results: 44 patients from 11 centres in Spain have been included up to date. 8 patients (17,8%) were diagnosed in geriatric age (equal or older than 65 years) (Age range for the complete cohort: 17-83 years) and 9 patients presented with high comorbidity, arbitrary defined as CIRS-G score >10. (Range for the complete cohort: 3-13) Age and comorbidity were poorly correlated (p= 0.0187, R-square 0.15) No differences in clinical presentation (Classic, PNH in the setting of another bone marrow failure syndrome or Subclinical PHN or high disease activity) when stratifying by age or comorbidity were observed. 4 patients had a concomitant myeloid clonal disorder (3 myelodisplastic syndrome and 1 myeloproliferative neoplasm), 3 of them (75%) in geriatric age.
Median follow up was 7,2 years. Both age equal or older than 65 years and CIRS-G >10 were associated to poorer overall survival (HR: 0.0134 and 0.045 & p= 0.0015 and 0.0103 respectively). Regarding PNH with high disease activity, 18 patients were identified, 4 of them in geriatric age. In 2 of them (50%), Eculizumab was used, which contrasts with eculizumab use in younger patients (78,6% in the same indication) Regarding comorbitidy impact on eculizumab therapy outcome, 2 patients had CIRS-G score >10 and had similar overall survival as patients with lower comorbidity in this cohort.
Summary/Conclusions: Age and comorbidity are associated with poorer overall survival in PNH. Older age and comorbidity may not preclude the use of effective treatment in PNH patients, including those with high disease activity. Prospective evaluation of comorbidity in PNH patients, regardless of age is warranted.

PB1759

A RARE ASSOCIATION: EBSTEIN-BARR VIRUS ASSOCIATED LYMPHOPROLIFERATIVE DISORDER AND PURE RED CELL APLASIA

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Background: Lymphoproliferative disorders (LPD) constitute a heterogeneous group of diseases related to expanding polyclonal or monoclonal lymphoid cells in the setting of immune dysfunction. Ebstein-Barr virus (EBV) has been implicated in the development of a wide range of B-cell LPD spectrum. EBV associated LPDs (EBV-LPD) are more commonly encountered after stem cell and organ transplantations.
Pure red cell aplasia (PRCA) is an uncommon disorder characterized by a severe normocytic anemia due to erythroblastopenia in an otherwise normal bone marrow. PRCA may be primary or develop secondary to viruses, autoimmune diseases, hematological malignancies, thymoma, solid tumors and drugs.
Aims: A case, who was diagnosed with EBV-LPD and developed PRCA during follow-up, is presented.
Methods: A 75-year-old woman with pain in upper and lower extremities applied to our center in February 2016. Her past medical history was unremarkable except for rheumatoid arthritis. On physical examination bilateral cervical, submandibular, axillary lymphadenopathies (LAP) and splenomegaly were detected. Laboratory tests revealed normochromic normocytic anemia, elevated serum lactate dehydrogenase and acute phase reactants. Positron emission tomography (PET) showed supra- and infradiaphragmatic malignant lymph nodes and splenic involvement. An excisional biopsy of cervical LAP was performed. Pathological examination showed CD20 (+) and CD30 (+) large B cells in the interfollicular area. EBV early RNA signals were ckecked by in-situ hybridization and viral transcripts were detected. Diagnosis of EBV-LPD was made. During diagnostic work-up deepening of anemia with reticulocytopenia, increased transfusion requirement and inadequate response to transfusion necessitated a bone marrow aspiration and biopsy. Pathological examination of the bone marrow was compatible with PRCA. Parvovirus IgM and DNA was negative; IgG was found to be positive. Because of the lack of response to steroids, Rituximab was given (375 mg/m2, weekly). Anemia and patient's clinical condition improved after 8 weeks of treatment.
Results: In the pathogenesis of LPD polyclonal lymphoid response to an antigenic trigger is thought to be followed by development of monoclonal neoplastic diseases. In our case, this trigger was thought to be EBV as it is known as one of the main causative agents for LPD in the literature. Clinical complaints and physical examination findings are common among all patients and frequently not leading to a definitive diagnosis in most of them as it is the case in our patient. Compared to the strong association of secondary PRCA with parvovirus B19 its association with EBV is rare. PRCA can develop before the diagnosis, during the course and after the remission of LPD. In our case we observed PRCA in the follow-up period of EBV-LPD.
Summary/Conclusions: On the basis of EBV-LPD being more common in transplant setting our case was thought to be unique due to the absence of transplantation or immunosuppression history. This case report points out to the possibility of oexistence of two rare diseases, EBV-LPD and PRCA.

Chronic lymphocytic leukemia and related disorders - Biology

PB1760

LDH AS PREDICTIVE PARAMETER IN TREATMENT-NAÏVE PATIENTS WITH TRISOMY 12 CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Patients affected by chronic lymphocytic leukemia (CLL) that have trisomy 12 (+12) on FISH analysis have unique clinical and biological features. In a prior analysis (*Autore F, ASH 2016*) of 487 patients with +12 compared to 816 patients with negative FISH, patients with +12 had a significantly higher prevalence of elevated LDH, β-2-microglobulin, ZAP70 positivity, CD38 positivity, CD49d positivity and unmutated IGHV as compared to patients with negative FISH. They also showed shorter progression free survival (PFS), treatment free survival (TFS) and overall survival (OS).
Aims: To identify clinical and laboratory features that predict disease progression, time to treatment and survival in treatment-naive patients with +12 CLL.
Methods: This study included 487 treatment-naive patients with +12 CLL from 16 academic centres, diagnosed between January 2000 and July 2016. A cohort of 250 patients with +12 CLL followed at a single US institution was used as external validation. Data were summarized as medians and 25th and 75th percentiles. Chi-square test or Fisher's exact test were used to compare categorical variables, while Wilcoxon-Mann-Whitney-Test was applied for continuous variables. The survival analysis was based on the Kaplan-Meier method and the log-rank test was used to compare survival curves. A Cox model was used for multivariate analysis of the impact of different factors on survival. P values lower than 0.05 were considered statistically significant (STATA 12.0) and reported as two-sided. We analysed also CLL-specific survival considering events deaths due to the haematological disease.

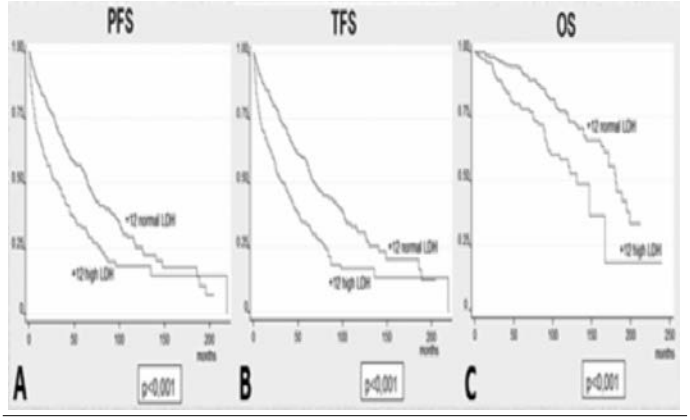

Figure 1.

Results: Parameters associated with shorter PFS, TFS, OS and CLL-specific survival on univariate analysis were IGHV, LDH, β-2-microglobulin and Rai stage; age, ZAP70 and CD38 associated with OS only; on multivariate analysis, high LDH and unmutated IGHV remained significantly with shorter PFS, TFS, OS and CLL-specific survival, higher Rai stage with shorter PFS and elevated β-2-microglobulin with shorter OS. Considering interestingly the association of a simple and new laboratory parameter such as LDH to the outcomes, confirmed on multivariate analyses for PFS (hazard ratio [HR] 1.55, 95% confidence interval [CI] 1.2 to 2.1; p=0.004), TFS (HR 1.62, 95% CI 1.2-2.2; p=0.002), OS (HR 1.69, 95% CI 1.1-2.7; p=0.034) and CLL-specific survival (HR 3.86, 95% CI 2.0-7.5; p<0.001), we divided our +12 CLL cohort according to LDH levels available at diagnosis: 103 patients showed LDH levels above the normal limit and 184 within normal range. Patients with high LDH levels showed shorter PFS (30 months *vs* 65 months, p<0.001; Figure 1A), TFS (33 months *vs* 69 months, p<0.001; Figure 1B), OS (131 months *vs* 181 months, p<0.001; Figure 1C) and CLL-specific survival with a rate of attributable mortality of 29% *vs* 11% (p<0.001). In the validation cohort, 104 patients had high LDH levels and 145 patients had normal LDH levels; factors significantly associated with PFS and TFS on univariate analysis were LDH, β-2-microglobulin, Rai stage and ZAP70; LDH, β-2-microglobulin and age associated with OS. On multivariate analysis high LDH was the sole parameter significantly associated with all shorter outcomes, along with elevated β-2-microglobulin, which associated with shorter OS.
Summary/Conclusions: Our study on 487 patients with +12 CLL and the analysis on 250 patients of the validation cohort showed that patients with +12 and elevated LDH have shorter PFS, TFS, OS and CLL-specific survival.

PB1761

THE PERCENTAGE OF CELLS WITH ABNORMALITIES IN FISH STUDIES CONFERS PROGNOSTIC INFORMATION IN CLL PATIENTS

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Background: Genomic aberrations detected by FISH have become one of the most important and widely used prognostic factor for chronic lymphocytic leukemia (CLL) patients. In addition several publications have described that patients with a higher percentage of abnormal nuclei have a worse outcome.
Aims: To analyze the effect of the percentage of abnormal nuclei detected by FISH (13q deletion (13q-), 11q deletion (11q-), 17p deletion (17p-) and trisomy 12 (+12)) in overall survival (OS) and time to first treatment (TTFT).
Methods: We studied a non-selected cohort of 650 consecutive CLL cases from a local database with a median follow up time of 50 months (0-346). The cut-off point for the percentage of abnormal nuclei for each alteration was detected by dividing the variable into deciles, and selecting the most efficient cut-point, and based on previous publications.
Results: FISH detected aberrations in 68% of the cases (442/650). The most frequent abnormality was 13q-, observed in 302 patients (47%), but as a sole alteration in 212 cases, followed by +12 (106 patients, 16%), 11q- (83 patients, 13%), and 17p- (33 patients, 5%). As expected, the group of patients with 13q- as a sole abnormality was the one with the better OS (195 months) followed by the group of patients with normal FISH (160 months), +12 (124 months), 11q- (56 months) and 17p- (46 months), consistent with the Dohner hierarchical classification (Döhner H *et al.* NEJM 2000). Similar results were observed in TTFT: 13q- as sole abnormality (106 months), normal FISH (112 months), +12 (29 months), 11q- (10 months), 17p- (10 months). The best predictive cut-off point that divided patients according to its prognosis was different for each alteration. We confirmed that a high percentage of cells carrying the deletion is associated with a significantly worse TTFT in cases with 17p, 13q, and 11q deletions, and a significantly shorter OS in cases with 17p deletion. We observed a similar trend for OS in cases with 13q and 11q deletions, probably not significant because of the low number of patients included, compared to previous studies. We observed the same trend in patients with +12. The Table 1 summarizes these findings. Probably with a higher number of cases and a longer follow up, it could have also been possible to reach statistically significant differences in the subgroups in which it was not objected.

Table 1.

FISH abnormality	Number of cases	Overall survival (months)	P	Time to first treatment (months)	P
13q-(sole abnormality) < 75% ≥ 75%	212	195 (CI 95%, 162-228) 275 (CI 95%, not reached) 157 (CI 95%, 126-188)	0.06	106 (CI 95%, 69-143) 122 (CI 95%, 91-153) 40 (CI 95%, 16-64)	0.001
11q- < 40% ≥ 40%	83	56 (CI 95%, 33-79) 71 (CI 95%, 50-92) 39 (CI 95%, 24-53)	0.08	10 (CI 95%, 3-17) 37 (CI 95%, 26-48) 7 (CI 95%, 4-9)	0.025
17p- < 20% ≥ 20%	33	46 (CI 95%, 24-68) 65 (CI 95%, 50-80) 32 (CI 95%, 22-41)	0.02	10 (CI 95%, 1-18) 40 (CI 95%, 16-63) 5 (CI 95%, 0-12)	0.026
+12 < 60% ≥ 60%	106	124 (CI 95%, 31-164) 130 (CI 95%, 66-194) 98 (CI 95%, 54-141)	0.9	29 (CI 95%, 18-40) 38 (CI 95%, 15-61) 24 (CI 95%, 11-36)	0.085

Summary/Conclusions: Not only the type of cytogenetic abnormality but also the percentage of abnormal nuclei detected by FISH are important factors in the prognosis of CLL patients.

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METHYLATION STATUS OF RAD21 GENE IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chronic Lymphocytic Leukemia (CLL) pathogenetic mechanisms have not been fully elucidated yet. However, genetic and epigenetic alterations seem to be involved in the pathogenesis and extensive clinical heterogeneity of the disease. DNA methylation in CpG sites of a gene promoter, which may affect the chromatin structure as well as gene transcriptional activity, is a crucial epigenetic modification in CLL. *RAD21* gene is involved in DNA repair and its encoded product acts as basic subunit of the Cohesin protein complex that regulates the cohesion and proper separation of sister chromatids during mitosis or meiosis.
Aims: We investigated the methylation status of *RAD21* gene promoter and its possible implication in CLL pathogenesis and the formation of CLL cytogenetic aberrations.
Methods: The study included 105 CLL patients and 17 healthy donors (controls)**.** Total genomic DNA extraction was performed from bone marrow or peripheral blood samples of all patients and controls. Methylation analysis of *RAD21* gene promoter was carried out using the new techology of MethylScreen™ in the CFX96Biorad Real-Time PCR system. For this purpose, we used EpiTect Methyl II PCR Assay which enables us to calculate the methylated and unmethylated fraction after simultaneous digestions with specific restriction enzymes. Karyotypic analysis was performed on unstimulated and stimulated with CpG-oligonucleotide DSP-30 bone marrow cells of CLL patients. FISH analysis was carried out using the commercial CLL set probes for detection of the most common abnormalities of the disease including deletions of 17p13 (TP53), 11q22.3 (ATM) and 13q14.3/13q34.3 (D13S319/13q34) regions and trisomy 12 (CEP 12).
Results: Among the105 CLL patients, 21 patients exhibited a normal karyotype also confirmed by FISH and 84 patients showed chromosome abnormalities detected by karyotypic or/and FISH analysis. Methylation study was successful in all healthy donors and in 101 out of 105 CLL patients. All healthy donors had non-methylated *RAD21* gene promoter. On the contrary, 25.74% (26/101) of CLL patients carried >10% cells with methylated CpG islands in *RAD21* promoter, which was significantly increased compared to controls (p=0.039, χ^2=4.25, *df*=1). RAD21 methylated cell fraction varied among patients. More specifically, 9.9% of patients (10/101) showed 11-50% methylation rate, 10.89% (11/101) 51-89% and 4.95% (5/101) showed high methylation rate score, >90% of the analyzed cells. Stratification of patients according to cytogenetic findings showed that the promoter of *RAD21* was methylated in 28.57% of patients (6/21) with normal karyotypes and 25% of patients (20/80) with abnormal karyotypes. In detail, methylation in *RAD21* promoter was present in 33.33% of patients (5/15) with abn(14q32), in 33.33% (4/12) with abn(8), in 31.25% (5/16) with -17/del(17p), in 27.78% (5/18) with trisomy 12, in 25.81% (8/31) with del(13q), in 20% (2/10) with del(6q) and in 12.5% (2/16) with del(11q). Based on karyotypic complexity, *RAD21* promoter was methylated in 18.18% (4/22) of patients with a single chromosome aberration, 26.09% (6/23) with two chromosome aberrations and 25.71% (9/35) of patients with complex karyotype (≥3 aberrations).
Summary/Conclusions: Methylation of *RAD21* gene promoter, which leads to transcriptional inactivation and consequently inhibition of *RAD21* expression, seems to be implicated in CLL pathogenesis and the formation of specific chromosome aberrations. Clarification of the epigenetic landscape of CLL may help in the design of new targeted therapeutic agents.

PB1763

ROLE OF KEAP1-NRF2 PATHWAY GENETIC VARIABILITY IN THE SUSCEPTIBILITY AND PROGNOSIS OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chronic lymphocytic leukemia (CLL) is the most prevalent leukemia in the western adult population. Although advanced age, white ancestry, and family history of hematologic malignancies are risk factors, the etiology of CLL still unknown. One of the mechanisms associated with the development of this pathology is related to the oxidative stress (OS) resulting from an imbalance between the production of reactive oxygen species (ROS) and their disposal by the antioxidant defenses. The nuclear factor erythroid 2-like gene - type 2 (*NFE2L2*) and its suppressor, the Kelch-like ECH-associated protein 1 (*KEAP1*) gene, plays a central role in ROS balance. Changes in these genes, whether due to somatic mutations or genetic variants (SNPs), have been associated with some hematological diseases. However, the role of *NFE2L2* and *KEAP1* genes polymorphisms in susceptibility and prognosis of CLL is not studied.
Aims: To assess the role of two SNPs in the NFE2L2 and KEAP1 genes on CLL susceptibility, their influence on prognosis/survival, and their correlation with clinical and laboratory characteristics of patients.
Methods: Genetic variants rs13001694 (NFE2L2) and rs11085735 (KEAP1) were genotyped by tetra-primers-AMRS-PCR in 176 patients with CLL and 261 controls. The role of these genes polymorphisms in CLL susceptibility and their association with clinical and laboratory characteristics as well as with therapy response was assessed by logistic regression analysis and/or by Fisher's exact test. The influence on prognosis and survival was performed through Kaplan-Meier curves by estimating the progression free survival (PFS) and the overall survival (OS).
Results: The results showed that individuals with the GG genotype (NFE2L2) are at higher risk of developing CLL [Odds ratio (OR): 2.032; 95% confidence interval (CI): 1,234-3,351; P=0.004]. In addition, the genotypic profile (GP) GG / CC (NFE2L2 / KEAP1) is a risk factor (OR: 2.186; 95% CI: 1.279-3.744; p=0.003) for the development of CLL while the AA / CC profile constitutes a protective factor (OR: 0.634, 95% CI: 0.407-0.984, p=0.037). In contrast, patients with genotype AG (NFE2L2) and/or CC (KEAP1) had a higher rate of complete response to rituximab therapy regimens (NFE2L2 AG: OR 1.6, 95% CI 1.063-3.933, p=0.037; KEAP1 CC, OR 1.2, 95% CI 1.041-3.477, p=0.045, NFE2L2 / KEAP1 AG / CC: OR 1.9, 95% CI, 1.843-4.485, p=0.017) and with fludarabine (NFE2L2 / KEAP1 AG / CC: OR 1.5, 95% CI, 1.119-3.887, p=0.026). Finally, the overall survival of CLL patients appears to be influenced by the genotypic profile of NFE2L2 / KEAP1 [GP AG / AC patients have a lower mean survival (72.5±13.8 months) than patients with other GPs (139.4±10.2 months, p=0.037)], while progression-free survival seems to be influenced by the KEAP1 genotype [patients with CC genotype have a longer mean survival (198.0±13.6 months) than patients with AA and AC genotypes (85.3±13.4 months; P=0.022)].
Summary/Conclusions: This study suggest that genetic polymorphisms in *NFE2L2* and *KEAP1* genes might be risk factors for CLL development and may constitute novel genetic markers for therapy response (namely regimes with rituximab and fludarabine) as well as prognostic markers, by influencing overall survival and progression free survival in CLL patients.
The authors declare no conflicts of interest.

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EVALUATION OF BASAL CHROMOSOME ABERRATIONS AND MICRONUCLEUS FREQUENCY IN UNTREATED PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA AND THEIR ASSOCIATION WITH PROGNOSTIC MARKERS

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Background: Chronic lymphocytic leukemia (CLL) is a heterogeneous disease, with variable clinical presentation and evolution. Two major subtypes can be distinguished, mutated (M) and unmutated (UM), characterized respectively by a high or low number of somatic hypermutations in the variable region of immunoglobulin genes and different outcome. Cytogenetic and FISH (fluorescence *in situ* hybridization) studies have proved to be important tools in the biologic characterization of this disease, allowing the identification of distinct risk groups. Genomic instability involves a process prone to the accumulation of chromosome alteration in somatic cells and is a major driving force of tumorigenesis. The analysis of chromosome aberrations (CA) and micronucleus (MN) represent different forms to evaluate genomic instability.
Aims: In this study, we have analyzed the basal frequency of CA and MN in untreated CLL patients. Results were evaluated in relation to different prognostic factors.
Methods: A total of 67 untreated CLL patients (36 males; mean age: 66.6 years; range: 42-83 years; Rai stage: 0: 27%; I-II: 59%; III-IV: 14%), and 6 normal controls, were studied. Chromosome analysis was performed on stimulated peripheral blood lymphocytes cultures. For each patient, CAs were evaluated on 50 cells stained with 10% Giemsa and the MN frequency was assessed on 250 interphase nuclei. FISH analysis was performed using the CLL panel according to manufacturer´s protocol. *IGHV* (immunoglobulin heavy chain variable region) mutational status was analyzed by RT-PCR and bi-directional sequencing. The study was approved by the local Ethics Committee. All individuals provided their informed written consent.

Results: An increased number of CAs, including chromatid breaks and dicentrics, in CLL patients (6.59±5.3%) compared to controls (0.25±0.04%) (p=0.021) was observed. A tendency to increased CA frequency in cases with abnormal (8.18±6.1%) compared to normal karyotypes (5.67±4.4%) (p=0.08) was also found. The analysis taking into account FISH risk groups showed a higher frequency of CA in patients with deletions 11q22 and/or 17p13 associated to poor outcome (8.54±6.1%), than those with no alterations or 13q14 deletion related to a better outcome (5.64±3.9%) and cases with +12 with an intermediate prognosis (4.54±3.5%). By MN analysis, an increased frequency in CLL patients (2.81±1.5%) compared to controls (0.67±0.3%) (p=0.0001) was found. Patients with +12 presented the highest percentage of MN compared to the other two groups (~1.3-fold), indicating the aneugenic effect of this alteration. The evaluation according to the *IGHV* mutational status showed similar frequencies for CAs and MN in M-CLL (6.2±5.2% and 2.82±4.9%, respectively) and UM-CLL (6.2±5.8% and 2.7±1.3%, respectively). No association between CA and MN frequencies and clinical parameters was found.
Summary/Conclusions: Our results confirm the presence of basal genomic instability in untreated CLL patients as measured by both CA and MN techniques. To our knowledge, this is the first analysis of these parameters taking into account prognostic factors of the disease. Cases with deletions 11q22 and/or 17p13 had the highest value of CA and those with +12 showed the highest frequency of MN, reflecting different mechanism of DNA damage.

PB1765

B CELLS RESISTANT TO CD20 MONOCLONAL ANTIBODIES DISPLAY SPECIFIC ALTERATIONS IN GENE EXPRESSION PROFILE

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Background: CD20 monoclonal antibodies (mAb) are a standard of care for B-lymphoid malignancies. Yet, their clinical efficacy is quite variable and many patients relapse, while their malignant cells express very low density of CD20 on the cell surface. In spite of being used for 20 years as a therapy target, little is known about the biology and regulation of CD20 inside the cell.
Aims: The aim of this proposal was to investigate the intracellular mechanisms regulating expression of CD20 antigen.
Methods: Diverse cell and molecular biology techniques were used, including flow cytometry analysis, real-time PCR and RNA sequencing.
Results: We show that treatment of B cells with different CD20 mAbs initiates a signaling cascade within the cells that is partially distinct from classical B-cell receptor signaling machinery and does not involve BCR proximal proteins. Importantly, it results in a prompt downregulation of CD20 expression. Through chronic exposure to gradually increasing doses of monoclonal antibodies, we have generated cell lines that are resistant to additional treatment with mAb. Notably, these cells are resistant also to any other of the available anti-CD20 antibodies even at very high concentrations as shown by dose-response experiments. This resistance is sustained for long period and maintained even upon many rounds of cell passages. We could confirm that these cells have downregulated CD20 protein from the cell surface and that this effect was not just due to its internalization. Instead, we detected a defect in CD20 transcription as measured by quantitative real-time PCR. Flow cytometry analysis of other surface markers showed a strong upregulation of CD55 and CD59, known inhibitors of complement activation. The combination of CD20 loss together with the increase of CD55 and CD59 is responsible for the complete resistance to the mAbs. We have then analyzed changes in overall gene expressions by performing RNA sequencing and quantitative real-time PCR. We have identified several interesting genes whose expression was altered in our resistant cells when compared to their control counterparts. Among the most interesting hits was a strong downregulation of the transcription factor NFkB, which was expressed more than 10-fold lower in the rituximab or ofatumumab resistant cells. We could confirm this result in multiple independent experiments. We have postulated that anti-CD20-triggered signaling results in the inactivation of NFkB and this may lead to the block in CD20 transcription. To confirm our hypothesis, we have treated the cells with phorbol ester PMA, which nonspecifically activates NFkB. Indeed, cells treated with PMA managed to rapidly upregulate CD20 on their cell surface.
Summary/Conclusions: In summary, CD20 triggering by therapeutic mAbs initiates complex intracellular changes that result in downmodulation of CD20 expression. Further analysis of detailed intracellular mechanisms regulating CD20 is warranted in order to propose novel interrogation nodes that might modulate CD20 surface density and thereby enhance the therapeutic potential of CD20 monoclonal antibodies.
This research has been financially supported by the Ministry of Education, Youth and Sports of the Czech Republic under the project CEITEC 2020 (LQ1601) and by the research grant AZV-MZ-CR 15-33561AA-4/2015 and grant MUNI/A/1106/2016.

PB1766

DIFFERENTIAL EXPRESSION PATTERNS OF CHEMOKINE RECEPTORS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chemokines and their receptors are involved in the regulation of cell recruitment, survival, proliferation, and trafficking, all these processes crucial in the pathogenesis of chronic lymphocytic leukemia (CLL). Comprehensive profiling of chemokine receptors in CLL and its subgroups according prognostic relevance is missing.
Aims: To characterize the chemokine expression pattern in CLL patients and subgroups according to clinical course and cytogenetic aberrations.
Methods: We studied the gene expression pattern of 16 canonical and 4 atypical chemokine receptors in peripheral blood mononuclear cells (PBMC) of CLL patients (n=88) and healthy subjects (n=34) by using SmartChip quantitative RT-PCR (WaferGen Bio-systems). The expression of CXCR3, CXCR4, CXCR5, CXCR7, and CCR7 was confirmed by flow cytometry.
Results: Among deregulated receptors, 5 receptors (CCR7, CCR10, CXCR3, CXCR4, CXCR5) were up-regulated and 9 receptors (CCR2-CCR6, CCR8, CCRL2, CXCR1, CXCR2) down-regulated in CLL; the expression of others did not differ between CLL and controls (P>0.05). In patients with del(17p) associated with a poor prognosis, we observed higher mRNA levels of CXCR6, CXCR7 and CCR10 comparing to del(13q). On protein level, the percentage of neoplastic B cells positive for CXCR4, CXCR5, and CCR7 was higher and percentage of CXCR7 lower than on normal B cells (P<0.05). In patients with CLL a marked increase in MFI of CXCR4 (P<0.001) and CCR7 (P<0.001) on CLL cells was detected comparing to healthy subjects.
Summary/Conclusions: Our results provide a complete picture of expression patterns of chemokine receptors in PBMC of CLL patients and prognostically relevant subgroups. Further studies are needed to clarify how chemokine receptor network affects neoplastic development and progression.
Grant support: MZ ČR VES16-32339A, IGA_LF_2017_009, IGA_LF_2017_001.

PB1767

RESIDUAL SERUM CONCENTRATIONS OF RITUXIMAB ARE ASSOCIATED WITH RELAPSE RISK IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Rituximab is an anti-CD20 chimeric monoclonal antibody approved in first-line treatment of patients with chronic lymphocytic leukemia (CLL), in association with chemotherapy. Rituximab displays a time-dependant pharmacokinetic with a high variability between patients that is primarily related to target mediated elimination.
Aims: Rituximab pharmacokinetics has been associated with clinical response but there is no data on its association with patients' evolution after immunochemotherapy, which is the aim of the present study.
Methods: Residual serum concentrations of rituximab were determined by an enzyme-linked immunosorbent assay (ELISA) for 35 CLL patients before each infusion, administrated every 28 days at T0, T1, T2, T3, T4, T5. Response and relapse criteria were evaluated according to the International Workshop on Chronic Lymphocytic Leukemia guidelines.
Results: Patients were assigned to two groups related to time to relapse. The first group (n=7), had an early relapse in less than 3 years, the second group (n=28), in more than 3 years. A lower residual serum rituximab concentration was observed in patients with an early relapse and statistical significance was reached for the values obtained after the 3rd cycle (T3) (p=0.02). Concerning the area under the curve (AUC), the difference was significant across all the cycles, an early relapse being associated with a low AUC (AUC_{mean}A=1.28 ±1.01 mg/L*day, AUC_{mean}B=2.79±1.93 mg/L*day, p=0.02). Additionally, the residual rituximab serum concentration between T2 and T5, superior at 70µg/ml, is associated with a long response time, with a sensibility of 100% and a specificity of 52%. Low residual serum rituximab concentrations in the early relapse group were associated with a higher expression of CD38 and a more frequent administration of the chemotherapy rituximab-bendamustine than rituximab-fludarabine-cyclophosphamide. On the other hand, there was no association with age, sex, cytogenetics, tumour burden or with FCGR3A-158VF polymorphism.
Summary/Conclusions: In conclusion, serum residual rituximab concentration in patients with CLL has an impact on clinical evolution after treatment. This study provides data that sustains the need of rituximab serum concentration adaptation in certain CLL patients, in order to reduce relapse risk.

PB1768

ACTIVITY OF THE CD19 ANTIBODY MOR208 IN COMBINATION WITH IBRUTINIB, IDELALISIB OR VENETOCLAX *IN VITRO*

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Background: CD19 is broadly expressed across B-cell malignancies, including chronic lymphocytic leukemia (CLL). MOR208 is an Fc-enhanced CD19 antibody mediating potent antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) and direct cytotoxicity. Single agent MOR208 has shown promising activity in clinical studies.
Aims: We investigated the *in vitro* cytotoxicity of MOR208 when combined with the tyrosine kinase inhibitors (TKIs), ibrutinib and idelalisib, and the BCL-2 inhibitor, venetoclax.

Methods: The CLL cell line MEC-1 was treated with 0.3–10 µM ibrutinib, idelalisib or DMSO (control) for 7 days or 3–10 µM venetoclax or DMSO for 24 hours. Inhibition of proliferation, cytotoxicity and impact on CD19 expression were then assessed. ADCC assays with MOR208 incorporated a fixed number of primary human natural killer cells from healthy volunteers as effector cells. By contrast, the number of target cells was reduced according to antiproliferative or cytotoxic effects of the TKIs or venetoclax. Dose-dependent ADCC activity of MOR208 was analyzed by flow cytometry. Cytotoxic effects were studied in at least three independent experiments.
Results: Ibrutinib and idelalisib induced only moderate direct cytotoxicity on MEC-1 target cells but had strong antiproliferative effects. In contrast, venetoclax induced strong cytotoxicity on MEC-1 target cells within 24 hours. Both effects led to reduced tumor target cell numbers in the subsequent ADCC assays. CD19 expression was largely unaffected by all three drugs. The addition of MOR208 to ibrutinib, idelalisib or venetoclax treated target cells resulted in enhanced maximum ADCC when compared with single agent MOR208. EC50 values remained unaltered in TKI or venetoclax treated conditions compared with the DMSO control. Calculations according to Chou-Talalay yielded combination indices below 1 for all three drugs, thus confirming synergistic activity.
Summary/Conclusions: The cytotoxic effect of MOR208 was synergistically enhanced when combined with ibrutinib, idelalisib or venetoclax *in vitro*. These promising data provide a strong rationale for combination of MOR208 with these agents in future clinical trials.

PB1769

LYMPHOCYTE EXHAUSTION AND THE NATURAL HISTORY OF CHRONIC LYMPHOCYTIC LEUKEMIA – FRIENDS OR FOES?

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Background: Chronic lymphocytic leukemia (CLL) is a disease characterized by the accumulation of morphologically mature monoclonal lymphocytes B with CD19+/CD5+/CD23+ phenotype in lymphoid tissue, peripheral blood and bone marrow. The course of CLL is chronic by default. Of note, however, is its heterogeneity. Programmed cell death protein 1 and its ligand 1 (PD-1, PD-L1) as well as CD200 and CD200 receptor (CD200R) are major inhibitory receptors regulating T cell exhaustion, *i.e.* a state of T cell dysfunction. The role of lymphocyte exhaustion in the natural history of CLL is still a matter of discussion.
Aims: The aim of this study was to determine the percentages and absolute numbers of exhausted lymphocytes B and T in peripheral blood and bone marrow of CLL patients. Moreover, we analyzed relationship between the number of PD-1-positive, PD-L1-positive, CD200-positive, and CD200R-positive lymphocytes and established prognostic factors in CLL.
Methods: The study included 60 untreated patients with CLL and 20 healthy subjects. The immunophenotype of peripheral blood mononuclear cells (in both groups) and bone marrow cells (solely in the CLL group) was determined by means of flow cytometry.
Results: Patients with CLL showed higher frequencies and absolute number of exhausted B lymphocytes CD19+PD-1+ ($p<0.0001$), CD19+PD-L1+ ($p<0.0001$), CD19+CD200+ ($p<0.0001$) and CD19+CD200R+ ($p<0.0001$), as well as higher frequencies and absolute number of exhausted T helper lymphocytes CD4+PD-1+ ($p=0.0021$), CD4+PD-L1+ ($p=0.0032$), CD4+CD200+ ($p=0.0027$), CD4+CD200R+ ($p=0.0062$), and exhausted T cytotixic lymhocytes CD8+PD-1+ ($p=0.0036$), CD8+PD-L1+ ($p=0.0029$), CD8+CD200+ ($p=0.0038$), CD8+CD200R+ ($p=0.0073$) than the controls in the peripheral blood. Similar observations were done in the bone marrow samples ($p<0.0001$, $p<0.0001$, $p<0.0001$, $p<0.0001$, $p=0.0134$, $p=0.0183$, $p=0.0263$, $p=0.0169$, $p=0.0261$, $p=0.0362$, $p=0.0293$, and $p=0.0379$, respectively). Enhanced exhaustion of peripheral blood and bone marrow lymphocytes was associated with higher Rai stage, increased concentration of lactate dehydrogenase and beta-2 microglobulin, and more rapid progression of the disease. The number of lymphocytes B CD19+ZAP-70+ correlated positively with the number of CD19+PD-1+ B cells, CD4+PD-1+ T cells, and CD8+CD200+ T cells.
Summary/Conclusions: The study confirmed the association between unfavorable prognosis and high expression of exhaustion markers in CLL patients. Determination of PD-1+, PD-L1+, CD200+ and CD200R+ lymphocytes T and B constitutes valuable diagnostic tool, completing cytometric evaluation of CLL.

PB1770

HSP70 AND HSF1 GO HAND IN HAND AND HAVE A ROLE IN THE SURVIVAL OF CHRONIC LYMPHOCYTIC LEUKEMIA NEOPLASTIC B CELLS

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Background: B-cell Chronic Lymphocytic Leukemia (CLL) is a neoplastic disorder characterized by the accumulation of clonal B cells in peripheral blood, bone marrow and lymphoid tissues. CLL is a clinically and biologically heterogeneous disease. As a consequence, novel biological and cytogenetic features have become increasingly important in predicting prognosis at the time of diagnosis and the research for molecules involved in apoptosis resistance and increased survival of neoplastic B cells is still ongoing.
Aims: We recently found that the Heat Shock Protein of 70kDa (HSP70) is overexpressed in Chronic Lymphocytic Leukemia (CLL) B cells. Considering the pro-survival role of HSP70 in cancer, we were aimed at characterizing this protein and its master regulator, the Heat Shock Factor 1 (HSF1), within the pathogenetic mechanisms leading to CLL.
Methods: HSP70 and HSF1 expression levels were evaluated by Western blotting (WB) analysis in leukemic and normal B cells. HSP70 and HSF1 protein levels were correlated to IGHV mutational status and ZAP70 protein expression in CLL patients. HSP70 and HSF1 levels were also analyzed in neoplastic cells obtained from patients undergoing Ibrutinib based regimen by WB analysis. Moreover, HSP70 and HSF1 localization was analyzed by subcellular protein fractionation followed by WB analysis. The effects of HSP70 and HSF1 inhibition (by Zafirlukast and Fisetin) were evaluated by Annexin V/Propidium Iodide flow cytometry test and WB analysis of PARP cleavage.
Results: We demonstrated that HSP70 and HSF1 are overexpressed in leukemic *vs* normal B cells and their expression levels correlate to poor prognosis in CLL. We also analyzed HSP70 and HSF1 levels in patients following *in vivo* Ibrutinib based regimen, observing a positive correlation between these two protein expression levels and moreover we observed that these two protein levels decreased after therapy. We found that at steady state both HSP70 and HSF1 are localized in the nucleus of CLL B cells. HSP70 and HSF1 inhibition was proved to be effective in inducing a dose-dependent *in vitro* apoptosis of CLL B cells.
Summary/Conclusions: HSP70 and HSF1 overexpression and correlation with poor prognosis in CLL patients underline their pivotal role in the regulation of leukemic B cell survival. HSP70 and HSF1 both correlation and reduction in CLL patients following *in vivo* Ibrutinib regimen let us hypothesize a role of these proteins in the progression of the disease. In normal B cells HSP70 and HSF1 are both localized into the nucleus after stress conditions, however we found both HSP70 and HSF1 localized into the nucleus of CLL B cells at steady state, suggesting a constitutive activation of these proteins in CLL. Although HSP70 has been extensively linked to cancer, little progresses have been made in bringing HSP70 inhibitors to the clinic, because of their potential off-target effects. For this reason we tried an alternative approach by targeting the HSP70 major regulator, HSF1. We observed that both inhibitors, Zafirlukast and Fisetin, lead to an *in vitro* dose dependent B cell apoptosis. These data demonstrate HSP70 and HSF1 involvement in the pathogenesis of CLL and identify HSP70/HSF1 axis as a target for new therapeutic strategies.

PB1771

OVEREXPRESSION OF GENE FOR HUMAN CONCENTRATIVE NUCLEOSIDE TRANSPORTER 3 IS A PREDICTOR OF RESISTANCE TO FLUDARABIN-BASED CHEMOTHERAPY IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Human concentrative nucleoside transporter 3 (hCNT3) belongs to a family of nucleoside transporters involved in fludarabine cellular uptake. It has been reportedthat overexpression of *SLC28A3* gene encoding hCNT3 predictspoor response to fludarabin-based chemotherapy.However, the mechanisms by which elevated expression of *SLC28A3* mediate fludarabine resistance are still elusive.
Aims: The aim of the study was to examine possible influence of *SLC28A3* gene overexpression on treatment response to fludarabine-cyclophosphamide therapy (FC) in patients with chronic lymohocytic leukemia.
Methods: We retrospectively analysed data from 54 CLL patients diagnosed and treated at Clinic for Hematology, Clinical Center of Serbia from 2003 to 2013. Blood samples were prospectively collected and analysed for biological and molecular features, as well as standard laboratory parameters. The expression of *SLC28A3* gene was analyzed in peripheral blood mononuclear cells by RQ-PCR methodology, using TaqMan chemistry and Abl as endogenous control gene. Quantification of target gene expression was made by comparative ddCt method, using HL-60 cell line as the calibrator. All analyses were done prior to any treatment.
Results: Median age at diagnosis was 57 years (range 38-75). All patients were treated with fludarabin-based chemotherapy, 45 (83%) in the first treatment line. Overall response rate to the first line therapy was 81%, equally distributed on complete and partial responses (CR and PR), while the remainder included the same number of patients with stable disease (SD) and progressive disease (PD) (5, 9.6%). Most of the patients (42, 78%) relapsed during the follow up. At the time of the last follow up 14 (26%) patients were still alive, 35 (65%) were dead, and 5 (9%) were lost to follow up. Median overal survival was 76 months.
In the group of patients who received FC in the first treatment line (43/54), median expression of *SLC28A3* mRNA in patients who experienced CR, PR, SD and PD was 0.036±0.030, 0.062±0.063, 0.030±0.025 and 0.157±0.257, respectively. The level of *SLC28A3* expression was not associated with the *IGHV* mutational status. Patients who experienced PD to FC treatment overexpressed gene for hCNT3 compared to patients who achieved CR (p=0.013) and PR (p=0.05). We detected a significantly higher level of *SLC28A3* expression in patients who experienced PD to FC treatment in comparison to patients who achieved CR (p=0.013) and PR (p=0.05).
Summary/Conclusions: Overexpression of *SLC28A3* gene is a predictor of resistance to treatment with FC chemotherapy. Further studies are warranted to confirm these findings.

PB1772

THE SPECTRUM OF TP53, SF3B1, AND NOTCH1 MUTATIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS EXPOSED TO IONIZING RADIATION DUE TO THE CHORNOBYL NPP ACCIDENT

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Background: Generally, chronic lymphocytic leukemia (CLL) is considered to be a non-radiogenic form of leukemia. We previously found some clinical and biological features of CLL in group of clean-up workers of Chornobyl NPP accident indicated unfavorable disease course, such as high frequency of solid tumors and Richter transformation, mainly unmutated status of heavy chain variable region (*IGHV*) genes with increased usage of *IGHV1-69* and *IGHV3-21* (Abramenko *et al.*, 2008). Analysis of genetic features of leukemic cells in IR-exposed CLL patients may provide an additional data on the possible causal relationship with IR.
Aims: the aim of the study was to analyze *TP53*, *NOTCH1 and SF3B1* mutations in CLL patients, sufferers of Chornobyl NPP accident to clarify the possible pathogenetic relationship between IR and CLL development
Methods: *TP53*, *NOTCH1*, and *SF3B1* mutations were analyzed in 106 CLL patients who have been exposed to ionizing radiation (IR) due to Chornobyl NPP accident (83 clean-up workers, 16 inhabitants of radionuclide contaminated areas, and 7 evacuees) and in 130 IR non-exposed CLL patients as the control group. *TP53* gene mutation analysis was performed for exons 3 to 10. *NOTCH1* mutations and *SF3B1* mutations were analyzed in the hotspot regions of these genes were the vast majority of CLL-specific lesions were reported: in c.7282_ 7680 region in exon 34 of *NOTCH1* gene, and in exons 14, 15 and 16 of *SF3B1* gene, correspondingly.
Results: We found *TP53* and *SF3B1* mutations with similar incidence in both groups – in 11.3% and 10.0% of IR-exposed patients, and in 12.7% and 11.5% of IR non-exposed CLL patients, respectively. In contrast, *NOTCH1* mutations were found with significantly lower frequency in IR-exposed patients in comparison with the control group (6.7% *vs* 17.7%; p=0.012). Some other features were found among IR-exposed CLL patients also. Specifically, *TP53* mutations were seen with equal frequency among mutated (11.1%) and unmutated (11.8%) *IGHV* cases in IR-exposed CLL patients, while the tendency to prevalence of *TP53* mutations in unmutated compared with mutated *IGHV* cases was found in the control group (14.1% and 5.6%, correspondingly; *p*=0.178). In IR-exposed group *SF3B1* mutations were combined with mutations in *TP53* almost in half of detected cases. In opposite, in the control group we observed reported earlier mutual exclusivity between *SF3B1* and *TP53* lesions (p=0.001 in comparison between observed groups). Among IR-exposed CLL patients we found two different cases with identical rare mutation of *TP53* gene - c.665C>T substitution leading to change proline for leucine at codon 222 (Pro222Leu). This substitution is very likely to represent inherited *TP53* mutation, which may influence CLL development under IR exposure.
Summary/Conclusions: In summary, our data suggest that *TP53* abnormalities are involved in CLL development in sufferers of the Chornobyl NPP accident and also a possible interaction between inherited IR sensitivity caused by mutation in *TP53,* radiation and CLL development.

PB1773

DRUG SENSITIVITY SCREENING IN CHRONIC LYMPHATIC LEUKEMIA AND MULTIPLE MYELOMA FOR PERSONALIZED CANCER THERAPY

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Background: Personalized Cancer Medicine is rapidly developing field that includes predictive medicine, preventive medicine and various personalized or individualized therapies, *e.g.* labeled "precision medicine". One particular challenge with cancer is that origin of each cancer is a clonal event evolving into tumor heterogeneity. We focus on Chronic Lymphocytic Leukemia (CLL), Multiple Myeloma and Follicular lymphoma (FL) that are currently considered incurable. Although current treatment regimens prolong life for patients, CLL and MM cancer eventually relapse. Current challenges in using therapeutics against CLL and MM includes design of optimal treatment for individual patients based on characterization the tumor and its intratumor heterogeneity as observed by whole genome sequencing. Efficient therapies require a personalized approach that combines targeting lymphoma cells and the tumor microenvironment by restoring the patient's own anti-tumor immunity. One solution to this challenge is the so-called "n-of-one" studies where protocols are organized with diagnostically based patient stratification to individualized treatments (n=1).
Aims: To introduce individualized treatment for patients against available therapies, we aim to established cell-based assays and drug sensitivity platform at NCMM, University of Oslo and Oslo University Hospital. To establish a pipeline for direct drug senstivity screening in CLL and MM (WP1-Path A). To Complement the results from WP1-Path A, with Signaling pathway analysis (WP2-Path B) towards testing in xenografted mice and implementing therapy in n-of-one clinical trials. To Offer patients with intractable CLL and MM individualized treatment with an effective combination of targeted therapies.
Methods: We culture CLL cells with combination of feeder cells that express CD40L, APRIL and BAFF for 24 hours stimulation. We perform drug sensitivity screening with Prestimulated CLL cells in 384 well formats without feeder cells. We culture MM cells in 384 well format for drug screening in response to T_{helper} cells prestimulation in the presence of IL2. To support high-throughput drug sensitivity screening , We use cell-based assays such as CellTiter-Glo® Luminescent Cell Viability Assay and CellTox™ Green Cytotoxicity Assay to define drugs that inhibit cancer cell growth. Additional methods such as cell proliferation assay, CellTox Green, apoptosis and oxidative stress (glutathione release) are also applied. We also use established cell barcoding on CLL/MM for flow cytometry (7-AAD/BrdU cell proliferation and Caspase8/9 apoptosis assay).
Results: Standard Curve for cell proliferation, CellTiter-Glo assay has been performed for MM/CLL cells. Time course measurement using cell proliferation, CellTox-Green assay for CLL cells (unstimulated and soluble CD40 ligand stimulated) has been performed. Time course measurement (0,24,48,72 hrs and 5 days) using cell proliferation, CellTox-Green assay for M2 cells has been performed. Benzalkonium chloride (BzCl) is used as Positive control. Endpoint measurement using CellTiter-Glo assay for CLL and MM cells was performed with cell density of 5000. Dose Response curve for 50 drugs has been generated for CLL patients (n=4) and MM (n=4) (Figure 1).

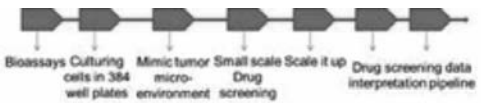

Figure 1.

Summary/Conclusions: We perform drug sensitivity screening to select potential drug candidates and pathway inhibitors through an approach where we directly assess patient samples. Selected drug candidates will first be validated by bioassays and flow cytometry to assess effects on intracellular mitogenic pathways (phosphoflow-based approach). We propose to use the drug sensitivity screening platform to identify and validate drug candidates for xenografting and "n-of-one" clinical trial studies.

PB1774

FCGR2A AND FCGR3A VARIANTS ARE NOT ASSOCIATED WITH RESPONSE TO RITUXIMAB IN PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chronic lymphocytic leukemia (CLL) is the most common form of adult leukemia in Western world with highly variable clinical outcome. Rituximab is a monoclonal chimeric anti-CD20 agent, that has demonstrated significant benefit for patients with different form of B cell lymphoproliferative disorders. Chemoimmunotherapy with rituximab, fludarabine and cyclophosphamide (R-FC) has shown to prolong progression free survival (PFS) and overall survival in CLL patients compared with chemotherapy alone. FCGR2A is polymorphic and has two alleles, FCGR2A-131H and FCGR2A-131R. This polymorphic variation is due to a single base substitution of nucleotide adenine for guanine in position 494. FCGR2A-H131 allele has a higher affinity for human IgG2, comparing to FCGR2A-R131. The gene for FCGR3A has also two polymorphic variant alleles: 158 valine (V158) and phenylalanine (F158) due to single base substitution of timidine to guanine at nucleotide position 559. FCGR3A-158V variant has higher affinity for Fc gamma receptor than 158F variant. These Fc gamma receptor polymorphisms may influence antibody-dependent cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC) and direct proapoptotic effect.
Aims: The aim of our study was to investigate a possible association of these two FCGR2A and FCGR3A variants with response to R-FC therapy in CLL patients.
Methods: We have analyzed these two polymorphisms in 90 patients with CLL treated with R-FC regimen. Median age of our patients was 62.7(36-78) and 63% were male. Number of patients with stage III/IV disease was 65(72%) and median WBC count at the start of treatment was 68.5(34-173x10^9/L). Percentage of previously treated patients was 51/90 (56.6%). Average numbers of R-FC cycles were 4.3 and median PFS was 35.1 months. Median time of observation after treatment was 3.6 years (range:6 months-8 years). Response was evaluated 2 months after therapy according to National Cancer Institute (NCI) criteria. Complete response (CR) was achieved in 24/90 (26.7%), partial response (PR) in 56/90 (62.2%) and no response in 10/90 (11.1%). DNA was isolated from peripheral blood mononuclear cells and genotyping was performed by using PCR/RFLP methods. The distribution of genotypes was compared by using a chi-squared test or Fisher's exact test.
Results: Distribution of genotypes in our patients was: 33% H/H, 49% H/R and 18% R/R for FCGR2A and 43% V/V, 40% V/F and 17% F/F for FCGR3A. Rate of CR and PR were similar irrespective of the FCGR variants and our results did not demonstrate significantly different genotype distribution for FCGR2A (p=0.8001) or FCGR3A (p=0.1019) in CLL patients with complete, partial or no response to R-FC treatment (Table 1).

Table 1. Genotype distributions for FCGR2A & FCGR3A in patients with CLL.

FCGR2A/FCGR3A	Complete Response n=24(26.7%)	Partial Response n=56(62.2%)	No Response n=10(11.1%)	p-values
FCGR2A 131H/R				
(131H/H)	8(26.6%)	18(60%)	4(13.4%)	0.8001
(131H/R)	10(22.7%)	29(65.9%)	5(11.4%)	
(131R/R)	6(37.5%)	9(56.3%)	1(6.2%)	
FCGR3A 158F/V				
(158 F/F)	8(20.5%)	29(74.4%)	2(5.1%)	0.1019
(158 F/V)	11(30.6%)	21(58.3%)	4(11.1%)	
(158 V/V)	5(33.3%)	6(40%)	4(26.7%)	

Summary/Conclusions: Our results are similar with previously reported results in other studies in CLL patients, but in contrast with the results for follicular lymphoma (FL), which showed that high-affinity FCGRA-158V/V variant was associated with the highest response rates in FL patients treated with rituximab. These findings could be explained with the different mechanism of action of rituximab in CLL compared to lymphoma patients or could be due to the variations in selected patient's population.

PB1775

MUTATIONAL STATUS, IMMUNOGLOBULIN HEAVY VARIABLE GENES PATTERN AND STEREOTYPED RECEPTORS REPERTOIRE OF MACEDONIAN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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Background: The mutational status of the immunoglobulin heavy variable (IGHV) genes is established as one of the most important prognostic molecular genetic markers in chronic lymphocytic leukemia (CLL). It divides the CLL patients into two subsets with a different clinical course, mutated (M-CLL) and unmutated (U-CLL). U-CLL is delineated with a cutoff value of 98% identity with the closest germ line of IGHV genes. The shaping of the CLL IGVH gene repertoire depends on a different genetic background and effects of the environmental factors. In addition, a strong bias in the use of individual genes and subgroups between normal and malignant B-cells and presence of highly homologous "stereotyped" heavy complementary-determining region 3 (VH-CD3) is shown, which suggests the role of a specific antigen in the pathogenesis of the disease.
Aims: In this study, we analyzed the mutation status and pattern of IGHV, IGHD and IGHJ gene usage in Macedonian CLL patients.
Methods: Ninety-seven consecutive CLL patients that presented at the University Clinic of Hematology –Skopje in the period between 2011-2013, were included in the study. IGHV mutation status and gene repertoire were analyzed using the reverse transcriptase– polymerase chain reaction (RT-PCR) and sequencing methodology. The mutational status of the IGVH genes was determined using two databases: IMGT/V-QUEST tool and IgBLAST software. The stereotyped subset assignment was performed using ARResT/AssignSubset tool (Bioinformatics Analysis Team).
Results: We found that 44.3% of the cases belonged to M-CLL and 55.7% to U-CLL, with a progressive disease dominant in the U-CLL subset. Both groups were comparable regarding the age and gender distribution. Only 39% of the M-CLL patients presented with a progressive disease, compared to 74% of the U-CLL patients (p<0.05).The comparison of median time to the first treatment (TTT) between M-CLL and U-CLL (39 months *versus* 8 months, respectively) showed a statistically significant difference between the groups (p<0.01). Most frequently expressed IGVH genes were: the IGHV3 subgroup (44.3%), followed by IGHV1 (28.9%), IGHV4 (23.7%), IGHV5 (2.0%), and IGHV2 (1.0%). Among 32 different IGVH genes, 8 genes were found (V1-46,V1-69,V3-21,V3-23,V3-30,V3-33,V3-48 & V4-34) in 58.8% of all cases, revealing a strong bias in IGVH gene expression in CLL. IGHV1-69 was the most frequently expressed gene of all genes (16.5%), and exclusively found in the U-CLL group demonstrating a frequency of 29.6%. The IGHV3-21 was detected with a low frequency of 4.1%, as reported for CLL patients from other Mediterranean countries. The distribution of IGHD subgroups was as follows: IGHD3, 52.6%; IGHD2, 17.5%; IGHD6, 13.4%; IGHD1 7.2%; IGHD4 7.2%; and IGHD5 2.09%. The most frequent IGHJ gene was IGHJ4 (49.4%), followed by IGHJ6 (239.2%), IGHJ5 (13.4%), IGHJ3 (11.4%) and IGHJ2 (3.8%), IGHJ1 (2.09%). In 10.1% of the cases, the VHCDR3 amino acid sequences belong to previously defined stereotyped clusters. Only one of the rearrangements with stereotyped VH-CD3 belonged to the M-CLL subset.
Summary/Conclusions: Our study showed a strong correlation between IGHV gene mutational status and clinical course of CLL. Results on IGHV-IGHD-IGHJ genes usage in our study are comparable to the previously reported from Mediterranean countries. The high frequency of V1-69gene and low frequency of IGVH3-21 in our CLL patients that originate from a small geographic region further promotes the geographic bias in the use of IGVH genes and points to an important role in antigen stimulation in the pathogenesis of the CLL subsets. Our findings indicated a lower expression of the stereotyped BCR region than those previously reported (~30%), but they were comparable with the results reported for the Serbian CLL patients (10.1% *versus* 15,3%, respectively), in the only previous published study of this kind from Western Balkans.

Chronic lymphocytic leukemia and related disorders - Clinical

PB1776

LAMBDA LIGHT CHAIN RESTRICTION – USEFUL FOR HAIRY CELL LEUKEMIA PROGNOSTICATION?

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Background: Hairy cell leukemia (HCL) patients have near-normal life expectancies since the introduction of purine nucleoside analogues. However, HCL remains a chronic, often relapsing disease in which maximizing treatment-free survival (TFS) is the main goal.
Aims: Prognostication is not standardized in HCL, emphasizing the relevance of the characterization of HCL populations.
Methods: We retrospectively analysed 40 patients (90% men), diagnosed between 1997 and 2016, with a median follow-up of 6 years.
Results: At presentation, the median age was 58 years and 69% of patients were symptomatic - fatigue (53%), B symptoms (50%), bleeding (14%), abdominal discomfort (6%) and severe infection (22%). The commonest cytopenia was thrombocytopenia (70%), with median platelet count being 66x10^9/L. Monocyte counts below 0.1x10^9/L were observed in 61% of the patients. Splenomegaly was observed in 83% of the patients and 21% had abdominal lymphadenopathies. The majority of the patients (88%) was treated with cladribine in first line, achieving an overall response (OR) rate of 100% and a complete response (CR) rate of 38%, of which 67% were classified as minimal residual disease (MRD)-negative CR. Retreatment was required in 33% of the patients, of which the majority received cladribine. The median time-to-next-treatment (TNT) from first to second line was 3 years. The OR rate for second-line treatment was 91%, 50% achieving CR, of which 33% were classified as MRD-negative CR. Only 5% of the patients required further treatment lines. Even the presence of scarce hairy cells in the bone marrow precluded classification of response as CR. This might have contributed to the low CR levels observed in our patients. As post-treatment bone marrow biopsies were available in only 24 patients, response analysis was restricted to these patients. All of these 24 patients had bone marrow fibrosis at diagnosis, which reverted when and in whom first CR was obtained. Median overall survival (OS) was not reached and, at 10 years, the OS was 90%. Four deaths occurred, all unrelated to HCL. Regarding prognostication, a trend to a longer TFS, albeit no statistically significant, was observed in patients achieving CR (namely MRD negative) and without thrombocytopenia at presentation. Excitingly, the 61% of patients with kappa (k) light-chain restriction (LCR) displayed a significantly higher TFS than the 39% with lambda (λ) LCR (p 0.034, Wilcoxon-Gehan test). To the best of our knowledge, there are no published reports on prognostic value of LCR in HCL (Figure 1).

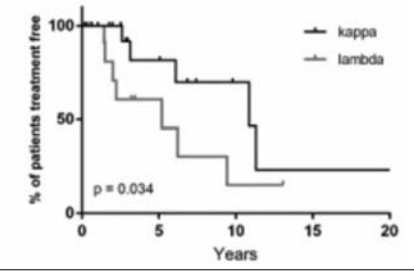

Figure 1.

Summary/Conclusions: If multicentre studies corroborate our findings, LCR may be of use in the prognostication/risk stratification of HCL. Similarly with multiple myeloma and other hematological malignancies, lambda (λ) LCR appears to correlate with worse prognosis, leading to a shorter TFS.

PB1777

CLINICAL EFFICACY AND LONG-TERM OUTCOMES OF SPLENECTOMY IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chronic lymphocytic leukemia (CLL) is often accompanied by splenomegaly. In certain cases the spleen may enlarge to a giant size, causing abdominal discomfort, regional portal hypertension, and becomes a place of malignant cells concentration. In 2.3-4.3% of cases CLL may be complicated by autoimmune cytopenias (autoimmune hemolytic anemia (AIHA), immune thrombocytopenia (ITP), Evans-Fisher syndrome). Accordingly, the effectiveness of steroid and chemotherapy in such cases may be impaired, raising the question of splenectomy advisability.
Aims: To analyze splenectomy effectiveness in patients with CLL.
Methods: Splenectomy was performed in 41 patients with CLL, 12 of which were patients with CLL and ITP, 9 with CLL and warm type AIHA, 5 patients with CLL and Evans-Fisher syndrome, along with 15 CLL patients without immune disorders. Among the patients there were 26 males and 15 females. Indications to splenectomy were following: massive splenomegaly with abdominal discomfort, immune cytopenia and regional portal hypertension. In one female patient the surgical intervention was performed urgently due to spontaneous splenic rupture and acute intra-abdominal bleeding.
Results: Splenectomy was effective in 37 patients (90.2%): abdominal discomfort disappeared, hemolysis stopped and hemoglobin levels normalized or increased, platelets numbers normalized or increased. Splenectomy was ineffective in 3 patients with CLL associated with ITP: amid elimination of abdominal discomfort the platelets number did not increase significantly (2 patients), while in 1 patient despite increase in platelets number leukemia progression was observed. One (2.4%) patient with CLL and AIHA died on 3rd day after surgery because of acute adrenal insufficiency. The analysis of late effects of splenectomy in patients with CLL showed that average life expectancy after the surgery comprised 111.6 months within observation period between 11 and 277 months. In patients with CLL with immune cytopenias the average life expectancy after surgery was shorter and equal to 60.7 months within the observation period between 2 and 361 months.
Summary/Conclusions: Splenectomy remains an effective method of treatment of patients with CLL accompanied by severe splenomegaly and immune cytopenia. Long-term results of splenectomy in patients with CLL without cytopenia are better than in patients with CLL and cytopenias. Aggressive hemolysis, large spleen covered in perisplenic adhesions, amid portal hypertension and thrombocytopenia are considered to be special surgical risk factors in this patients.

PB1778

MONOCLONAL B-CELL LYMPHOCYTOSIS IN THAI POPULATION: PREVALENCE AND IMMONOPHENOTYPIC CHARACTERISTICS

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Background: Monoclonal B-cell lymphocytosis (MBL) is characterized by the presence of <5X10^9 clonal B-cells/L in peripheral blood (PB) in otherwise healthy subjects, in the absence of symptoms and signs of a B-cell lymphoproliferative disorder (LPD). MBL is considered a precursor to chronic lymphocytic leukemia (CLL) and other B-cell malignancies.
Aims: To study the immunophenotypic features and prevalence of MBL in healthy Thai individuals.
Methods: Peripheral blood (PB) samples from 616 healthy Thai individuals (313 female), 18-80 year-old with normal lymphocyte counts were immunophenotyped using high-sensitive flow cytometry, based on 5-color staining and the screening for >5 × 10^6 total PB leukocytes. The initial PB samples were screened for clonal B cells using MultiMix Triple-Color Reagent (Kappa Light Chains/FITC, Lamba Light Chains/RPE and CD19/RPE-Cy5). In those cases in which a clonal B cell population was detected by imbalanced of sIgK:sIgL ratio of >3:1 or <1:3, were further tested for CD5, CD23, CD20 and CD79a expression.
Results: Of total 616 subjects, MBL was found in 8 cases (1.2%) including 3 and 5 female and male cases respectively. Among 40 years or older, MBL was found in 5 out of 448 cases (1.1%). Compared with non-MBL group, subjects in MBL group were significantly older (median age of 78 years *versus* 50 years; p=0.01) and had a significant higher number of absolute and B-lymphocyte count (median 3.1 *versus* 1.6 X 10^9/L; p=0.03 and 0.35 *versus* 0.16 X10^9/L; p=0.02, respectively) while the median white blood cell count was not different between 2 groups. Also, there were more subjects in MBL group who had family history of lymphoproliferative diseases (LPD; 37% *vs* 0%; p<0.01) and influenza vaccination within 2 years (50% *vs* 8.7%; p=0.003). Among 8 cases with MBL clone, 6 cases had low-count MBL (<0.5X10^9 clonal B-cells/L) while only 2 cases had high-count MBL (>0.5X10^9 clonal B-cells/L). All 8 cases had persistent positivity of MBL clone after tested was repeated within 3 months after the initial test. In the follow up test, only 1 case with initial high-count MBL had decrease number of B cell clone and became low count MBL. There was not significant different in age between subjects in low and high-count MBL group. Six cases had typical CLL phenotype MBL clone (CD5+, CD23+, CD20+/dim and light chain restriction). Whereas 1 case had atypical CLL phenotype MBL (CD5+, CD20+ and light chain restriction but CD23-) and 1 case had non-CLL phenotype MBL (CD20+ but CD5-). In univariate analysis, age (RR 4.19; 95%CI 1.0-17.7; p=0.049), absolute lymphocyte count (RR 2.76; 95%CI 1.01-4.87; p=0.047), family history of LPD (RR 122; 95%CI 51.1-293.4; p<0.001) and

influenza vaccination (RR 10.47; 95%CI 2.54-43.07; p=0.003) were associated with increase risk of developing MBL. After adjusted for age, only history of influenza vaccination and family history of LPD were an independent risk factor for developing MBL with age adjusted RR of 9.75 (95%CI 2.3-40.5; p=0.002) and 92 (95%CI 56.3-149.5; p<0.001), respectively.
Summary/Conclusions: MBL prevalence in Thai population is much lower than previously reported. It more frequent in elderlies and associated with family history of LPD and influenza vaccination. Although uncommon, the presence of high-count MBL warrants further investigations to define the biological and clinical significance in term of LPD transformation and long-term survival.

PB1779

SPONTANEOUS CLINICAL REGRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA: CLINICAL AND BIOLOGIC FEATURES OF 9 CASES FROM THE ERIC REGISTRY

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Background: Spontaneous clinical regression in chronic lymphocytic leukemia (CLL) is rare (1% per year). We previously reported on the clinico-biologic features of 9 Binet stage A CLL patients from our Center in Rome who experienced a persistent spontaneous clinical regression of the disease at a median time of 11 years from diagnosis, maintained after 5 more years of follow-up. The lymphocyte count at CLL regression was 3.16 x 10^9/L (1.3-4.9), with a persistent small CLL clone (CD19+/CD5+/CD23/light chain restricted: 44%, range 5-60%). Biologic features included negative CD38, mutated IGHV, often with VH3-30 and Vk4-1 usage, and a distinctive gene expression profile.
Aims: To conduct a retrospective collection of clinical data and basic biologic information on CLL spontaneous regressions and to make them accessible for future research.
Methods: A registry of spontaneous CLL regressions (absence of lymphadenopathy, splenomegaly or constitutional symptoms, peripheral blood (PB) lymphocytes <4 x 10^9/L, in the absence of any previous treatment) was launched within the ERIC consortium.
Results: So far, 9 CLL patients showing a spontaneous regression have been reported and 8 have been formally registered, 7 from Italy and 2 from Sweden. Six were males and 3 females, with a median age of 57 years at diagnosis (range 51-82), stage Binet/Rai A/0 in 6, A/I in 2 and B/II in 1. The median lymphocyte count at diagnosis was 14.1 x 10^9/L (5.3-51.9). Biologic features included: mutated IGHV in 8/8 with VH3-30 (2), VH3-21, VH3-15, VH3-23, VH4-31, VH4-34, VH4-59; CD38 <30% in 6/6; ZAP70 <20% in 4/6; FISH (7 cases): del13q in 4, negative in 3, +12 in 1 case. No patient had undergone treatment, except for one diagnosed in 2009 who received FCR for disease progression in 2013 (lymphocytes 107 x 10^9/L), obtained a PR and 18 months later developed a Richter's syndrome - a diffuse large B-cell lymphoma clonally unrelated to CLL - with the concomitant disappearance of the CLL clone from the PB and bone marrow, that has lasted up to January 2017 (lymphocytes 3.5 x 10^9/L, CLL 0.035 x 10^9/L). An additional case diagnosed in 2013 (stage A/I, lymphocytes 37.2 x 10^9/L) reached the highest lymphocyte count 19 months later (91.2 x 10^9/L) and subsequently started a spontaneous reduction in lymphocytosis down to 39.6 x 10^9/L in 2015 and to 8.9 x 10^9/L in January 2017 in stage A/0, indicative of a partial but ongoing CLL regression. Excluding the latter cases, in the other 7, all in stage A/0, the highest lymphocyte count was 16.0 x 10^9/L (8.9-76.0), the lowest at the last follow-up was 2.8 x 10^9/L (1.8-4.4), with 0.66 x 10^9/L CLL cells (0.085-3.0) in the 4 evaluable cases. The median time from diagnosis to clinical regression was 4 years (range 2-17) and this has been maintained for 2 further years (range 0.5-7). One of these cases (mutated VH3-21, +12) seems the most dramatic: in 2008 at diagnosis, the lymphocytes were 51.9 x 10^9/L, in 2009 a peak at 76.0 x 10^9/L was recorded; in 2011, when the CLL regression started, the patient underwent several mild viral upper respiratory infections; the CLL complete regression (1.8 x 10^9/L) persists up to the last follow-up. In 5/9 cases one event - mild viral infections, a cerebral hemorrhage, a stroke, a pelvis fracture and a Richter's syndrome - occurred before the spontaneous regression, but no relevant drug intake was recorded.
Summary/Conclusions: Clinicians should be aware that spontaneous regression is a possibility, albeit infrequent, in the natural history of CLL. The collection and study of such cases within the ERIC registry may shed light on mechanisms leading to spontaneous regression and critical pathways in immunosurveillance in CLL.

PB1780

CLINICAL AND LABORATORY CHARACTERIZATION OF PLATELET DYSFUNCTION DURING IBRUTINIB TREATMENT IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA. MONOCENTRIC EXPERIENCE

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Background: Ibrutinib (IBR) is a potent and irreversible inhibitor of Bruton's tyrosine kinase (Btk) approved by FDA for the treatment of patients (pts) affected by chronic lymphocytic leukemia (CLL) with del 17p or *TP53* mutation or for pts with relapsed/refractory (R/R) CLL. IBR is associated with bleeding events usually mild (Common Toxicity Criteria (CTC) grades 1-2), rarely severe (grade 3-4). A defect of platelet function, namely an inhibition of Btk-mediated signaling by platelet glycoproteins (GP) GPVI and GPIb, has been hypothesized to cause these bleedings. IBR associated bleedings and platelet dysfunction may be relevant in CLL pts who are usually elderly and with comorbidities requiring antithombotic therapies.
Aims: To investigate and characterize the effect of IBR on platelet function in pts with CLL.
Methods: We enrolled from May 2014 to December 2016 twenty pts with CLL treated with orally administered 420 mg daily of IBR; 18 R/R CLL pts received IBR in monotherapy and 2 pts with previously untreated CLL received IBR in association with anti-CD20 MoAb. Median age was 68 years (57-84); 13 pts had unmutated IgVH and 2 had 17p deletion. The median number of prior therapies in R/R CLL pts was 3 (2-7). Five pts discontinued IBR therapy: 2 for Richter's transformation, 1 for progressive CLL, 1 underwent allogeneic HSCT, 1 for heart disease. The platelet function was studied before and during IBR by light transmission aggregometry (LTA) using platelet-rich plasma and the following agonists: ADP 2-4 uM, PAR1-AP 25 uM, Collagen 10-3.3-2 ug /mL, arachidonic acid 1 mM, ristocetin 0.6-1.2 mg/mL. Also measurements of von Willebrand factor antigen (vWF:Ag) and ristocetin cofactor activities (RiCo) by chemiluminescent immunoassay were performed. All pts had measurements of the platelet function at the baseline and after 1, 3, 6 months initiation of IBR and then every 3 months up to 24 months. Median observation period was 9 months. No patient received concomitant antiplatelet or anticoagulation therapy.
Results: Nineteen pts achieved a partial response and an increase of hemoglobin and platelet count. We recorded CTC grade 1 or 2 bleedings (bruising, petechiae, conjunctival and retinal hemorrhage, rectal bleeding) in 15 pts; no patient needed IBR interruption or dose reduction. All pts displayed severe impairment of collagen induced aggregation upon IBR. Reduction of maximal aggregation (35.6+/- 32% *vs* 70.6+/- 21% baseline) and prolongation of the lag phase (261+/- 54 sec *vs* 72+/- 26.8 sec baseline) by 2 ug/mL collagen was measured in all pts during IBR. In 10 pts a significant improvement of the aggregation by 2 uM ADP (71+/- 31.8% *vs* basal 48.6+/- 31%) and 4 uM ADP (84+/- 11% *vs* basal 64+/- 25%) was found during IBR. The aggregation by 25 uM PAR1-AP, 1.2 mg/ml ristocetin and 1 mM arachidonic acid was unchanged before and under IBR. Finally, in 9 pts the vWF:Ag and RiCo were high at the onset of the disease (163+/- 59.8% and 181.6+/- 82.5%) and reduced up to normal values under IBR (118+/- 71% and 145+/- 65%).
Summary/Conclusions: Our study showed minor bleedings in pts treated with IBR. A severe impairment of collagen-induced aggregation was caused by IBR, which was counteracted by amelioration of ADP-induced aggregation, that could explain, at least partially, the mild clinical phenotype in treated pts. The assessment of platelet function in IBR treated CLL pts could help to predict and monitor the bleeding risk, and to guide pts through invasive procedures. In addition, pts under anticoagulant or antiplatelet treatment might need be carefully monitored by clinical and laboratory evaluation.

PB1781

HAIRY CELL LEUKEMIA :A SUMMARY OF CLINICAL DATA ON 202 PATIENTS AND THE RESULTS OF THERAPY WITH CLADRIBINE IN ISRAEL

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Background: Hairy cell leukemia (HCL) accounts for approximately 2% of all leukemias and is associated with pancytopenia, splenomegaly, and recurrent infections. Therapy with the purine analogues cladribine (2CdA) or pentostatin (2'deoxycoformycin), has been most effective and both agents have achieved equivalent results in HCL. In this regard cladribine given as a single course, achieves a high response rate. Several alternative dosing schedules have been reported for 2CdA including subcutaneous (SC) or intravenous (IV) delivery, either as a "fixed daily dose" or "weight based dose" for 5 or 7 days. Seeing that excellent results are obtained using 2CDA in all schedules used, it now seems very important to focus on reducing therapy induced toxicity, related mostly to development of neutropenia, immunosupression and severe infections.
Aims: In this retrospective study, we have summarized the Israeli experience with HCL over the past 30 years, and analyzed demographic data, relevant laboratory and clinical parameters with special emphasis on outcome after first line treatment with cladribine.
Methods: We collected retrospective data on patients with HCL from 12 medical centers in Israel, followed and treated during 1985-2015. The study was approved l by local institutional IRBs of each medical center
Results: Data from the medical records of 202 patients with HCL was summarized. Mean follow up was 7.5 years (0.1-40), with a 5 and 10 years' overall survival of 96% and 90.62% respectively. The median age at diagnosis was 53 years, and most (81.77%). were males. In terms of ethnicity: 88.3% of patients were Jews with (52.2% Ashkenazi and , 36.1% Sephardic Jews) while d 11.7% were Arab, Druz or others. First line therapy with cladribine was given to 159 patients (80.71%); other therapies 9.14%, while 1.1% did not receive any treatment. The median time from HCL diagnosis to treatment with 2CdA was 5.9 years. IV therapy was given to 62% of patients and 38% received it SC. Complete remission rates, progression-free survival and overall survival were not significantly different between the two schedules. In univariate analysis: Sex, ethnicity, dose, patient weight, and treatment duration (5-7 days) had no impact on outcome, but patients older >65 years had a shorter survival. Infectious complications requiring hospitalization was reported in 50.3% of all treated patients (54%, post IV and 47% post SC delivery: p=0.4). Median days of hospitalization were 8 for both groups (0-45) (p=0.55), and the length of NADIR was 18 and 20 days for IV and SC delivery respectively (p=0.33).
Summary/Conclusions: This study is the first comprehensive summary of the national Israeli experience involving a large cohort of HCL patients with long follow up. These results serve as validation of previous reports relating to HCL and confirm that the excellent outcome achieved after a single course of treatment with 2CdA is independent of schedule and method of drug delivery. In addition, patient ethnicity was insignificant

PB1782

CHRONIC LYMPHOCYTIC LEUKEMIA: CHANGES IN CLINICAL STAGE DISCRIMINATE PATIENTS WITH DIFFERENT OUTCOME WITHIN THE IWCLL PARTIAL RESPONSE CATEGORY

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Background: Over the last decades, progress in chronic lymphocytic leukemia (CLL) treatment has resulted in an impressive increase in overall survival (OS). In CLL, as in other tumors, response to therapy overcomes negative prognostic factors and is the most important predictor of survival. Clinical stages reflect tumor load and correlate with OS both at diagnosis and over the course of the disease (Rai et al, Blood 1975).
Aims: To determine whether changes in clinical stage discriminate patients with different outcome within IWCLL response categories, particularly the heterogenous partial response (PR) group (Hallek et al, Blood 2008).
Methods: Two-hundred twenty-nine patients with CLL were retrospectively evaluated. Median follow-up was 91 months (range, 2-390). CLL diagnosis was based on IWCLL criteria. Endpoints were time to next treatment (TTT) and OS. TTT and OS curves were estimated by the Kaplan-Meier method and differences were evaluated with the log-rank test. Response to therapy was determined according to IWCLL recommendations and by changes in clinical stage A landmark analysis was performed in ninety-two patients in whom a PR was achieved at any time during the course of the disease, using the time when a PR was achieved as "time 0".
Results: From the whole series of 229 patients, those who achieved a better IWCLL degree of response after first line of therapy had a better OS than those with an inferior response (p<0.001). With a median follow up of 91 months (range, 2-390), the median survival in patients who achieved complete remission (CR) was 214 (95% CI: 123-305) *vs* 134 (95% CI: 79-189) and 91 (95% CI: 84-98) months in those who achieved PR and failed to therapy, respectively (Figure 1.A). Among patients in PR (n=66), after a median follow-up of 42.5 months (range 1-201), those patients with stage A disease at the time of response evaluation (PR Binet A) had significantly better outcome than those whose stage was Binet B/C (median survival 63 *vs* 43 months; p= 0.047). Interestingly, when the analysis was restricted to response assessment after first line therapy (n=229), patients who achieved PR Binet A did not have significant differences in OS compared to those patients who were in CR (median survival were 164 and 214 months respectively; p<0.001); on the contrary, patients in PR Binet B/C had a similar outcome than those who did not respond to treatment (median survival 81 and 91 months respectively) (Figure 1.B). Similar results were observed in the outcome of patients with PR subclassified according to Rai clinical stage.

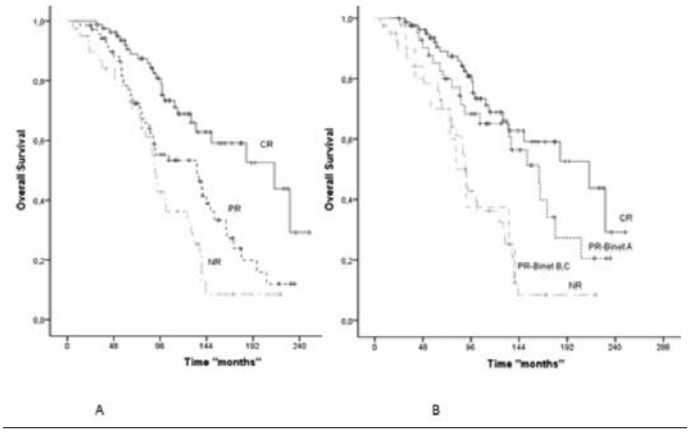

Figure 1.

Summary/Conclusions: Changes in clinical stage provide reliable information on the degree of response to therapy in patients with CLL, particularly those in the IWCLL PR category. This study supports the use of clinical stages as a complementary and simple tool to assess response in patients with CLL, both at the end and over the course of treatment.

PB1783

INCIDENCE OF THYROID GLAND DISORDERS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Frequency of autoimmune complications like immune anemia or immune thrombocytopenia has increased in patients with chronic lymphocytic leukemia (CLL). However, there is no data in the literature investigating the relation of the other autoimmune disorders including thyroid gland diseases with CLL.
Aims: We aimed to investigate the presence, features and frequencies of thyroid disorders in patients with CLL.
Methods: Thyroid function tests, thyroid autoantibodies (antithyroglobulin antibody [anti-TG], antithyroid peroxidase antibody [anti-TPO]), thyroid ultrasonographies (USG) and scintigraphies of CLL patients were performed. Demographic data, Rai-stages, and established thyroid disorders were recorded.
Results: One hundred CLL patients were included into the study (65 male, mean age was 62,9±10,4). Free T3 (fT3) was within normal limits in 96 cases (96%), was low in 2 cases (2%), was high in 2 cases (2%); free T4 (fT4) was normal within normal limits in 89 cases (89%), was low in 7 cases (7%), was high in 4 cases (4%).TSH was within normal limits in 90 cases (90%), was low in 7 cases (7%), was high in 3 cases (3%). Anti-TPO and anti-TG were positive in 10 cases (11.8%) and in 18 cases (21.2%), respectively. While USG was normal in 36 cases, multinodular goiter (MNG) in 21, chronic thyroiditis in 20, MNG associated with thyroiditis in 10, uninodular goiter (UNG) in 8, UNG associated with thyroiditis in 4, and diffuse goiter in 1 case were determined by USG. Toxic adenoma in 3 cases, toxic MNG in 2 cases, and thyroiditis in 1 case were determined in 6 patients in whom thyroid scintigraphy was performed for hyperthyroidism. After evaluation of all the tests; while no thyroid disease was determined in 33 of the cases (33%), MNG in 25 (25%), thyroiditis according to the results of USG in 12 (12%), UNG in 11 (11%), Hashimoto thyroiditis in 9 (9%), toxic MNG in 3 (3%), subclinical hyperthyroidism in 3 (3%) cases, subclinical hypothyroidism in 1 case (1%), lymphocytic thyroiditis in 1 case (1%), toxic UNG in 1 case (1%), and euthyroid sickness syndrome in 1 case (1%) were determined. The patients were divided into 2 groups according to their Rai-stages and ages. Accordingly; Rai-stage 0 - I - II (n=80) and Rai-stage III - IV (n=20), <65 years (n=56) and ≥65 years (n=44). Anti-TPO positivity was similar in 2 Rai-stages groups and in both sexes (p=0,999, p=0,167, respectively). Anti-TG positivity was also similar in 2 Rai-stages groups and in both sexes (p=0,507, p=0,223, respectively). However, anti-TPO positivity was statistically different between age groups; anti-TPO was positive in 3 patients in <65 years old age group, and was positive in 7 patients in ≥65 years old age group (p=0.049). Anti-TG was positive in 7 patients in <65 years old age group,

and was positive in 11 patients in ≥65 years old age group (p=0.053). There was not a statistically difference in thyroid function tests according to the Rai-stages, ages and sexes.
Summary/Conclusions: We determined that incidence of hypothyroidism or hyperthyroidism associated with all reasons do not increase in patients with CLL when compared with general population. However, we also determined that the incidence of Hashimoto thyroiditis was higher than general population (incidence of Hashimoto thyroiditis in general population is 2-5%). Anti-TG positivity was also higher than general population (positivity of anti-TG in general population is 5-20%). In addition, the positivity of 2 antibodies increased with advanced ages. Patients with CLL -especially the elderly cases- in both sexes and in all Rai-stages should be examined for thyroid gland disorders, mainly for Hashimoto thyroiditis.

PB1784

CLINICAL-BIOLOGICAL CHARACTERISTICS, TREATMENT OUTCOME AND SURVIVAL OF SMALL LYMPHOCYTIC LYMPHOMA PATIENTS: A REAL-LIFE EXPERIENCE

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Background: Studies of B-SLL published to date have included heterogeneous groups of patients(pts) and did not use modern diagnostic criteria, or included pts who had in fact chronic lymphocytic leukemia. Outside the context of clinical trials, SLL pts are treated heterogeneously and thus there are no data concerning the impact of different treatment approaches on response and survival. In the updated WHO classification it is pointed out that there are a subset of cases with lymph node(LN) involvement by SLL in which proliferation centers(PCs) were not observed and pts in whom lymphadenopathy was <1.5 cm showing a better prognosis
Aims: To: a)record clinical, biological features and treatment strategy in a series of SLL pts diagnosed in our centers b)correlate clinicopathological characteristics and treatment with response and survival c)detect possible differences in terms of response and survival between SLL pts according to LN characteristics (size of LN and presence of PCs)
Methods: Pts diagnosed with SLL from 2007 up to now fulfilling the diagnostic criteria of SLL according to the 2008 WHO classification were included. Clinical and biological data were recorded at diagnosis as well as treatment related variables, such as type of treatment, response and patient survival. Moreover, LN features such as the size, and the presence of PCs were also studied. PCs were evaluated in hematoxylin and eosin sections and defined as pale areas containing prolymphocytes and paraimmunoblasts, surrounded by a dark background of small lymphocytes.
Results: 47 pts were analysed. Pts' median age was 69y (range, 40–87) with no gender predominance (24male/23female). According to Binet staging system 12, 19 and 9 were classified as A, B and C stage respectively while according to Ann Arbor system 42(89%) had advanced disease stage. 11 pts presented with bulky lymphadenopathy, 11 had splenomegaly and 4 had B-symptoms. LN biopsies were performed in 37 out of 47 pts. All pts underwent bone marrow (BM) biopsy with a median BM infiltration of 45% (0-97%). PCs were identified in 19 out of 24 pts in whom data were available, while 31 pts were presented with LN >1,5 cm as measured in CT. The estimated 10 y -OS was 60% while median TFS was 5,3 mos. Age and ECOG performance status were the only parameters that were statistically significant in terms of survival (p=0,019 and p=0,013 respectively). Pts with LN<1,5cm and pts in whom there were no detectable PCs tended to have better survival. 24 pts (51%) were in need of therapy and most of them were treated with mild immunochemotherapy [13 received Rituximab(R)-Chlorambucil(Chl), 3 RCVP, 2FCR, 1RBendamustine, 2 Chl, 2R as monotherapy and 1 received corticosteroids].23 pts were assessable for response and among them 4 entered CR,17 PR and one had stable disease.
Summary/Conclusions: Outside the context of clinical trials SLL pts were treated mostly with lymphoma immunochemotherapeutic protocols while mild treatment approaches resulted in significant responses. LN features such as size and presence of PCs tended to have prognostic significance. Further analysis in larger series of pts is on the way.

PB1785

HEMINSIGHT TO ASSESS PATIENT REPORTED OUTCOMES O F PATIENTS AFFECTED BY CHRONIC LYMPHOCYTIC LEUKEMIA IN DAILY CLINICAL PRACTICE

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Background: Chronic lymphocytic leukaemia (CLL) is the most common leukemia in Western Countries, with a median age at diagnosis between 67 and 72 years. The therapeutic landscape of CLL is changing rapidly with the advent of small molecules acting as B-Cell Receptor (BCR) signaling inhibitors. In this setting, long term oral therapy may lead to the reduction in compliance, with a possible impact on effectiveness. Moreover, long-term follow-up may highlight complications, such as drug-related adverse events that, together with the disease itself, may impact quality of life (QoL). Patient Reported Outcomes (PROs) in daily clinical practice is a resource-intensive procedure and may be affected by low adherence, risk of recall bias and difficulties in establishing reproducible procedures. HemInsight, a project conceived in 2010 for myeloproliferative neoplasms in haematological centres in Denmark, enables patients to periodically submit PROs online to be combined to the medical records.
Aims: HemInsight was implemented at our Centre to collect PROs from CLL patients in daily practice.
Methods: HemInsight incorporated the EORTC QLQ-C30, EORTC QLQ-CLL 16, SF-36, and the eight-item Morisky Medication Adherence Scale (MMAS-8) questionnaires to collect PROs and their changes during various stages of CLL (diagnosis - progression – treatment). PRO assessments were scheduled for the patients who received regular reminders by email to complete the tasks. The following measurements will be assessed: system attraction (percentage of CLL patients adhering to the project); patient compliance in filling out questionnaires; system efficiency (number of alerts related to QoL worsening and number of questionnaires not submitted) and system effectiveness (significant differences in changes in QoL scores from diagnosis to response/relapse, changes of therapeutic approach/action following an alert, changes in adherence of therapy).
Results: At the time of the present report, 74 patients with a CLL diagnosis have been enrolled, 15 of whom were newly diagnosed. Fourteen patients underwent cytoreductive therapy and 2 are under treatment with novel oral drugs. System attraction: the study was proposed to 91 consecutive patients, independently of age, level of education and internet accessibility, and 72.5% of patients agreed to participate to the study. The main reason of refusal was older age and scant internet/technology knowledge. In 3 cases with no access to internet, but with interest to participate in the project, the questionnaires were administrated through tablet, before the scheduled visit, by a dedicated nurse. Patient compliance: a global response of 58.2% was observed; 48 patients responded at least once and 23 at all scheduled time points. In each case, all questionnaires were fully completed. At this timepoint we cannot yet evaluate system effectiveness as the study is still ongoing. However, *ad interim* data (Table 1) suggest that patients who interrupt questionnaires fulfilling are those with younger age, more intense working activity and experiencing no changes in disease status (*e.g.* untreated cases or those in remission). In particular, patients who were under treatment during the questionnaire administration period, showed a higher adherence compared to those in follow up, both previously treated or not (80% *versus* 26%, p<0.05).

Table 1.

PROs adherence according to treatment status in CLL patients

Adherence to PROs	Treatment naive	Previously treated	Under treatment	Total
No N(%)	17 (74)	6 (26)	0 (0)	23
Partial N(%)	19 (76)	5 (20)	1 (4)	25
Full N(%)	13 (59)*	5 (23)*	4 (18)*	22

PROs: patients reported outcome. *p<0.05

Summary/Conclusions: In conclusion, HemInsight is a useful tool for QoL evaluation in CLL patients. Provisional data suggest a higher compliance of those patients who feel that they need a closer contact with the clinician, both for individual disposition or disease status.

PB1786

HEALTHCARE COST OF MEDICARE PATIENTS WITH PREVIOUSLY TREATED CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chronic lymphocytic leukemia (CLL) is the most prevalent form of leukemia in adults in western countries, accounting for 20% to 30% of all leukemia cases. CLL affects mainly elderly patients, with a median age at the time of diagnosis reported to be 71 years. Although CLL is not curable, disease symptoms and progression may generally be controlled with adequate pharmacologic treatments. Bendamustine-based regimens have long time been used in the management of CLL patients but few studies have analyzed the comorbidity- and/or adverse event (CAE)-related healthcare costs in elderly patients receiving these regimens in a real-world setting.
Aims: To describe all-cause and CAE-related healthcare costs of elderly patients with CLL treated with a bendamustine-based regimen in second or later lines of therapy in a real-world setting.
Methods: A retrospective cross-sectional cohort study design was used. Adult patients who received a bendamustine-based regimen in second or later lines of therapy on or after January 2010 were identified from the Medicare Limited Data Set (LDS) 5% Standard Analytic Files (data availability: 1999–2014). The index date was defined as the initiation date for the first of the studied bendamustine-based regimens. Selected patients were required to be continuously enrolled in their Medicare plan for ≥6 months before and ≥3 months after the index date – unless the patient died during the first 3 months after the index date. Patient cohorts were determined based on the treatment initiated on the index date (index treatment); the two most prevalent bendamustine-based regimens were analyzed, *i.e.*, (1) bendamustine and rituximab in combination (BR cohort) and (2) bendamustine monotherapy (bendamustine cohort). Healthcare costs, including inpatient, emergency room, outpatients and CLL-drug costs, incurred while treated with the index treatment were described for each cohort. For each medical cost component, all-cause and CAE-related costs were summarized. Healthcare costs were adjusted for inflation (2016 USD) and reported per-patient-per-month (PPPM).
Results: A total of 275 patients were included in the BR cohort and a total of 100 patients in the bendamustine cohort. Most patients (61,8% in the BR cohort and 65,0% in the bendamustine cohort) were male and the mean age was approximately 75 years old. During the 6 months prior to the index date, patients in the BR and bendamustine cohorts were similar in terms of commorbidity profile; mean Charlson comorbidity index was 3.53 in the BR cohort *versus* 3.66 in the bendamustine cohort (p=0.580). During treatment, total all-cause healthcare costs were $14,520 PPPM for the BR cohort and $13,125 PPPM for the bendamustine cohort – outpatient costs (mainly driven by CLL-drug costs) represented the largest cost component. CAE costs accounted for a relatively large portion of the total all-cause healthcare costs; 58.3% for the BR cohort and 66.9% for the bendamustine cohort.
Summary/Conclusions: In this population of elderly patients previously treated for CLL, healthcare costs incurred during relapsed treatment with bendamustine-based regimens were high and a large portion of the costs were driven by comorbidity and/or adverse event-related costs. Results also suggest that the addition of rituximab to bendamustine does not appear to be a major cost factor.

PB1787

THE ROLE OF MAINTENANCE THERAPY IN THE TREATMENT OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: The inclusion in the treatment program of new drugs (including new monoclonal antibodies and targeted therapies) allowed the majority of patients with chronic lymphocytic leukemia (CLL) to achieve disease remission (complete or partial) after combined therapy. So, at now, the urgent task is long-term preservation and the deepening of the therapeutic response, if it is possible. This problem can be solved by intensification of therapy (including autologous transplantation of hematopoietic stem cells) or maintenance therapy (MT).
Aims: To estimate the importance of maintenance therapy in the treatment of patients with CLL.
Methods: The study included 198 patients. Male to female ratio - 1.3:1. We have used NCI revised guidelines (Hallek M, *et al.*, 2008) for treatment initiation, assessment of response and minimal residual disease (MRD). Induction chemotherapy was conducted under the following programs: RB, FC, RFC, R-CHOP, Ibrutinib-RB, Ibrutinib-R. Evaluation of MRD was performed using 5-color flow cytometry of the bone marrow cells. The maintenance therapy was conducted 144 (72.7%) patients: Rituximab 500 mg/m2 intravenously every 8 weeks (n=116) for 2 years; Ibrutinib 420 mg, orally, daily (n=28) continuously. The remaining patients (n=54) were under dynamic observation without therapy.
Results: The increasing the depth of response (from partial (PR) to complete remission (CR)) was observed only in group of patients receiving MT – 10.4% (15/144) (p=0.013). The frequency of increase the depth of remission in the patients treated with MT of Ibrutinib was 28.6% (8/28), MT of Rituximab – 6.0% (7/144) (p=0.0005). The medians of PFS and duration of response were a longer in the patients with MT *versus* in the patients without MT: PFS – 48 months and 37 months, respectively (p=0.03); duration of response – 44.0 months and 25.5 months, respectively (p=0.0006). The median of duration of response in the patients with MT of Ibrutinib was not reached, in the patients with MT of Rituximab – 41.9 month, in the patient without MT – 25.5 month (p=0.004). The frequency of relapses in the group of patients with MT was 39.6% (57/144), in the group of patients without MT – 66.7% (36/54) (p=0.0007). Recurrence of the disease occurred more frequently in the group of patients treated with MT of Rituximab, compared with Ibrutinib: 45.7% (53/116) and 14.3% (4/28), respectively (p=0.002). The median duration of observation in the group with rituximab was 22 months, while in the group with Ibrutinib – 11 months. MRD was not detected after 6-12 months of MT in 23.5% (12/51) had previously MRD-positive patients. Among patients with MRD-negative CR relapse is less common than in patients with MRD-positive CR – 20.0% (4/20) *versus* 62.5% (10/16), respectively (p=0.009). Significant differences in the incidence of infectious complications between patients with MT and without of MT were not detected (p>0.05) (Figure 1).

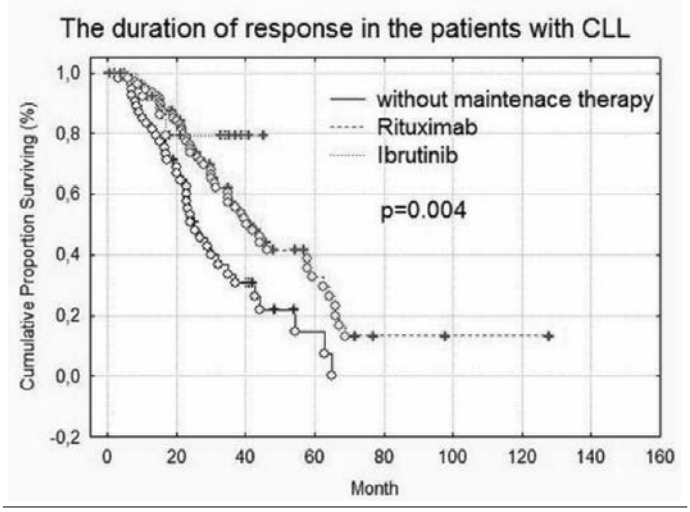

Figure 1.

Summary/Conclusions: The conducting of MT patients with CLL allows to achieve increasing the depth achieved remission and increase the duration of its preservation. MT may be a means of control over the minimal residual disease and the method of its eradication

PB1788

MULTISITE PERFORMANCE EVALUATION STUDY OF THE BD ONEFLOW LYMPHOID SCREENING TUBE

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Background: The BD OneFlow solution for diagnostic screening of chronic lymphoproliferative disorders (CLPDs) includes a standardized flow cytometry approach based on the EuroFlow (EF) Consortium liquid reagent system. The BD OneFlow solution enables reproducible identification and discrimination of normal from aberrant mature cell populations by combining standardized assays, setup reagents, and protocols. The BD OneFlow LST (Lymphoid Screening Tube) is intended for flow-cytometric immunophenotyping of normal and aberrant mature lymphocyte populations of B, T, and NK lineages in specimens (peripheral blood, bone marrow, and lymph node) from patients with hematological disorders. BD OneFlow LST acquisition and analysis template version 1.0 was revised to version 2.0 to include determination of lymphocytes as a percentage of total leucocytes. The FCS files from evaluable specimens of the original LST clinical trial were regressed using BD OneFlow LST template v2.0.
Aims: The objective of this study was to regress the FCS files from all the evaluable specimens previously collected using LST template v1.0 in the original clinical study to demonstrate equivalency between the investigational BD OneFlow LST system and the comparator EF liquid reagent system on a BD FACSCanto II flow cytometer with the 4-2H-2V CE-IVD configuration and LST template v2.0.
Methods: The FCS files using LST v1.0 template from the original clinical study included de-identified remnant peripheral blood (n=123), bone marrow (n=53), and lymph node (n=31) specimens from patients and healthy donors. Specimens

were collected in EDTA or heparin anticoagulants or PBS (for lymph nodes) at three external study sites. Informed consent was not required in this clinical study. All specimens in the original study were simultaneously stained with investigational BD OneFlow LST and comparator EF liquid reagents within 24 hours of collection and were acquired within 60 minutes of staining. In the current study, analyses were performed on a BD FACSCanto II instrument using LST v2.0 template with BD FACSDiva software v8.0.1. For primary endpoints, specimens were categorized as normal or follow-up needed. If follow-up was needed, specimens were categorized as B-, T-, NK-, or other-cell lineage. Overall agreement, negative agreement, and positive agreement, along with their one-sided lower 95% confidence limits, were calculated. For quantitative (percent) comparison of defined cell populations, Deming regression (slope and intercept analysis) was performed between the BD OneFlow method and the EF method.
Results: The BD OneFlow LST system compared to the EF system gave 100% (207 of 207) overall agreement (lower 95% CI: 98.6%) in delineating patients into normal (no follow-up) or follow-up, and 100% overall agreement in identifying the B-, T-, NK-, and other-cell lineages (lower 95% CI: 98.6%). There was 100% positive agreement and 100% negative agreement between BD OneFlow and EF for follow-up *vs* no-follow-up (normal) and for all cell lineages from specimens that required follow-up. Furthermore, compared to the EF system, the BD OneFlow LST system met the acceptance criteria for the quantitative assessment (Deming regression) of the defined cell populations.
Summary/Conclusions: The multisite performance evaluation of the BD OneFlow LST system and the comparator EF liquid reagent system was concordant in identifying abnormal from normal mature populations in patients with CLPDs. BD OneFlow LST is for *in Vitro* Diagnostic Use; CE Marked to the European *In Vitro* Diagnostic Medical Device Directive 98/79/EC. 23-19566-00.

PB1789

IMMUNOGLOBULIN HEAVY/LIGHT CHAIN ASSAY DETECT IMMUNE DYSREGULATION IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chronic lymphocytic Leukemia (CLL) is frequently accompanied by immune dysregulation. Hypogammaglobulinemia is the most important associated immune defect and all three classes of immunoglobulins (IgG, A and M) are involved. Recently, a novel assay for detecting heavy/light chain (hevylight) and their ratios has been described (HLC), which improves immunoglobulin detection and monitoring in plasma-cell dyscrasias by quantitating the different light chain types of each immunoglobulin class. The frequency and biological role of this assay has as yet not been studied in CLL.
Aims: To study the frequency of abnormal Heavy Light chain assay, in CLL patients.
Methods: This is an observational, multi-center study performed in collaboration with the Israeli CLL Study Group involving 10 medical centers in Israel. The cohort included patients with CLL as well as healthy volunteers**.** All patients studied had complete clinical database available and all medical records were examined and then summarized. Serum samples were analyzed for levels of: IgG1, IgG2, IgG3, IgG4. Isoforms of heavy/light chain: IgG kappa, IgG lambda, IgA kappa, IgA lambda, IgM kappa, IgM lambda and Free light chain: kappa (K) and lambda (L), ratio of K/L and calculation of ratios of monoclonal/polyclonal immunoglobulin (HLC ratio).
Results: The total cohort consisted of 126 "treatment - naïve", patients with CLL and 26 healthy volunteers**.** Median age was 64 years, 64% were males and 78% had Binet stage A, while 19% and 3% were stages B or C respectively. Significantly reduced levels of immunoglobulins associated with lambda chain (IgG-L, IgA-L and IgM-L) were identified in CLL patients compared to healthy controls (p value of 0.001, 0.005 and 0.001 respectively). Abnormal IgG-lambda values were evident in 15 patients (10%) and associated with more pronounced leucocytosis (p=0.005), higher B2mg levels (p=0.022) and the presence of 17p deletion (0.028). IgA and IgM lambda were abnormal in 38% and 56% of cases respectively compared to 8 and 9% in the controls. For IgG subclasses: both IgG2 and IgG4 levels were significantly lower in CLL patients than in healthy controls (p= 0.001 and p<0.01 respectively). In addition , IgG2 and IgG4 were also confirmed to be significantly lower in CLL patients than in controls (p<0.001), while abnormal IgG2 levels were associated with more advanced Binet stage and elevated LDH levels. Abnormal lambda FLC was observed 26 (21%) patients and only in one (4%) healthy control. Abnormal free light chain ratio (FLC) was present in 39 patients (32%), with a mean value of 4.22 (0.11-62.15) while only 1 (4%) was observed in healthy controls (mean value of 1.76 (0.25-2.3)) and was also associated with more advanced Binet stage and elevated LDH (p=0.003.).
Summary/Conclusions: CLL cells may produce light chains, or as shown here abnormal intact immunoglobulins (heavy and light chains). HLC levels were associated with advanced stage and adverse prognostic parameters. These findings lend support for the considerable potential of the HLC assay in the evaluation of clinical status in patients with CLL

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INFLUENCE OF TREATMENT ON CONCENTRATION OF CYTOKINES IN BLOOD OF PATIENTS WITH HAIRY CELL LEUKEMIA

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Background: A pathogenic role and prognostic value of cytokines in treatment of patients (pts) with hairy cell leukemia (HCL) are not finally established.
Aims: to define the concentration of cytokines such as TNFα, IL-6, sIL-2R, TGFβ1 in serum of HCL pts before and after treatment with IFNα or 2-CdA and to estimate the relationship with blood count indexes in HCL pts.
Methods: The study group consisted of 26 primary pts with the classic variant of HCL (median age - 47 years). A control group consisted of 12 healthy persons (median age - 50 years). The concentration of cytokines was measured using a validated commercial ELISA kits.
Results: Median of TNFα content in serum of HCL pts before treatment was substantially lower (3.57 pg/ml) than in healthy persons (8.56 pg/ml; p=0.275), however treatment with IFNα or 2-CdA did not influence TNFα level. Median of TGFβ1 concentration in serum of HCL pts was also significantly lower, than in healthy persons (265.52 and 1568.22 pg/ml respectively; p=0.0004). Reliable increase of TGFβ1 concentration was observed only after 2-CdA therapy (928.33 pg/ml; p=0.281). Cross-correlation relationship was revealed between the TGFβ1 concentration and the level of haemoglobin (τ=0.23; p=0.1) as well as with leucocyte count in HCL pts (τ=0.24; p=0.09). Median of IL-6 content in serum of HCL pts before treatment was higher, than in healthy persons. Therapy with IFNα or 2-CdA reduced IL-6 level to the control values. Certain cross-correlation relationships were revealed between the IL-6 level and percentage of lymphocytes in bone marrow (τ=0.35; p=0.01) as well as amount of lymphocytes in peripheral blood of HCL pts (τ=0.24; p=0.09). Median serum concentration of sIL-2R (24.73 ng/ml) in HCL pts more than 20-fold exceeded such in control group (1.15 ng/ml; p=0.0000005). Cross-correlation relationship was revealed between the percentage of hairy cells in bone marrow and sIL-2R level in serum (τ=0.27; p=0.08). Obtained results may be an evidence of predominant secretion of sIL-2R by tumor cells in HCL pts.
Summary/Conclusions: New data regarding pathogenetic relationship between production of certain cytokines and features of hematopoiesis in HCL pts was obtained. Between the blood level of some cytokines in HCL pts and efficiency of 2-CdA treatment a reliable relationship was revealed, which is possible to use for prediction of clinical course of this disease. Moreover sIL-2R level in blood possibly can serve as a marker of tumour activity in classic type of HCL.

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PROGRESSION-FREE SURVIVAL AND OVERALL SURVIVAL IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA – CLINICAL BENEFITS OF ACHIEVING A DEEP RESPONSE TO FIRST-LINE THERAPY

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Background: In recent years, there have been advances in the treatment of CLL with the approval of several novel oral agents that show improvement in PFS and OS. Additionally, some agents induce a deep response indicated by complete remission (CR) and/or minimal residual disease negativity (MRD-). However, there is limited information on the longer-term clinical benefits of achieving a deep response in a real-world setting.
Aims: This study aimed to characterize PFS and OS for patients who achieved a deep response to first-line therapy for CLL.
Methods: Patient-level data were collected between July and August 2016 from 93 oncologists/hematologists in the United States. Oncologists/hematologists provided patient level clinical data obtained from patient charts among CLL patients who initiated first-line therapy for CLL between January 2010 and December 2014. Selected patients were categorized into 2 cohorts based on their best response: patients who achieved CR and patients who did not achieve CR (non-CR). The non-CR cohort included patients with partial remission (PR), stable disease (SD) and progressive disease (PD). iwCLL 2008 criteria were provided to guide physicians' assessment of treatment response. The target sample size for each response type was *a priori* determined based

on distribution of response in clinical trials. Data on disease progression and mortality was provided by the treating oncologist/hematologist. PFS and OS were compared using univariate and multivariate Cox proportional analyses between the CR and non-CR cohorts (OS multivariate analyses were not conducted due to the small number of events). An additional analysis was conducted to examine the benefits of achieving MRD- *versus* not achieving MRD- among patients who achieved CR or PR.

Results: Data was collected on 330 CLL patients, including 179 patients in the CR cohort and 151 patients in the non-CR cohort (120 patients with PR, 25 with SD, and 6 with PD). Most patients were male, in their early sixties, and had an ECOG status of O/1 at the time of initiating first-line therapy. The median observation period was approximately 30 months. There were 43 (24%) patients in the CR cohort and 75 (50%) patients in the non-CR cohort who progressed/died (Table 1). Patients in the non-CR cohort had an >2-fold higher hazard of progression/death (adjusted hazard ratio [HR]=2.30, p<0.05) and death (unadjusted HR=2.61, p<0.05) compared to patients in the CR cohort. Among patients who achieved CR or PR, 84 patients achieved MRD- and 62 patients did not; 14(17%) patients who achieved MRD- and 27 (44%) patients who did not achieve MRD- progressed/died. Patients who did not achieve MRD- had an over three-fold higher hazard of progression/death compared to patients who achieved MRD- (adjusted HR=3.75, p<0.05). No death events were observed among patients who achieved MRD- while 4 (6%) events were observed among those who did not achieve MRD-.

Table 1.

Proportion of Patients with Progression/Death During the Observation Period

Event, N (%)	CR N= 179	Non-CR N=151	MRD- N=84	No MRD- N=62
Progression/Death	43 (24%)	75 (50%)	14 (17%)	27 (44%)
Progression	38 (21%)	69 (46%)	14 (17%)	23 (37%)
Death	7 (4%)	15 (10%)	0 (0.0%)	4 (6%)

Summary/Conclusions: Findings from this real-world study suggest that achieving CR is associated with improved PFS and OS compared to patients who do not achieve CR. Furthermore, significantly better outcomes were observed in patients who achieved MRD- compared to those who did not achieve MRD- but still achieved CR or PR. This suggests that deep response may be an important clinical parameter to consider in the treatment of CLL.

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ANTI-CD ANTIBODY MICROARRAY FOR MORPHOLOGY EXAMINATION OF CIRCULATING LEUKEMIA AND LYMPHOMA CELLS

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Background: Matching the morphology with immunophenotype for individual leukocytes is a major issue in diagnostics of leukemia and lymphoma due to the absence of a method for simultaneous cluster of differentiation surface antigen detection and full leukocyte morphology analysis. This problem can be solved by using a leukocyte-binding antibody microarray.

Aims: We describe an anti-CD antibody microarray on a transparent support for leukocyte sorting and a method for preparation of the microarray-bound cells for high-resolution morphology analysis. The aim of the work was to demonstrate, that the leukocyte binding is highly specific and that the microarray-bound peripheral blood mononuclear cells both from healthy donors and patients with B-cell leukemias and lymphomas are morphologically identical to the same cells in blood smears.

Methods: Anti-CD antibodies were immobilised on plastic coverslips in spots 2 mm in diametre. In order to study the peripheral blood mononuclear cells (PBMC) the mononuclear fraction separated by density gradient from peripheral blood are incubated with the microarray in non-mixing conditions at 4 ^{0}C. After the unbound cells are washed away the microarray-bound cells are dried in a cytocentrifuge and stained after May-Grünwald-Giemsa for morphology examination. Using this technique we have studied the PBMC from 55 healthy donors and 77 patients with different leukemias and lymphomas: chronic lymphocytic leukemia (CLL, 37 patients), hairy cell leukemia (HCL, 22 patients), splenic marginal zone lymphoma (SMZL, 7 patients), mantle cell lymphoma (MCL, 2 patients), follicular lymphoma (FL, 1 patient), 5 patients with multiple myeloma (MM), 2 patients with large granular lymphocytic (LGL) leukemia and one patient with acute myeloid leukemia (AML M2).

Results: Nonspecific cell binding both inside and outside the spots is below 5%. Due to the non-mixing incubation the density of the cells bound to an anti-CD antibody permits to determine the proportions of cells positive for the corresponding CD antigen with high correlation with flow cytometry. The patterns of the binding densities of the anti-CD-captured PBMC for CLL, HCL and SMZL patients clearly differ both from those for normal PBMC and from each other and agree well with the reported immunophenotypes of corresponding neoplastic cells. Both normal and pathologic microarray-bound PBMC after the proprietary drying procedure are morphologically identical to the same cells in a smear. In cases when pathologic cells are morphologically and/or cytochemically distinct, the anti-CD antibody microarray permits to determine their percentage and immunophenotype by analysing the relative amount of these cells captured by the antibodies against all the CD antigens. The results of such analysis of neoplastic PBMC for the patients with leukemias and lymphomas agree with flow cytometry results for the same patients including expression of CD2 in HCL, CD2 and CD11c in CLL, CD56 in MM. The amount of hairy cells determined morphologically on the microarray varied from 20 to 97 of all anti-CD19-captured cells and 2 to 80% of all lymphocytes and was in good agreement with the percentages of cells with CD19/CD103 and CD19/CD11c coexpression determined in the peripheral blood of the same patients by flow cytometry.

Summary/Conclusions: The microarray works as a "sorted smear" with cells positive for certain surface CD antigens localised in a predetermined area and permitting to apply any standard smear-oriented technique to the microarray-captured cells. Combined analysis of the pathologic cells' immunophenotype, morphology and cytochemistry on the microarray permits to arrive at preliminary diagnosis and can be used in cases of any controversies between morphology, cytochemistry and immunophenotyping. The work is partially supported by 16-34-01030 and 16-04-00282 grants from RFBR.

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COMPARATIVE ANALYSIS OF INTERNATIONAL PROGNOSTIC INDEX FOR CHRONIC LYMPHOCYTIC LEUKEMIA, PROGRESSION-RISK SCORE AND MD ANDERSON CANCER CENTER 2011 SCORE: REAL WORLD DATA FROM A SINGLE INSTITUTION

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Background: In recent times, several powerful prognostic scores have been developed in order to predict time to first treatment (TTFT) and overall survival (OS) of patients with chronic lymphocytic leukemia (CLL). The international prognostic index for chronic lymphocytic leukemia (CLL-IPI) developed by The International CLL-IPI working group was found to predict OS and TTFT, while the rest of two scores- progression-risk score (PRS) and MD Anderson Cancer Center Score 2011 (MDACC 2011) have been developed for prediction of TTFT in early stage CLL patients.

Aims: The aim of this study was to compare CLL-IPI, PRS and MDACC 2011 prognostic scores based on their impact on TTFT, treatment response (TR), progression-free survival (PFS) and OS of 54 treated CLL patients.

Methods: We retrospectively analyzed data from 54 consecutive CLL patients diagnosed and treated at Clinic for Hematology, Clinical Center of Serbia from 2003 to 2013. Blood samples were prospectively collected and analyzed for biological and molecular features (IGHV, FISH and *TP53*), as well as standard laboratory parameters. The three scores were retrospectively calculated using formulas from the original articles (International CLL-IPI working group, Lancet Oncol 2016, for CLL-IPI; Gentile et al, Leukemia 2016, for PRS; and Wierda et al, J Clin Oncol 2011, for MDACC 2011 score), and, than, correlated with TTFT, TR, PFS and OS of patients from the studied cohort.

Results: Median age at diagnosis was 57 years (range 38-75). All patients were treated with fludarabin-based chemotherapy, 45 (83%) in the first treatment line. Overall response rate to the first line therapy was 81%, equally distributed on complete and partial responses. Most of the patients (42, 78%) relapsed during the follow up. At the time of the last follow up 14 (26%) patients were still alive, 35 (65%) were dead, and 5 (9%) were lost to follow up. Median overal survival was 76 months. Lower score values for all the three scoring systems (CLL-IPI, PRS, and MDACC 2011) correlated with longer TTFT (p<0.05 for all). Cox regression analysis revealed that CLL-IPI and PRS are significant predictors of TTFT (p=0.003, RR=1.4, 95%CI 1.1-1.7 and p=0.019, RR=1.4, 95%CI 1.1-1.9, respectively), while MDACC 2011 was of borderline significance (p=0.052). In the multivariable analysis PRS emerged as the most significant predictor of TTFT among the three examined scores (p=0.041, RR=1.35, 95%CI 1.01-1.81). Regarding TR, only PRS appeared to have borderline statistical significance (p=0.052), showing that patients with lower score value may achieve better TR. Lower CLL-IPI can predict longer PFS after the first line treatment (p=0.007, RR=1.7, 95%CI 1.2-2.57), as well as PRS (p=0.039, RR=1.8, 95%CI 1.03-3.1). MDACC 2011 has not shown to have influence on PFS. Multivariable analyisis confirmed PRS to have the strongest predictive value of all the three scores regarding duration of PFS (p=0.039, RR=1.8, 95%CI 1.02-3.1). Furthermore, CLL-IPI and PRS were found to be significant predictors of OS (p=0.005, RR=1.4, 95%CI 1.1-1.8 and p=0.037, RR=1.5, 95%CI 1.03-2.24, respectively).

Summary/Conclusions: CLL-IPI and PRS were identified as significant predictors of TTFT, as well as of duration of TR and OS. Further studies are warranted to confirm these findings.

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MULTISITE PERFORMANCE EVALUATION STUDY OF THE BD ONEFLOW B-CELL CHRONIC LYMPHOPROLIFERATIVE DISORDERS T1 (B-CLPD T1) PANEL

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Background: The BD OneFlow solution for B-cell chronic lymphoproliferative diseases (B-CLPDs) incorporates a standardized flow cytometry approach based on the EuroFlow (EF) Consortium liquid reagent system. The BD OneFlow solution enables reproducible identification and discrimination of distinct cell populations by combining standardized assays, setup reagents, and protocols. The previously launched BD OneFlow LST (Lymphocyte Screening Tube) is intended for flow-cytometric immunophenotyping of normal (no follow-up required) and aberrant (follow-up required) mature lymphocyte populations of B, T, and NK lineages in specimens from patients with hematological disorders. The BD OneFlow B-CLPD T1 is being developed to work in conjunction with BD OneFlow LST for the immunophenotyping of B cells and distinguishing chronic lymphocytic leukemia (CLL) from other B-CLPDs such as atypical CLL, follicular cell lymphoma, mantle cell lymphoma, etc.
Aims: The objective of this study was to demonstrate equivalency (accuracy) between the investigational BD OneFlow LST and BD OneFlow B-CLPD T1 system and the corresponding comparator EF liquid reagent system on the BD FACSCanto II flow cytometer using BD FACSDiva software.
Methods: De-identified remnant peripheral blood (PB) (n=70) and bone marrow (BM) (n=31) patient specimens were collected in EDTA or heparin anticoagulants at four external study sites and tested within 26 hours of draw. Informed consent was not required in this clinical study. Specimens were stained with BD OneFlow LST in combination with OneFlow B-CLPD T1 tubes and comparator EF liquid reagents. Acquisition and analysis were performed on a BD FACSCanto II instrument using BD OneFlow LST and B-CLPD T1 templates in BD FACSDiva software v8.0.1. Categorization of samples with abnormal B-cell populations into CLL (typical) or other B-CLPDs, overall agreement, negative agreement, and positive agreement, along with their one-sided lower 95% confidence limits, were calculated. For qualitative categorization of relative fluorescence intensity (positive or negative) of the aberrant cell populations, overall agreement with one-sided lower 95% confidence limits was calculated.
Results: All evaluable specimens were identified by the OneFlow LST as having B-cell populations requiring follow-up by both methods. Compared to the EF system, the BD OneFlow LST in combination with the BD OneFlow B-CLPD T1 system gave 100% (101 of 101) overall agreement in classifying patients as having CLL (54 of 54 concordant) and in identifying patients with other B-CLPD diseases (47 out of 47 concordant) with a lower 95% CI of the overall agreement of 97.4%. The BD OneFlow B-CLPD T1 system, compared to the EF system, gave 100% (101 of 101 concordant) agreement for the qualitative assessment of the relative fluorescence intensity of CD45+CD19+ aberrant populations for CD20+, CD200+, and CD23+ subsets and 99.1% agreement for the CD79b+ subset.
Summary/Conclusions: The multisite performance evaluation between the BD OneFlow system (LST and B-CLPD T1) and the comparator EF liquid reagent system was concordant in distinguishing abnormal B-cell populations in patients with CLL from patients with other B-CLPDs, including presumptive cases of atypical CLL. The BD OneFlow B-CLPD T1 is a fully standardized and validated system for aiding in the diagnosis of CLL from other B-CLPDs in PB and BM specimens.
BD OneFlow LST and BD OneFlow B-CLPD T1 are CE Marked according to the European *In Vitro* Diagnostic Medical Device Directive 98/79/EC. 23-19567-00

PB1795

COMBINED PATTERNS OF IGHV REPERTOIRE AND MOLECULAR ALTERATIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA- SINGLE CENTER EXPERIENCE

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Background: The specific determining factors for malignant progression in Chronic lymphocytic leukemia (CLL), remaining unknown.
Aims: To investigat the potential existence of unique cytogenetic profiles associated with specific IGHV repertoires that could be associated with an increased risk of progression in CLL.
Methods: For this purpose, molecular analysis of well-established cytogenetic alterations of chromosomes 11, 12, 13, 14 and 17 together with the pattern of rearrangement of the IGHV genes were performed in 100 CLL cases.
Results: Our results based on molecular analysis from 100 subjects living in the same geographical area, show the presence of three major groups of clones with distinct but partially overlying configurations of IGHV gene usage, IGHV mutational status and cytogenetic alterations. These included a group which mainly consisted of clinical advanced stage CLL with a skewed but different CLL-associated IGHV gene repertoire (VH1-69 associated with HD3 gene and HJ6 gene), frequently associated with complex karyotypes and poor-prognosis cytogenetic alterations , a second group enhanced in clones expressing specific IGHV subgroups (VH3-23 associated with HD2 genes and HJ6 gene) with no or isolated good-prognosis cytogenetic alterations and a third group of clones with intermediate features, with prevalence of mutated IGHV genes, and higher numbers of del(13q)+ clonal B-cells.
Summary/Conclusions: These findings suggest that the specific IGHV repertoire and IGHV mutational status of CLL B-cell clones may adjust the type of cytogenetic alterations acquired and their clinical significances. Further long-term follow-up studies investigating the IGHV gene repertoire of CLL clones in distinct geographic areas and microenvironments are required to validate our findings and discard or confirm the potential role of some antigen-binding BCR specificities contributing to clonal evolution.

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PROGNOSTIC SIGNIFICANCE OF SERUM BAFF, APRIL, TACI AND BCMA LEVELS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: B-cell chronic lymphocytic leukemia (B-CLL) is characterized by the accumulation of $CD5^+$ B cells in the peripheral blood and bone marrow. Prognosis of B-CLL is highly variable which depends on certain prognostic parameters. Novel prognostic markers and risk assessment models are fundamental to identify high risk patients who may need early treatment. The two tumour necrosis factor family proteins BAFF (TNFSF13B) and APRIL (TNFSF13) and their receptors [BAFF-R (TNFRSF13C), TACI (TNFRSF13B), BCMA (TNFRSF17)] play a critical role in the survival of normal B cells.
Aims: In this study, we aimed to investigate the impact of serum BCMA, TACI, BAFF and APRIL levels on prognosis of B-CLL.
Methods: A total of 129 newly diagnosed CLL patients [median age: 64(39-88); M/F: 85/44] and 26 healthy volunteers were enrolled in this study. Serum BCMA, TACI, BAFF and APRIL levels were measured at diagnosis using enzyme-linked immunosorbent assay (ELISA). The association with conventional prognostic markers and impact on survival were evaluated.
Results: Serum BAFF, TACI and BCMA levels were significantly lower in the patient group (p<0.001), while serum APRIL level did not differ significantly between the patient and control groups (p>0.05) (Table 1). Serum BAFF [(p=0,008; r=-0,236)] and BCMA [(p=0,042; r=-0,183)] levels were negatively correlated with Rai stage and serum BAFF level was higher in low-risk patients based on modified Rai staging system (p=0,059). Serum TACI level was higher in CD38 positive patients [(p=0,06; 0,17(0,1-0,86) *vs* 0,13(0,1-1,07)]. Age (p=0,002), Rai stage (p=0,005) and Modified Rai stage (p=0,051) were the significant factors which had an impact on overall survival in multivariate analysis.

Table 1.

	Patient Group	Control Group	p value
BAFF (ng/ml)	0,08(0,05-2,08)	1,46(0,24-2,95)	p<0,001
BCMA (ng/ml)	0,16(0,12-1,93)	1,43(0,87-3,03)	p<0,001
TACI (ng/ml)	0,14(0,1-1,07)	0,28(0,24-0,87)	p<0,001
APRIL (ng/ml)	0,28(0,2-0,65)	0,26(0,24-0,65)	p=0,089

Summary/Conclusions: As BAFF and APRIL display their main biological effects once they bind to their receptors and pass through the intracellular compartment, we consider that it may be more feasible to measure the intracellular levels of these molecules which may be more predictive for B-CLL prognosis. The association of TACI and CD38 expression may indicate the notable balance between proliferation and apoptosis, as CD38 is considered to be a proliferation marker in B-CLL. Further large and prospective studies analyzing the intracellular levels of these molecules are essential to validate the prognostic role of these particular biomarkers in CLL.

PB1797

EXPERIENCE OF IBRUTINIB IN RELAPSED/REFRACTORY B-CELL CHRONIC LYMPHOCYTIC LEUKAEMIA AND MANTLE CELL LYMPHOMA IN A U.K. DISTRICT GENERAL HOSPITAL

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Background: Constitutive activation of B-cell receptor signalling appears to be essential for the proliferation of malignant B cells. Bruton's tyrosine kinase (BTK) has been identified as an essential component of the B-cell receptor signalling pathway. Ibrutinib is an orally administered BTK inhibitor that antagonises B cell receptor, chemokine & integrin mediated signalling.
Aims: We report our experience of using ibrutinib to treat relapsed/refractory B-cell chronic lymphocytic leukaemia (B-CLL) and mantle cell lymphoma (MCL) in a busy U.K. District General Hospital (DGH) serving a population of 600,000
Methods: 26 patients were commenced on ibrutinib for relapsed/refractory B-CLL or MCL between August 2014 & December 2016. 16 patients had B-CLL and 10 patients had MCL. Patients with B-CLL were commenced on 420mg daily; those with MCL received 540mg daily. The median age at which ibrutinib was commenced was 71.1 years (range 50-85). The median age of patients with B-CLL was 71.1 years (range 50-80) and for MCL was 71.6 years (range 54-85). The median number of prior lines of therapy decreased over the time period from 3.2 in 2014 to 1.2 in 2016. The mean interval between diagnosis and commencement of ibrutinib was 6.7 years (B-CLL) and 4.8 years (MCL). The average number of co-morbidities in both groups was similar: 1.4 in B-CLL and 1.5 in MCL. After May 2015 all patients received aciclovir and co-trimoxazole prophylaxis. Response to ibrutinib was assessed by clinical examination and blood results; imaging and bone marrow examination were conducted at the clinician's discretion.
Results: The median follow up was 15.5 months for B-CLL patients and 8 months for MCL patients. The median survival of all patients who did not receive anti-viral and pneumocystis prophylaxis was 5 months and the median survival for those who did receive prophylaxis was not reached (p <0.0001). The median survival of patients in both groups who had received more than 1 prior line of treatment was 17 months; the median survival in those who had received just one prior line of treatment was not reached (p= 0.0085). In the B-CLL cohort there was no difference in survival between those with and without 17p / p53 deletion. 11/26 patients experienced side effects: 8 had grade 1 and 2 side effects (diarrhoea, drug rash, cardiac arrhythmias) which were easily controlled. 3 patients had grade 4 side effects (1 severe arthropathy, 2 intracranial haemorrhage - one of which was fatal). 4 of the 16 (25%) with B-CLL and 5 of the 10 (50%) with MCL died during the period of follow-up. Causes of death were: intra-cerebral haemorrhage (1), unrelated cancer (1), disease progression (2), disease progression+sepsis (2), sepsis alone (3). Of the remaining 17 patients, 14 continue to receive ibrutinib, 2 (B-CLL) were switched to idelalisib+Rituximab (for grade 4 toxicity) & 1 went on to have an allogeneic transplant (MCL).
Summary/Conclusions: Though our cohort of patients is small, our experience shows that the use of prophylaxis with co-trimoxazole and aciclovir is associated with significantly improved overall survival. Moreover, patients who received fewer lines of prior treatment had a better survival. Patients with 17p/p53 deleted B-CLL responded as well as those without a deletion. Ibrutinib is a very effective therapeutic option in patients with relapsed CLL and MCL.

PB1798

THE VALUE OF RITUXIMAB ADDITION TO CHEMOTHERAPY TREATMENT OF REAL-WORLD CLL PATIENTS: A 15 YEAR SINGLE CENTER EXPERIENCE

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Background: The addition of the monoclonal antibody rituximab to chemotherapy has been shown to improve progression free survival and overall survival in prospective trials in CLL patients. However, CLL patients participating in clinical trials may not be fully representative of the overall patient population in clinical practice as there is selection due to study availability, willingness to participate and various in- and exclusion criteria. To date, the efficacy of rituximab added to standard chemotherapy in first line and relapsed CLL patients has been poorly validated in observational studies in unselected real-world CLL patients.
Aims: To evaluate the efficacy of rituximab-chemotherapy (R-CTX) compared to chemotherapy (CTX) in a real-world CLL population.
Methods: All patients from a large teaching hospital diagnosed with immunophenotypically confirmed CLL in the period from 1-1-2000 up to 1-9-2015 were analyzed for this study and were categorized into two groups (1) those treated with CTX and (2) those who received R-CTX. The clinical outcome of patients was evaluated based on the "treatment-free interval" (TFI), defined as the time from stop of chemo(immuno)therapy to start of next treatment. Patients who did not need next treatment were censored at time of last follow-up or death. In addition, overall survival (OS) for patients treated in the period when rituximab had become available in our center was compared to patients treated before the rituximab era (before and after 1-1-2006, respectively).
Results: A cohort of 375 CLL patients was studied, of whom 124 CLL patients (33%) required treatment in the observation period. The median age at first-line therapy was 67 years; 55% and 45% of these patients received first line CTX or R-CTX, respectively, and 47% of these patients required a second or later line of (R-)CTX. In total 221 treatment periods of (R-)CTX were studied with respect to treatment-free interval, 124 first-line, and 97 courses of retreatment. In the first-line treatment group 12 (10%) and 24 patients (19%) were treated with purine-analogue-based schedules without or with R respectively, *i.e.* (R-)fludarabine or (R-)fludarabine plus cyclophosphamide, 55 (45%) and 31 patients (25%) were treated with chlorambucil/CVP-based regimens without or with R respectively, and two patients (2%) were treated with CHOP and R-bendamustin. The median TFI for patients treated with CTX was 31 months (95%CI; 20 – 42 months) and was significantly better in the R-CTX group where the median TFI was not reached during the observation time (hazard ratio 0.40, 95%CI 0.22 – 0.73). In second or later lines of treatment 15 (15%) and 11 patients (11%) were treated with purine-analogue-based schedules without or with R respectively, *i.e.* (R-)fludarabine or (R-)fludarabine plus cyclophosphamide, 25 (26%) and 31 patients (32%) were treated with chlorambucil/CVP-based regimens without or with R respectively, and 15 patients (15%) were provided with other treatment modalities, *i.e.* (R-)CHOP or (R-)bendamustin. The median TFI for CTX was 27 months (95%CI; 18 – 52 months) *vs* 55 months for R-CTX (95%CI; 41 months – NR), HR 0.47 (95%CI 0.15 – 0.90) for subsequent lines. OS for patients treated in the R era was 48 *vs* 35 months for patients treated before the introduction of rituximab (p=0.02).
Summary/Conclusions: Our study shows that the addition of rituximab improved treatment free interval in first- and subsequent lines and prolonged overall survival in a cohort of CLL patients receiving treatment in routine clinical 'real world' practice.

PB1799

RICHTER SYNDROME: SERBIAN LYMPHOMA GROUP EXPERIENCE

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Background: Richter syndrome (RS) represents transformation of chronic lymphocytic leukemia/ small lymphocytic lymphoma (CLL/SLL) into more aggressive B-cell lymphoproliferative disorder, most commonly, diffuse large B cell lymphoma (DLBCL). vera rarely classical Hodgkin lymphoma (HL). In some point of disease course, 2-10% of all CLL/SLL population develope RS, usually exhibiting chemoresistance and survival within a year after diagnosis.
Aims: The aim of the study is to evaluate clinical, laboratory and histopathological features of patients with RS at transformation, and their impact on the outcome.
Methods: We processed data from the medical records of 36 CLL and SLL patients with RS diagnosed and treated in four institutions in Serbia from 2003 to 2016: Clinic for Hematology, Clinical Center of Serbia; Clinic for Hematology, Medical Military Academy; Clinical Hospital Center Zemun; and Clinic for Hematology, Clinical Center Kragujevac. In all of RS patients, diagnosis was established after histopathological and immunohistochemistry analysis of biopted tissue (lymph node, bone marrow, Waldeyer's ring, maxillary sinus, spleen or liver).
Results: In four institutions RS was diagnosed in 36/1250 CLL/SLL patients (2.8%). Median age was 57.5 years (range 41-79). In 16 (44%) patients RS was confirmed in lymph node sample, in 13 (35%) patients in bone marrow, in 4 (11%) patients in Waldeyer´s ring, in 2 (5%) patients in maxillary sinus, in 2 (5%) patients in liver or/and spleen, while in 3 patients in more than one location. Histopathological findings of all patients in transformation were consistent with DLBCL, except one, showing pattern of HL. Prior to the transformation, 26 (72%) patients received chemotherapy (Chlorambucil 6 patients, Fludarabin based regimens 11 patients, CHOP 3 patients, COP/RCOP 4 patients, other modality 3 patients), 4 (11%) of them were on the "watch and wait" strategy, while 6 (17%) patients were diagnosed with RS at presentation, and treated as RS. Median time to transformation was 36 (0-180) months, At the time of transformation median LDH and beta-2 microglobulin levels were significantly higher than on presentation (p=0.035 and p=0.010, respectively). The majority of patients received CHOP (20/36, 55%) and RCHOP (7/36, 19%) as initial therapy for RS. The reminder of patients received other treatment modalities, such as ESHAP, FC, high dose corticosteroids, COP, RCOP and radiotherapy. After excluding 6 patients with SLL from the group, Cox regression analysis showed that high LDH and low Hb levels at the time of transformation are significant predictors of shorter survival after diagnosis of RS (HR=1.001; 95% CI 1.000-1.001; p<0.001 and HR=0.978; 95% CI 0.961-0.995; p=0.011, respectively). Bone marrow as a site of transformation did not reach statistical significance as a predictor of shorter survival after transformation (p=0.087). Median survival after diagnosis of RS was 8 months (range 0-133) (Figure 1).

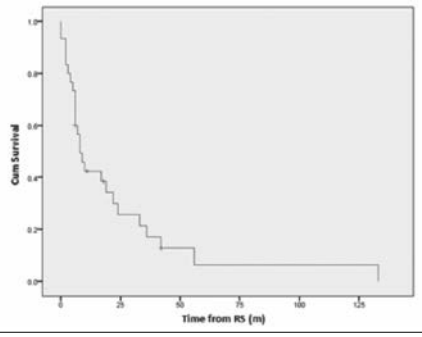

Figure 1.

Summary/Conclusions: Incidence of RS in our study is partly coherent with literature data. Levels of LDH and Hb at the time of transformation are significant predictors of outcome of patients with RS. Real number of patients with RS is probably higher, but commonly bad condition of these patients on diagnosis of RS probably influences the decision of a clinician not to indicate biopsy.

PB1800

INFECTIOUS COMPLICATIONS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA TREATED WITH IBRUTINIB

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Background: Chronic lymphocytic leukaemia (CLL) is characterised by frequent co-existent infectious complications. They stem from, among other things, hypogammaglobulinemia, which is connected with CLL, and correlates with the disease duration and severity, as well as T-lymphocyte function disorders. The application of innovative therapies (chemoimmunotherapy) on the one hand facilitates considerable improvements in treatment outcomes and on the other hand it increases the risk of life-threatening infectious complications. The introduction of a new drug, ibrutinib (Bruton's kinase inhibitor), has created a unique opportunity for CLL patients, especially those with prognostically unfavourable genetic aberrations (del17p), or in the case of whom previous chemotherapies have failed to give satisfying results. Previous observations indicate the risk of side effects (*e.g.* bleeding, infectious complications, heart rhythm disorders) which might sometimes limit the applicability of ibrutinib in some CLL patients.
Aims: The aim of this paper was to evaluate the risk of infectious complications in persons with CLL, and to determine potential correlations between possible infectious complications and selected clinical, morphological and biochemical parameters.
Methods: The study comprised 43 CLL patients aged 48-82 years (average age 67 years), 18 women and 25 men. At the beginning of the ibrutinib therapy the patient's disease was at the 2-4 clinical stage, according to Rai *et al.* Usually they were individuals who had received a couple of previous chemotherapies (from 1 to 7) which contained, inter alia, purine analogues, and the monoclonal antibodies (rituximab, alemtuzumab, ofatumumab). Ibrutinib was administered at a dose of 420 mg/d.
Results: Infectious complications were observed in 16 patients (37.2%). These included, for example, upper respiratory tract infection, bronchitis, pneumonia, urinary-tract infections, pharyngitis. The conducted analysis showed a statistically significant correlation between the concentration of IgM in the blood serum (before ibrutinib administration) and infectious complications during these therapy ($p<0.05$). The average IgM concentration in patients with complications was considerably lower when compared to people who did not experience any complications. The patients (n=3; 6,98%) with complications at the moment of the CLL diagnosis also had them during the ibrutinib treatment. This phenomenon was confirmed in 13 patients (33%) in the other group. The correlation was borderline significant ($p=0.09$). Infectious complications were observed more frequently in the patients with 3-4 stage CLL (according to Rai *et al.*) than in the individuals at the less-advanced clinical stages of the disease (0-2), and this correlation also showed borderline significance ($p=0.08$). No significant correlation was detected between the risk of infectious complications and earlier therapy with purine analogues and neutropenic episodes during the ibrutinib therapy.
Summary/Conclusions: Ibrutinib is considered to be a real breakthrough in CLL treatment; but it has to be borne in mind that the drug gives possible side effects which might occur during therapy. They include infectious complications which are among the main causes of death in this group of patients. The results obtained by us indicate that the risk of infection during ibrutinib therapy relates mainly to patients with low IgM concentration in the blood serum and at more advanced clinical stages of the disease. In this case the occurrence of previous complications (before ibrutinib administration) is also relevant. We are aware of the limitations of our work related to the small number of patients. Yet, even at this stage, it is possible to select CLL patients with increased risk of such usually life-threatening complications.

PB1801

MONOCLONAL B-CELL LYMPHOCYTOSIS AND PROSTATE CANCER: AN UNEXPECTED, POSSIBLE ASSOCIATION

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Background: Monoclonal B-cell lymphocytosis (MBL) is a recently recognized entity characterized by the presence, in the peripheral blood, of a monoclonal B-cell population lower than 5000/µl, in the absence of any type of clinical features. MBL clones may have: a) chronic lymphocytic leukemia (CLL-like) phenotype (CD5+, CD19+, CD23+, CD20 dim); b) atypical CLL phenotype (CD5+, CD19+, CD23- or CD20 bright); c) non-CLL phenotype (CD5-). MBL can be also distinguished in "low-count" (<500/µl) and "high-count" (>500/µl) subtypes. High-count MBL frequently shows typical CLL phenotypic/genetic features and require adequate follow-up in order to detect their possible evolution into symptomatic CLL. MBL showing a clonal B-cell count higher than 1000-1500/µl are usually defined "clinical" MBL. Using highly sensitive (*i.e.* >6 colors and >500000 events) flow cytometry approaches, CLL-like MBL clones have been found at a frequency of 7-12% in healthy subjects, showing, however, very low median counts of clonal B-cell (10-170/µl), with only 0,14% being clinical MBL. Though several studies have described the association between CLL and various types of neoplastic disorders, only few data exist about the risk of non-hematologic cancer in individuals with MBL; in particular, no association between MBL and prostate cancer (PC) has been so far reported.
Aims: To study prospectively the frequency of CLL-like MBL clones in patients affected by PC compared to healthy males of the same ages, after our previous occasional observation of an apparently increased MBL incidence at baseline in a cohort of patients with PC originally studied to detect lymphocyte abnormalities possibly induced by radiotherapy (RT).
Methods: We enrolled 34 consecutive patients affected by PC (mean age 74 years, range 58-91), naïve for chemotherapy (sixteen previously treated with hormone-therapy). All patients were planned to receive whole-pelvis RT with radical (n. 23) or salvage (n. 11) intent. Fifty-four healthy males (mean age 71 years, range 58-87) represented the control group. Immunophenotypic analysis of peripheral lymphocytes before RT was performed by BD FacsCanto II flow cytometer, using a 5-6 colors approach and the following antibody combinations: CD19 FITC/CD5 PE/CD45 PerCP/CD20 PE-Cy7/CD23 APC; Kappa FITC/Lambda PE/CD19 PerCP-Cy5.5/CD20 PE-Cy7/CD5 APC/CD45 APC-Cy7. For each sample, 100000 events were collected. CD45+ lymphocytes were gated on CD45 *vs* SSC dot plot, then B cells were isolated by gating on CD19 and CD19+ CD5+ cells were interrogated for intensity of CD20. Finally, CD19+ CD5+ CD20dim selected population was analyzed for light chain clonality and CD23 expression.
Results: Median (range) absolute counts of white blood cells (WBC), total lymphocytes and B-cells, as well as absolute single values of MBL clones are reported in Table 1. In PC patients we found 3 MBL (8,8%), two of which were "high count/clinical" MBL (5.8%). In contrast, in healthy subject group, only one "low count" MBL (1.8%) was detected, showing a very small clone (8 cells/µl). Such a difference was not statistically significant ($p=0.2$).

Table 1.

	Median WBC/µl (range)	Median Lymphocytes/µl (range)	Median B-cells/µl (range)	MBL n. (%)	Absolute MBL clone values/µl
Prostate cancer (n. 34)	7404 (5100-12900)	2304 (1200-4900)	264 (52-1984)	3 (8.8%)	294, 1254, 1970
Controls (n. 54)	7577 (4090-12380)	2237 (800-5100)	201 (28-484)	1 (1.8%)	8

Summary/Conclusions: The preliminary results of our prospective study, performed using a routine, not highly sensitive flow cytometry approach, highlight a possible association between (clinical?) MBL and PC, never described before and probably warranting further investigation in a larger number of patients.

Chronic myeloid leukemia - Biology

PB1802

IDENTIFICATION OF NOVEL MUTATIONS IN CANCER-RELATED GENES IN HUMAN ERYTHROLEUKEMIA K562 CELL LINE BY NEXT-GENERATION SEQUENCING

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Background: Chronic myeloid leukemia (CML) is a clonal hematopoietic stem cell disorder characterized by reciprocal chromosomal translocation t(9;22)(q34;q11), resulting in the formation of the *BCR-ABL1* fusion oncogene. One of the most common CML *in vitro* model is the K562 BCR-ABL1-positive human erythroleukemia cell line derived from a female patient with CML in blastic phase (CML-BP) and representing an important tool for the studies of malignant hematopoiesis in last decades. Although K562 karyotype was described several times, detailed genomic analysis of this cell line is not yet available and to our best knowledge there are no publications yet describing complex genomic landscape of K562 cells.
Aims: The aim of our study was to determine the mutational landscape of K562 cell line using next-generation sequencing (NGS). Additionally classical fluorescence *in situ* hybridization (FISH) with BCR and ABL1 probes was performed to confirm cytogenetics.
Methods: The K562 cell line was purchased from DSMZ (Braunschweig, Germany). We analyzed almost 1300 genes implicated in human cancer using custom designed capture (SeqCap EZ, NimbleGen, Roche) followed by high-throughput sequencing on Illumina HiSeq 1500. Common variants (>1%) gathered in ESP6500 and 1000 genomes projects and our internal exome database were filtered out and the subsequent analysis was focused on putative protein damaging variants with the frequency in the database from NHLBI GO exome sequencing project less or equal to 0.01. We used different bioinformatic tools for variant effect prediction (eg. PolyPhen-2, SIFT, IntOGen). Mutations were confirmed with Sanger sequencing. FISH was performed using commercially available probes (Vysis, Abbott, USA), that identifies *BCR-ABL1*/*ABL1-BCR* fusion genes.
Results: Sequencing and bioinformatical analysis revealed 88 variants with potential biological significance. We detected Q136fs*13 mutation in *TP53,* which has already been described in K562 cell line previously by ATCC, but we have also identified several new mutations in genes involved in tumorigenesis and drug resistance (Table 1). Moreover, cytogenetic analysis showed both multiplication of the *BCR* and *ABL1* genes and amplification of the *BCR-ABL1* fusion gene (Ph chromosome is present in at least four additional copies).

Table 1. Selected prominent mutations identified in K562 cells.

Mutation	NCBI Reference
TP53 p.*Q136fs*13 (known)*	NM_001126114.2
ASXL1 p.Y591*	NM_015338.5
MLH1 p.K175fs	NM_001258271.1
BIRC6 p.A3622V	NM_016252.3
AKT3 p.G37*	NM_001206729.1
BRCA1 p.I540V	NM_007297.3

Summary/Conclusions: We describe several new mutations in such genes as *ASXL1*, *BRCA1* or *MLH1* in one of the most frequently used cell line in leukemia research, K562 erythroleukemia. Our results confirm high level of genomic instability in the blastic phase of CML represented by the K562 cell line and add new, valuable information for researchers who want to employ this cell line. The awareness of the genomic aberrations present in the K562 erythroleukemia cell line is essential for further studies as those aberrations may have a significant impact on the observed results.

PB1803

INVESTIGATION OF POLYMORPHISMS RELATED TO MIR-608 IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background: Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by the expression of the BCR-ABL oncoprotein, which is essential for the pathogenesis of the disease. Imatinib, an ATP-competitive selective inhibitor of BCR-ABL, has unprecedented efficacy for the treatment of CML. Several cellular and genetic mechanisms of imatinib resistance have been proposed, including overexpression of the BCR-ABL gene, the tyrosine kinase domain mutations, pharmacokinetic and pharmacodynamic factors.
Aims: The purpose of this study was to investigate miRNA-608 role in response to therapy with tyrosine kinase inhibitors (Imatinib). In this study, we analyzed rs9762 SNP located in a miRNA-608 binding site of 3′UTR of BCR-ABL gene and rs4919510 SNP in the mature sequence of miR-608 in CML patients with different response to tyrosine kinase inhibitor therapy. These polymorphisms disrupt the negative effect of mir-608 on its target BCR-ABL.
Methods: In our study 76 CML patients at the age of 15−65 were involved. Genomic DNA was extracted from peripheral blood leukocytes by standard phenol-chloroform method. Genotyping was performed by the PCR-RFLP technique.
Results: Combination of genotypes affecting mir-608/BCR-ABL1 interaction (*GG in mir-608 binding site and/or *GG in mature miRNA itself) was revealed with 81% in CML patients with uneffective therapy. We suggest that mir-608 could possess oncosuppressing activity as mir-203 but it should be confirmed by further experiments.
Summary/Conclusions: miRNAs could be a perspective tool for therapy and polymorphisms affecting its regulation should also be considered.

PB1804

TARGETED STRATEGY FOR ABL TYROSINE KINASE INHIBITOR RESISTANT PHILADELPHIA CHROMOSOME POSITIVE LEUKEMIA CELLS

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Background: Although ABL tyrosine kinase inhibitors (TKIs) such as imatinib have demonstrated the potency against Philadelphia chromosome (Ph)-positive leukemia patients, resistance to ABL TKI can develop in chronic myeloid leukemia (CML) patients. Therefore, new approach against ABL TKI resistant cells may improve the outcome of Ph-positive leukemia patients. It has already reported that ABL kinase domain mutations have been implicated in the pathogenesis of ABL TKI resistance, however, it is fully not known the molecular mechanism of drug resistance including second (nilotinib and dasatinib) and third generation (ponatinib) ABL TKIs.
Aims: As leukemia is a genetic disease driven by heritable or somatic mutations, we hypothesized that ABL TKI resistance may often happen due to additional somatic mutations in the oncogene.
Methods: We established several TKI-resistant *in vitro* cell line models. We also investigated model to evaluate the next-generation sequencing (NGS) panel, NGS platform to screen mutational hotspots in 50 leukemia-related genes.
Results: We established ABL TKI resistant cell lines (K562 imatinib-R, K562 nilotinib-R, K562 dasatinib-R, K562 ponatinib-R, Ba/F3 T315I and Ba/F3 ponatinib-R) in this study. We conducted fluorescence *in situ* hybridization (FISH) analysis on parental K562 and ABL TKI resistant K562 cells. BCR-ABL expression levels were not increased in ABL TKI resistant K562 cells compared to parental K562. We next investigated the BCR-ABL point mutation by direct sequence analysis. We could not detect the BCR-ABL point mutation in ABL TKI resistant K562 cells. However, the exon 4 deletion in the BCR-ABL gene was found in K562 ponatinib-R cells. In contrast, compound mutations in BCR-ABL were found in Ba/F3 ponatinib-R cells. K562 ponatinib-R cells were also highly resistant to imatinib, nilotinib and dasatinib. We examined the intracellular signaling of ABL TKI resistant K562 cells. Phosphorylation of BCR-ABL and Crk-L was reduced in K562 dasatinib-R cells, however, MAPK activity was increased. In K562 ponatinib-R cells, MAPK activity was reduced. We next evaluated the NGS panel (GeneRead DNAseq Targeted Panels V2) to investigate the mutation. We found that several somatic mutations in TET2, FLT3, RB1, TP53, SETBP1, ASXL1, and BCORL1 in parental K562 cells. We also found that additional somatic mutations in K562 imatinib-R (IDH1 and KRAS), K562 dasatinib-R (IDH1) and K562 ponatinib-R (SF3A1). We could not detect additional mutation in K562 nilotinib-R cells. We next investigated the MEK inhibitor and IDH1 inhibitor activity against K562 imatinib-R and K562 dasatinib-R cells. MEK inhibitor or IDH1 inhibitor did not induce cell growth inhibition directly. However, combined treatment of ABL TKI resistant K562 with imatinib or dasatinib and MEK inhibitor or IDH1 inhibitor caused more cytotoxicity than each drug alone. Because aberrant activation of PI3K signaling pathway and deregulation of HDAC activity may be a cause of malignant disease in humans, we examined the PI3K and HDAC inhibitor in ABL TKI resistant cells. We found 72 h treatment of oral inhibitor of class I PI3K as well as class I and II HDAC enzymes, CUDC-907 exhibits cell growth inhibition ABL TKI resistant K562 cells and Ba/F3 ponatinib-R cells in a dose dependent manner. In the mouse study, a dose of 20 mg/kg/day p.o of ponatinib and 30 mg/kg/day p.o of CUDC-907 inhibited tumor growth of T315I mutant cells compared with control mice and induced apoptosis in tumor samples.
Summary/Conclusions: Our study indicated that leukemia cells have acquired resistance through somatic mutation or exon 4 deletion in the BCR-ABL gene, suggested that individual based investigations may be important to evaluate the ABL TKI resistance. We also provide the promising clinical relevance as a candidate drug for treatment of ABL TKI resistant leukemia patients.

PB1805

FLUORESCENCE *IN SITU* HYBRIDIZATION SIGNAL PATTERNS AND INTRACHROMOSOMAL BCR-ABL1 AMPLIFICATION ANALYSIS IN IMATINIB-RESISTANT CHRONIC MYELOGENOUS LEUKEMIA PATIENTS USING TRICOLOR DUAL FUSION PROBE

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Background: Conventional cytogenetics is a common modality for tyrosine kinase inhibitor (TKI) response assessment in chronic myelogenous leukemia (CML) patients. There is no consensus regarding the use of conventional bone marrow (BM) cytogenetics or peripheral blood (PB) interphase fluorescence *in situ* hybridization (I-FISH) during follow-up. The routine dual colour FISH probes are less sensitive to reliably identify der(9) deletions during follow-up. BCR/ABL/ASS1 tri-colour dual fusion (TCDF) probe is highly sensitive and specific in identifying der(9) deletions.
Aims: Our aim was to identify the I-FISH fusion patterns of BCR/ABL/ASS1 TCDF probe and correlate the patterns with patient-specific molecular genetic parameters.
Methods: This was an ethically approved study conducted at a government-funded tertiary care institute. From January 2015 to June 2016, PB I-FISH analysis was performed on European LeukemiaNet defined imatinib-resistant CML patients using BCR/ABL/ASS1 TCDF probe (Abbott Laboratories, Abbott Park, Illinois, USA). The residual *BCR-ABL1* transcript load was monitored in international scale (*BCR-ABL1*^{IS}) using an automated cartridge-based GeneXpert system (Cepheid, Sunnyvale, CA, USA).
Results: On analyzing 37 adult patients, all had residual Philadelphia (Ph) chromosomes (100%). Classic Ph fusion pattern was seen in 33 (89%), derivative chromosome 9 [der(9)] deletions in 25 (67.5%) and supernumerary Ph chromosomes in 11 (30%) patients. Coexistence of classical fusion and der(9) deletions were seen in 21 patients (57%), whereas 8 patients (22%) had a mutual existence of classical fusion, der(9) deletions and supernumerary Ph chromosomes. None had Ph amplification. Figure 1 demonstrates the I-FISH patterns seen in a 43-year-old male diagnosed with CML-CP and had progressed to blast crisis at his 72nd month of imatinib therapy. In this Figure red, yellow and white arrows indicate blast cells without Ph chromosome, Ph+ blast with a loss of residual *ABL1* on der(9)classical and random signal overlap, respectively. A mean (± S.D) of 29% (± 30) and 18% (± 17) der(9) deleted cells were seen amongst patients with b2a2 and b3a2 *BCR-ABL1* transcript types, respectively and this difference was statistically significant (p=0.008). There was also a significant difference in the disease transformation status according to the percentage of der(9) deleted cells (p=0.03). In this regard, patients with progressive disease (accelerated phase/ blast crisis progression) had a mean (± S.D) of 47% (± 35) der(9) deleted cells in comparison to 19% (± 20) such cells in patients without disease transformation. In addition, patients with Ph duplication/triplication had a mean (± S.D) *BCR-ABL1*^{IS} levels of 49.478% (± 40.184), in comparison to *BCR-ABL1*^{IS} levels of 16.00% (± 19.993) in patients without these anomalies and this difference was also statistically significant (p=0.029).

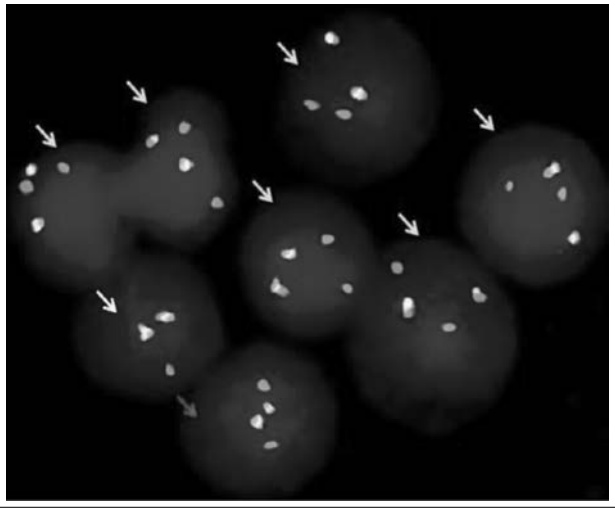

Figure 1.

Summary/Conclusions: Our work would be an appropriate reference material for I-FISH signal interpretation using BCR/ABL/ASS1 TCDF probe. We have demonstrated a high frequency of der(9) deletions, clonal heterogeneity and absence of *BCR-ABL1* amplification in an imatinib-resistant Indian CML cohort. For the first time, a significant association of der(9) deleted cell percentage with b2a2 transcript type and disease transformation status has been identified and the same has to be tested in a larger cohort.

PB1806

ARE YOU ACTUALLY SUSPECTING A CHRONIC MYELOID LEUKEMIA WHEN ORDERING A BCR/ABL RT-PCR?

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Background: Chronic myeloid leukaemia (CML) is a myeloproliferative neoplasm (MPN). It is characterized by a reciprocal t(9:22)(q34;q11.2) resulting in the fusion oncogene *BCR/ABL* in a hemopoietic stem cell. Clinical features are absent in nearly 20-40% of patients at diagnosis time. Hence, laboratory suspicion is crucial. Peripheral blood shows leukocitosis with left shift and "myelocyte bulge", absolute eosinophilia, and absolute basophilia invariably present[1-3]. The demonstration of the Philadelphia (Ph) chromosome with cytogenetic analysis, or *BCR/ABL* fusion gene by qRT-PCR will confirm the diagnosis (typical CML).
Aims: In order to gain accuracy when *BCR/ABL* PCR is ordered, we review myeloproliferative hematimetric parameters, with special focus in basophilia, before performing molecular analysis.
Methods: We retrospectively reviewed 299 *BCR-ABL* PCR requests received at our laboratory between January 1, 2015 and January 1, 2017. 80% of the total requests were ordered by haematologists physicians, 13.46% by other medical specialities (11.5% internal medicine) and 7.7% from the laboratory. Complete blood cell count (CBC) were analysed by ADVIA 2120. Neutrophilia was defined in our laboratory as absolute neutrophil count of >7.7x10 9/L, and basophilia was defined as absolute basophil count of >0.2 x10 9/L. A total of 299 requests for PCR of *BCR-ABL* were reviewed by laboratory hematology staff, before performing them, according to 2008 WHO Classification of Hematologic neoplasms criteria. We reviewed clinical history, previous CBC and PBM if necessary for this screening. We performed 235 test (78.6%) and 64(21.4%) were considered inadequate according former criteria. qRT-PCR p210 was performed and if a negative result was obtained with high CML suspicion qRT-PCRp190 and qRT-PCRp230, such as cytogenetic studies were performed.The statistical analysis was performed with STATA.
Results: 235 *BCR/ABL* by PCR tests were performed and 24 (10.21%) resulted positive. 167 (71.06%) were placed for neutrophilia; 41 (17.87%) for thrombocytosis and 26 (11.07%) for other criteria (eosinophilia, monocytosis, splenomegaly or combined).Among 24 positive cases 100% presented basophilia at diagnostic time and 91.66% (22/24) presented basophilia and neutrophilia. Two cases without neutrophilia at diagnosis were CML with extreme trombocytosis. We found 33 cases with basophilia among 235 patients. 24 cases (72.73%) were diagnosed of CML and 9(27.27%) resulted in other MPN Ph- or unclassifiable MPS/MDS neoplasm. Our results show that when CML is suspected, basophilia>0.3 x10 9/L has a 100% sensitivity and 95.75% specificity. ROC curve for basophilia as a screening test before performing *BCR/ABL* PCR is 0.984 (Figure 1).

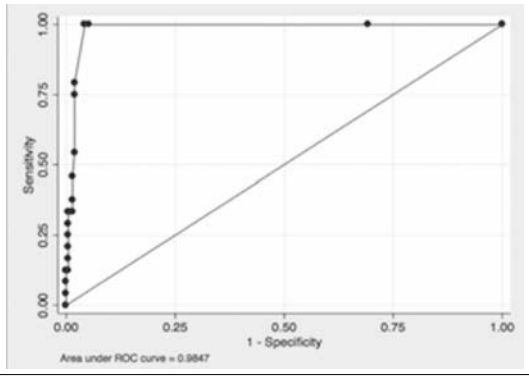

Figure 1.

Summary/Conclusions: Our results show that basophilia should be carefully investigate when CML is suspected, with high sensibility (100%) and specificity (95.75%). In cases no CML with basophilia >0.3 x10 9/L, further investigation should be performed in order to diagnose a MPN Ph- or MDS/MPN.Even basophilia is well stablished as nearly universal in CML [1,3,4], this study reveals it is not always pursue enough, when clinicians ask for a molecular study.

PB1807

BCR-ABL DEL. C.1086-1270 (P.R362FS*21) AND TKI RESISTANCE IN CML PATIENTS FROM RUSSIAN FEDERATION

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Background: Data concerning the impact of BCR-ABL del. c.1086-1270 on TKI resistance in CML is still controversial. This mutation was first described by Curvo *et al.* (2008) and was thought to confer TKI resistance. However computer modeling performed by Meggyesi N. *et al.* (2012) revealed disruption of ATP binding site in mutated tyrosine kinase therefore abrogating enzymatic activity. Nevertheless pathogenic effect of BCR-ABL p.R362fs*21 could be attributed to the formation of heterodimer with "wild type" Bcr-Abl p210 as described by Poulikakos P.I. *et al.* (2011).
Aims: To assess the impact of BCR-ABL del. c.1086-1270 (p.R362fs*21) on TKI resistance in CML patients from Russian Federation.
Methods: 33 male and 49 female CML patients (age 24-80) with BCR-ABL transcript level >0.1% were included in the study. BCR-ABL del. c.1086-1270 was estimated by nested PCR followed by Sanger sequencing. Initial screening for deletions was performed by means of fragment analysis (Applied Biosystems 3130).
Results: 92 RNA (cDNA) samples isolated from peripheral blood of 82 CML patients were tested. BCR-ABL del. c.1086-1270 (p.R362fs*21) was found in 32 patients (39%). 15 out of 32 (47%) patients with deletion were TKI sensitive while 17 (55%) were TKI resistant. In one TKI resistant case BCR-ABL del. c.1086-1270 was accompanied by BCR-ABL c.844G>C p.E282Q point mutation not described so far (Figure 1). This mutation was found in BCR-ABL del. c.1086-1270 transcript only and was absent in "wild type" Bcr-Abl p210 transcript amplified from the same patient.

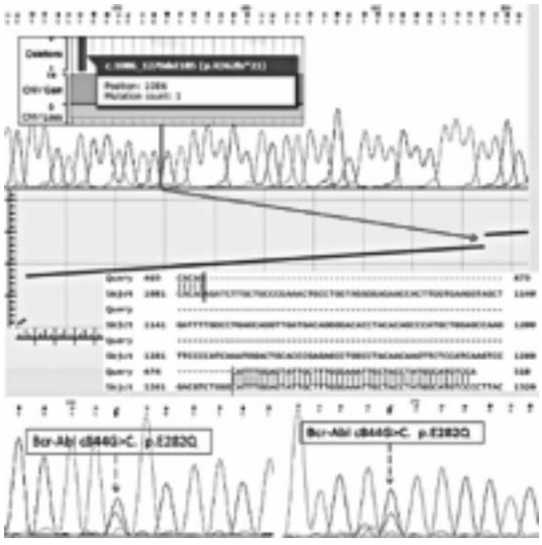

Figure 1.

Summary/Conclusions: BCR-ABL del. c.1086-1270 could be found in almost half of CML patients and have no evident impact on the induction of big molecular response in TKI sensitive cases. Our observation that independent c.844G>C p.E282Q point mutation expressed on the same BCR-ABL transcript with deletion c.1086-1270 (p.R362fs*21) being absent in "wild type" transcript strongly contradicts the hypothesis, that del. c.1086-1270 could be generated by alternative splicing of "wild type" BCR-ABL transcript.

PB1808

PEROXIREDOXIN II ACTIVITY HAS IMPORTANT ROLES TO CONTROL ABL TYROSINE KINASE ACTIVITY IN STIS TREATED CML PATIENTS AND ITS POTENTIAL APPLICATION IN IMATINIB RESISTANCE

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Background: Therapies targeting the redox environment such as over-expression of antioxidants or antioxidant treatment, could inhibit tumor cell growth even resistant cells. Bcr-Abl oncogene is known to induce high levels of intracellular ROS which may further induce genomic instability with malignant transformation and even imatinib (IM) resistance. Variable expression of antioxidants enzymes in leukemia, with limited studies with variable results so far. Altered redox biology in leukemia also has implications for therapeutics.
Aims: We investigated the roles of PRX II in CML primary cells at diagnosis and remission during signal transduction inhibitor (STIs), and tested the same roles in Ph+ cell lines.
Methods: Three BCR-ABL1 positive cell lines with different resistance to TKI and generating IM-resistant K562 cells by chronic exposure of increasing concentrations of IM were compared with cell growth by MTT assay, BCR/ABL expression by western blot analysis, changes of intracellular ROS level and antioxidant enzymes such as peroxiredoxin (Prx) 1, 2, 3 using immunoblot assay according to different concentrations of IM between 0 to 10 μM in time dependent manner (24 hours/48 hours). We also repeatedly investigated the effects of IM therapy using PRXII overexpressed K562 cells by transfection.
Results: Three BCR-ABL1 positive cell lines showed significant change in cell viability, Intracellular ROS level, eradication of BCR/ABL oncogene and levels of Prx2 during IM treatment with different response each other in degree and pattern by IM exposure. The levels of BCR-ABL1 oncogene were slightly decreased in Prx2 overexpressed K562 cells. Moreover, Prx2 overexpressed K562 cells showed further down-regulation of Bcr-Abl oncoprotein by IM treatment.
Summary/Conclusions: Our findings may contributes to find a new pathway on which TKIs are working besides the mechanisms of ATP binding competitively, blocking the binding of ABL-BCR kinase and substract resulting apoptosis of Ph+ cells. In addition develop the new strategies to overcome the situation of the Imatinib resistance in P210 BCR-ABL positive disease in the future. The importance of the roles of ROS and its PRX II, antioxidant enzymes in CML is further established by our work.

PB1809

FUNCTIONAL CHARACTERISTICS OF ERYTHROID PROGENITOR CELLS OF PATIENTS WITH CHRONIC MYELOID LEUKEMIA TREATED WITH IMATINIB AND NILOTINIB

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Background: It is believed that chronic myeloid leukemia arises as a result of myeloid progenitor cell malignancy. There are changing of proliferative activity in granulocyte-macrophage and erythroid hematopoiesis germs in patients bone marrow. Currently we don't have definitive results regarding tyrosine kinase inhibitors influence on erythropoietic cell characteristics of patients with CML.
Aims: the aim of study was to determine functional characteristics of erythroid progenitor cells of patients with chronic leukemia treated with Imatinib and Nilotinib.
Methods: We studied 300 bone marrow samples from 75 patients: with initial diagnosis of CML (n=7), patients receiving drug imatinib (n=47) and patients who taking nilotinib (n=21). We provide studying of erythroid mononuclears in semisolid *in vitro* and *in vivo* cultures. For *in vivo* culture we used special gel capsule, allowing cytokines and growth factors of mouse body affect human mononuclear cells. For *in vitro* culture we added 20% fetal calf serum, 30 ng/ml erythropoietin, and 20 ng/ml mlinterleykin-6 and interleukin-9. Cultivation was provided 14 days, then counted the number of erythroid colonies and provided their morphological studies.
Results: The results showed that the increase of erythroid progenitor cells proliferation rates and the reduction of differentiation rates as a result of the parallel cultivation of patients' bone marrow cells *in vivo* and *in vitro* happen irrespective of the presence of cytokines and growth factors in a normal microenvironment of these cultures. In addition, we showed that bone marrow cells of CML patients form erythroid colonies, when placed in the animals' body without previous anemia. Moreover, correlative relationship was found between the number of erythroid colonies and the number of leukemic cells in the patients bone marrow. It was established that the acquisition of leukemic clone cells resistance to TKI is characterized by increased proliferative activity irrespective of soluble microenvironment factors as well as the culture medium in the erythropoietin presence.
Summary/Conclusions: The normal microenvironment factors not effect on the erythroid progenitor cell proliferation independence of the response to TKI therapy. This may explain the fact that we don't have an increase the number of erythroid cells in patient bone marrow compared to culture *in vitro.* In addition, the ability of erythroid progenitor cells to form colonies in the absence of erythropoietin in culture can serve as an additional prognostic factor in the formation of resistance to TKI.

PB1810

DEVELOPMENT OF FRAGMENT ANALYSIS MULTIPLEX-PCR METHOD TO DETECT TRANSCRIPTS OF BCR-ABL FUSION GENE IN CHRONIC MYELOID LEUKEMIA

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Background: Chronic myeloid leukemia (CML) is a myeloproliferative, clonal and acquired hematological disease that is included within myeloproliferative neoplasms (WHO 2016). Its main characteristic is the presence (95% of the

cases) of a small chromosome denominated Philadelphia chromosome, coming from the reciprocal translocation between the chromosomes 9 and 22. Depend where the break-point occurs, different isoforms of the fusion gene BCR-ABL may appear. For the diagnosis of CML, detection of BCR-ABL rearrangement is crucial; and molecular biology techniques, such as RT-PCR, may be the only data at that point, but most current RT-PCR methods for detecting BCR–ABL are designed and optimized for detecting the majority forms (e14a2 and e13a2) without distinguishing between them. Characterization of the transcript is not necessary for the diagnosis but permits follow-up at the molecular level and differentiate between different BCR-ABL isoforms at the time of the CML diagnosis could be taken into account in future studies to investigate its role into the prognosis.
Aims: To develop a new multiplex RT-PCR method coupled to fragment analysis by capillary electrophoresis to identify different BCR-ABL isoforms: e13a3, e19a2, e14a3, e6a2, e1a3, e13a2, e14a2, e1a2 and e8a1.
Methods: 34 CML patients BCR-ABL positive by qRT-PCR and 1 negative control for BRC-ABL fusion gene were included in this study. Three hundred nanograms of total RNA from leucocytes were used for retro-transcription (SuperScript ® IV). Subsequently, Multiplex PCR reactions were assessed using primers described by Burmeister in 2008 [ABL-3 primer labeled with carboxyferrescein (FAM)]. G6PD was chosen as endogenous gene control using G6PD-F labeled with hexachloro-fluorescein phosphoramidite (HEX). Capillary electrophoresis of the multiplex RT-PCR reaction was done in an ABI3130XL analyzer, using ILS600 as marker.
Results: BCR-ABL fusion RNAs were detected in all patients (34/34), on the other hand we did not detect BCR-ABL on the negative control. The main isoform identified was e14a2 (detected in 22 out of 34 patients, 64.7%). Twelve patients were positive for e13a2 BCR-ABL isoform (35.3%). Interestingly we identified 7 patients (20.5%) with co-expression of e14a2 and e13a2 isoforms, being in all these cases the e14a2 isoform mainly expressed.
Summary/Conclusions: RT-PCR combined with capillary electrophoresis is revealed as a sensitive technique for the detection of different isoforms of BCR-ABL and may be included as a BCR-ABL first screening. Quantification with qRT-PCR might only be done in positive samples. Unfortunately we could not detect any isoform besides the majority ones, due to the size of our cohort. Finally, our study validates previous studies on the main BCR-ABL isoforms (e14a2 and e13a2) percentage detected in CML patients.

PB1811

Abstract withdrawn.

PB1812

PDGF AND BDNF PLASMA LEVELS IN CML PATIENTS BEFORE AND AFTER INITIATION OF TKI THERAPY

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Background: Chronic myeloid leukemia (CML) is a malignant myeloproliferative neoplasm, which is characterized by t(9;22)(q34.1;q11.21) translocation, also known as the Philadelphia chromosome (Ph). The resulting fusion gene *BCR-ABL1* encodes a constitutively active tyrosine kinase that dictates the pathophysiology of CML. Tyrosine kinase inhibitors (TKIs) have been shown to efficiently inhibit not only the Bcr-Abl kinase, but also act on other cell surface tyrosine kinase receptors, such as the platelet-derived growth factor receptor (PDGF-R). Similar receptors are vital in neurotrophin-mediated signaling pathways, for example TrkB receptor for brain-derived neurotrophic factor (BDNF). PDGF is a potent mitogen for cells of mesenchymal origin and plays a significant role in angiogenesis, a process which has recently been recognized as crucial in growth and survival of neoplastic cells of the hematopoietic system. BDNF acts on certain neurons of the central nervous system and the peripheral nervous system and has a wide role in neuroprotection and neuroregeneration. However, the exact roles of PDGF, BDNF and their receptors in normal and malignant hematopoiesis remain unclear.
Aims: In this study we aimed to investigate the levels of PDGF-AA and BDNF in plasma from CML patients and, where possible, to identify how TKI treatment affects these proteins levels.
Methods: Peripheral blood samples were obtained from newly diagnosed CML patients (n=5), CML patients treated with TKIs (n=5) and healthy controls (n=10). Informed consent was obtained from all subjects included in the study. Plasma PDGF-AA and BDNF levels were analyzed using Luminex technology with Human Neurodegenerative disease Panel 3 kit (Merck Millipore, Billerica, USA).
Results: We have observed that PDGF-AA levels were elevated in CML group (both before and during TKI treatment) compared to controls. Interestingly, we have noticed that PDGF-AA level for newly diagnosed CML patients was higher compared to TKI-receivers ($p < 0,05$). In case of BDNF, we have observed subtle changes between the tested groups: BDNF level in newly diagnosed CML subjects was lower compared to controls ($p < 0,05$), but in TKI-receivers the level was comparable to control group ($p > 0,05$). We have also tested one patient in subsequent time points (at diagnosis, 3 months with TKIs, 6 months with TKIs) for both PDGF-AA and BDNF - we have observed PDGF levels drop and BDNF rise with time.
Summary/Conclusions: In our study we have demonstrated that PDGF-AA and BDNF are feasible targets for plasma proteomic analysis in CML patients, both for studying general patterns of protein expression and also for identifying proteins differentially expressed before and during TKI treatment. We have shown that PDGF level drops down after TKI treatment, while on the opposite BDNF level in plasma raises with time in CML patients receiving TKIs. We have also monitored these proteins levels over time in the same subject, but this requires a larger study group to draw meaningful conclusions. Further studies are required to elucidate the PDGF, BDNF and possibly other growth factors, neurotrophins and their receptors role in normal and malignant hematopoiesis.

PB1813

A CASE OF ATYPICAL CHRONIC MYELOID LEUKEMIA WITH LATE DISCOVERY OF JAK2

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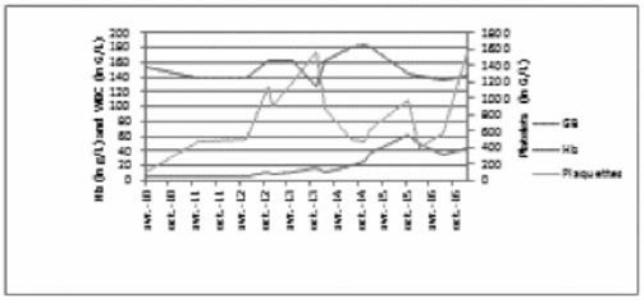
Background: Myeloproliferative neoplasms (MPN) include on the one hand chronic myeloid leukemia defined by the presence of Philadelphia chromosome and BCR-ABL remodeling, and on the other hand MPNs without Philadelphia chromosome (Polycythemia vera [PV], essential thombocythemia [ET] and primary myelofibrosis [PMF]). V617F JAK2 mutation is the main recurring genetic abnormality in these pathologies (1). It can be found in 95% of PV and 50% of ET and PMF (2). The 2016 WHO classification makes no proposal of an entity which would include BCR-ABL+and V617F JAK2+CML. However 28 of those cases were described in a 2013 literature review (9). Most patients developed either a V617F JAK2 mutation during treatment by tyrosine kinase for a BCR-ABL+CML; or a BCR-ABL+CML during treatment for a V617F JAK2+MPN (3,4,5,6,7). A very small number of patients showed coexistence of those two mutations (8).
Aims: We report a 62y old woman patient with chronic myeloid leukemia with late discovery of JAK2.
Methods: *Clinical presentation:* A 62-year-old man with no notable medical history was admitted in 2009 for CML. After failure of a first line treatment by Imatinib in 2009 (poor tolerance and incomplete molecular response), treatment by Nilotinib was initiated in 2012 allowing for, to this day, good molecular response despite poor digestive tolerance in the form of dyspepsia. Ever since 2012, the patient has had thrombocythemia (platelets: 826 G/L) followed then by polycythemia (Hb: 16.7–19 g/dL) that were first attributed to hemoconcentration and inflammation due to recurring bacterial urinary tract infections. Neither infiltration of the lymph nodes nor organomegaly had been noted. In 2014, the patient complained of abundant sweating in the absence of any fever and, despite the ongoing complete molecular response, hyperleukocytosis was observed (see Figure 1A). In 2015 the patient, then aged 68, signaled weight loss of 10 kg despite decent overall state of health. Tomodensitometry found evidence of hepatosplenomegaly. Taking into account the symptoms and persisting blood count abnormalities (WBC 27.7 G/L, Hb 182 g/L, Platelets 479 G/L, Neutrophils 22.4 G/L, erythromyelemia) a second MPN was suspected. V617F JAK2 mutation was found positive and treatment by Hydrea for essential thrombocythemia was initiated. Adaptation of Nilotinib posology was decided to avoid possible cytopenia due to its association with Hydrea.
Results: *Evolution:* (see Figure 1B) As of the last follow-up consultation in 2017, BCR-ABL remains undetectable and the overall state of health was preserved. Hyperleukocytosis as well as myelemia were persistent on the blood count whereas hemoglobin and platelets had normalized. To determine whether or not V617F JAK2 mutation was present at the time of CML diagnosis, a 2009 sample, in which JAK2 V617F had been estimated at less than 1%, was reanalyzed by means of molecular biology in January 2017.
This exam found the mutation in quantities below the clinical significance threshold (1%). But this positivity, however small (0.19%), shows preexistence of the pathological clone.
Summary/Conclusions: This patient's case can be integrated in the series of cases described in 2013 by Park *et al.* (9) as it consists of V617F JAK2 positive ET onset during treatment for a BCR-ABL positive CML. The physiopathology of those two pathologies has not yet been genetically determined (8). Are those two independent pathologies or do they share a common tumoral clone? In this case JAK2 and BCR-ABL evolved in negative correlation and as such it is surmised that there were in fact two independent diseases, with two preexisting pathological clones at the time of the first diagnosis, treatment of the first pathology having been responsible for the proliferation of the second clone.

A) evolution of white blood cells count, hemoglobin and platelet count from April 2010 to October 2016



B) evolution of BCR-ABL transcript and V617F JAK2 mutation from 2009 to 2016

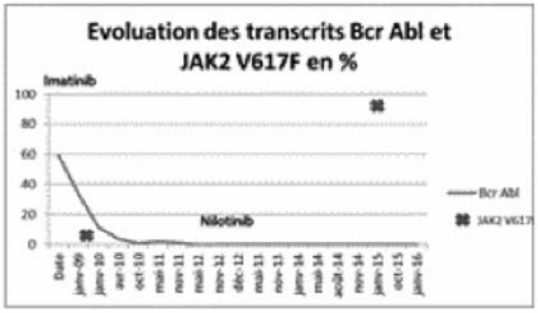


Figure 1.

Chronic myeloid leukemia - Clinical

PB1814

E14A2 TRANSCRIPT IS ASSOCIATED WITH HIGHER PROBABILITY OF DURABLE TREATMENT FREE REMISSION IN CML PATIENTS

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Background: TKIs discontinuation in CML-CP patients with deep molecular response (DMR) are feasible, safe and 40-60% of them maintain treatment free remission (TFR); sokal risk score and duration of TKI-therapy were significantly associated with molecular relapse, according to Euro-Ski and STIM1 trials. While it is known that patients with e14a2 achieve earlier, deeper and more durable responses compared to those with e13a2, few information is available on the influence of the type of bcr-abl transcript on TFR duration
Aims: Here we describe our single center experience of TKI discontinuation in CML-CP patients with sustained DMR
Methods: Bcr-abl transcripts were determined by RQ-PCR analysis performed in accordance with EAC protocol (Gabert et al, Leukemia 2003) and to the standards of the Italian national network Labnet. All 174 CML-CP patients presently followed at our institution according to ELN guidelines and treated with 1st or 2nd TKIs were analysed: 103 (59%) had e14a2 and 69 (40%) e13a2 transcript (in 2 pz bcr-abl were not detectable). Criteria for TKI discontinuation was sustained DMR (MR4 or better) for at least 2 years. After TKI withdrawal, RQ-PCR for BCR-ABL was performed every month during the first year and every 2 months thereafter. TKI treatment was reintroduced immediately if DMR loss occurred. TFR was defined as the time between the date of TKI cessation and the date of restarting treatment for DMR loss or, if TKI was not resumed, the date of the last contact.
Results: Forty-nine patients, 25 male and 24 female, discontinued TKI treatment. At the time of discontinuation median age was 63 years (43-85), median time from TKI start 113 months (30-172), median duration of sustained DMR 60 months (24-153). Sokal distribution was 49%, 29% and 20% for low, intermediate and high risk (one patient was not evaluable). Among our 174 patients 39% (40/103) of all e14a2 patients and 13% (9/69) of all e13a2 discontinued TKI (P 0.0002, chi square). Thirty-six patients discontinued imatinib (11 of them with previous INF treatment), 13 stopped nilotinib (8 in first line, 5 in second line treatment). Median follow up after treatment discontinuation was 19 months (3-76), including 31 patients with follow up > 12 months. Thirteen (26%) patients lost DMR. Median time off-therapy for these patients was 3 months (2-8), and only 1 lost DMR after 6 months. Therapy was restarted in all 13 patients (2 in MR1, 4 in MR2, 7 in MR3), 10 achieved a second DMR after a median interval of 2 months (1-7); 2/13 patients are in M3 after 7 and 12 months, 1 patient is not yet evaluable. Univariate analysis showed no difference in relapse risk according to age, gender, type and duration of TKI, duration of stable DMR and sokal score risk. Ten out of 11 patients treated with INF before imatinib remained in TFR. Of note, the type of bcr-abl transcript was significantly linked to DMR loss: after TKI discontinuation, 32/40 e14a2 patients (78%) maintained DMR *vs* 4/9 e13a2 patients (42%) (p 0.03). After 12 months 78% (+/-6% CI95%) of e14a2 and 41,6 (+/-17% CI95%) of e13a2 patients were still in TFR (log-rank: P=0.033) (see Figure 1). Using multivariate analysis the type of bcr-abl transcript and previous INF treatment correlated with DMR loss (p 0.012 and p 0.033). One patient died during follow up in DMR for CML-unrelated cause.

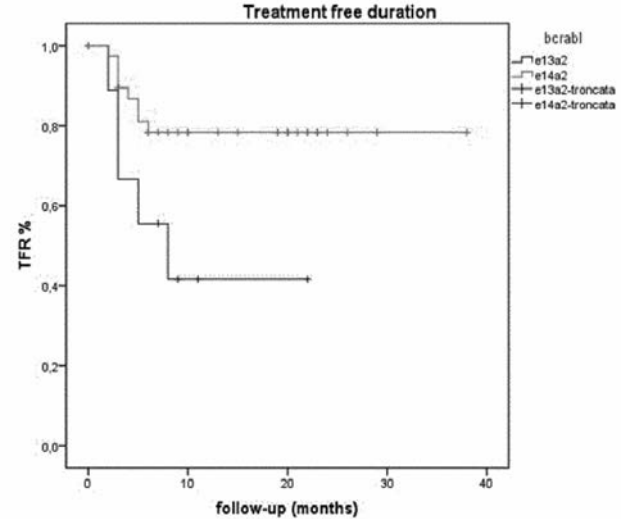

Figure 1.

Summary/Conclusions: In e14a2 CML patients the probability of discontinuation for sustained DMR is significantly higher as compared with e13a2 patients. Moreover, after discontinuation, e14a2 have significantly lower probability of DMR loss than e13a2. These data confirm that e14a2 transcript is associated with a more favorable CML disease profile than e13a2 (Jain *et al.*, Blood 2016); in addition they show that e14a2 is a favorable prognostic factor for TFR maintenance

PB1815

COMPARATIVE ANALYSES OF NILOTINIB *VS* HIGH-DOSE IMATIB *VS* SUSTAINING STANDARD-DOSE IMATINIB IN PATIENTS WITH CP CHRONIC MYELOID LEUKEMIA FOLLOWING SUBOPTIMAL MOLECULAR RESPONSE TO FIRST-LINE IMATINIB

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Background: Imatinib (IM) and its generic form are widely used as one of the standards of care for chronic phase (CP) chronic myeloid leukemia (CML). Although 7-year data by the IRIS demonstrated the long-term prognostic value of molecular response at specific time points, achieving major molecular response (MMR) at 18 months showed minimal event-free survival (EFS) benefit, compared with not achieving MMR but having complete cytogenetic response (CCyR). In addition, the best treatment for these patients remains less clear.
Aims: In this study, we investigated the efficacy of nilotinib (NIL) *versus* high-dose IM *versus* sustaining standard-dose IM for the patients in CCyR with suboptimal molecular response to first-line IM therapy.
Methods: Early CP CML patients who have achieved CCyR but not MMR after 18 to 24 months on first-line IM therapy at a daily dose of 400 mg were divided into the three treatment groups; nilotinib (NIL) 400mg BID (800 mg/day; group 1) *vs* IM 400 mg BID (800 mg/day; group 2) *vs* IM 400mg QD (400mg/day; group 3). Group 1 and 2 patients were selected in the RE-NICE multicenter study, in which crossover to the alternate treatment arm was allowed for patients failing to achieve a MMR at 12 months and for intolerant patients, and for patients who lost MMR at any time of treatment. Group 3 patients who have achieved CCyR but not MMR after at least 18 months of first-line IM therapy were selected from the Asia CML Registry (ACR) database system with the same inclusion criteria of RE-NICE. The efficacy endpoints are MMR rate by 12 months and MMR rate and undetectable molecular residual disease (UMRD) rates by 36 months.
Results: With a data cut-off date of 07 Dec 2016, a total of 108 patients were evaluated; 28 patients in NIL group (group 1), 28 patients in high-dose IM group (group 2), and 52 patients in standard-dose IM group (group 3). Median follow-up duration from enrollment was 36 months (range, 1-36), 45 months (range, 21-63), and 90 months (range, 14-159) for each group, respectively. All patients in group 1 remained NIL treatment, 18 patients in group 2 crossed over to NIL 400mg BID due to intolerance (n=4) and lack of response (no MMR after 12 months; n=14), in group 3, 22 patients switched to other treatment due to intolerance (n=7), lack of response (no MMR; n=12), failure (n=1), or treatment-free remission trial (n=2) and 2 patients lost to follow-up. When data on patients who crossed over to the other treatment was included, cumulative incidence (CI) of MMR by 36 months was significantly higher in group 1 than group 3 (83.1% *vs* 57.1%, P=0.021), but there was no different in group 1 *vs* 2 (P=0.195) and group 2 *vs* 3 (P=0.297). CI of $MR^{4.5}$by 36 months showed a trend of higher in group 1 than the other two group (11.7% *vs* 0% *vs* 2.6%, group 1 *vs* 2, P=0.066, group 1 *vs* 3, P=0.099, group 2 *vs* 3, P=0.405).
Summary/Conclusions: NIL 400mg twice daily treatment showed better efficacy than standard-dose IM for the treatment of patients who have suboptimal molecular response to first-line IM. Additionally, a switch to NIL in suboptimal molecular responder to IM had a trend for achieving a $MR^{4.5}$more frequently, suggesting the potential benefit of a treatment-free remission.

PB1816

COMPARATIVE ANALYSIS OF PULMONARY HYPERTENSION IN THE 105 CML PATIENTS TREATED WITH IMATINIB, NILOTINIB AND DASATINIB

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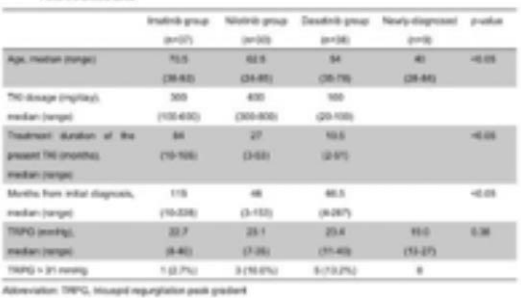
Background: Pulmonary hypertension (PH) has been reported as a serious adverse event in chronic myeloid leukemia (CML) patients treated by dasatinib. French group reported incidence of PH diagnosed by cardiac catheterization as 0.45% (13 of 2,900 patients) in symptomatic patients treated with dasatinib. Dasatinib-related PH usually resolves after cessation of treatment, but it can be fatal, as two deaths in France and one in Japan have been documented.
Aims: To clarify the incidence of tyrosine kinase inhibitor (TKI)-related PH, we noninvasively screened CML patients who have been given imatinib, nilotinib or dasatinib by echocardiography.
Methods: 105 patients with CML in chronic phase (CP) who received TKI were enrolled in this study between 2014 and 2015. Nine patients with newly diagnosed CML in CP prior to TKI treatment were added as control. Patients underwent echocardiography to evaluate values of tricuspid regurgitation pressure gradient (TRPG), which relates to severity of PH. Patients with TRPG values >31mmHg were suspected of PH onset according to European Society of Cardiology criteria. All patients gave informed consent.
Results: Patients were divided into 3 groups by the TKIs they used at the time of study enrollment; 37 patients on imatinib, 30 on nilotinib and 38 on dasatinib (Table 1). In imatinib group, patients' age was significantly higher, and duration of treatment was also longer than those of the 2nd generation TKIs. Echocardiography revealed mean values of TRPG as 22.7, 23.1 and 23.4mmHg in imatinib, nilotinib and dasatinib groups, respectively (p=0.887), and these values were higher than that in the newly diagnosed CML patients (19.0mmHg), though without significance (p=0.38). Nine of the 105 patients (8.6%) presented with an elevated TRPG>31mmHg, suggesting the presence of PH; 1 of 37 (2.7%) in imatinib group, 3 of 30 (10.0%) in nilotinib group, and 5 of 38 (13.2%) in dasatinib group. Three patients complained of dyspnea, while the remaining 6 were asymptomatic. We found no apparent risk factors associated with TRPG elevation, however, there were trends toward correlation of age and TRPG values in nilotinib and dasatinib treated patients, and treatment duration and TRPG values in nilotinib treated patients. Imatinib dosage tended to inversely correlate with TRPG value, suggesting that imatinib might decrease pulmonary arterial blood pressure in a dose-dependent manner.

Table 1.



Patient characteristics					
	Imatinib group (n=37)	Nilotinib group (n=30)	Dasatinib group (n=38)	Newly diagnosed (n=9)	p-value
Age, median (range)	73.5 (38-83)	62.5 (34-85)	54 (26-79)	40 (28-84)	<0.05
TKI dosage (mg/day), median (range)	300 (100-400)	400 (300-800)	100 (20-100)		
Treatment duration of the present TKI (months), median (range)	84 (10-105)	27 (3-53)	10.5 (2-97)		<0.05
Months from initial diagnosis, median (range)	119 (10-338)	46 (3-113)	66.5 (4-267)		<0.05
TRPG (mmHg), median (range)	22.7 (8-40)	23.1 (7-35)	23.4 (11-40)	19.0 (13-27)	0.38
TRPG > 31 mmHg	1 (2.7%)	3 (10.0%)	5 (13.2%)	0	

Abbreviation: TRPG, tricuspid regurgitation peak gradient

Summary/Conclusions: PH is a rare but life-threatening adverse event for dasatinib-treated patients, and its definitive diagnosis is made by cardiac catheterization. However, cardiac catheterization is too invasive for PH screening of the many patients with TKIs who do not have any symptoms. Our study, by using echocardiography, detected TRPG elevation not only in dasatinib treated patients (13.2%) but also in imatinib (2.7%) and nilotinib (10%), including patients without any symptoms. This indicates possible PH onset among patients treated with imatinib or nilotinib, as well as with dasatinib. Although TRGP values obtained by echocardiography might not be fully compatible with those by cardiac catheterization, the results suggested that noninvasive echocardiography is sensitive for screening PH and is also effective for easily screening groups of patients with suspect subclinical PH among patients treated with any available TKIs. Careful screening with echocardiography is necessary especially for older patients who have received TKIs for a long time.

PB1817

DYNAMICS OF BCR-ABL1 MUTATION ACQUISITION AND LONG-TIME MUTATION ASSOCIATED RESISTANCE PROGNOSIS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA TREATED BY TYROSINE KINASE INHIBITORS: RUSSIA, 2006-2016

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Background: While chronic myeloid leukemia (CML) can successfully be treated with tyrosine kinase inhibitors (TKIs), mutations in the BCR-ABL1 kinase domain are the most prevalent cause of TKI resistance. More than 100 *BCR-ABL1* kinase domain point mutations with various frequencies of incidence, domain positions and implications on TKI response in CML are associated with

TKI resistance. Here we present our data concerning prognostic significance of *BCR-ABL1* kinase domain mutations dynamics in Russian CML patients according the follow-up study having been performed during the last 10 years.
Aims: To determine the frequency dynamics of *BCR-ABL1* mutations in CML patients and its prognostic significance.
Methods: In this study we have included 1077 TKI resistant CML patients from 112 hospitals of 77 Russian cities having been observed during the period from 2006 to 2016. *BCR-ABL1* kinase domain point mutations in mRNA samples from peripheral blood cells were analyzed by means of PCR followed by Sanger sequencing. Statistical analysis was performed using SPSS 22.0 (IBM, USA) and Excel 2013 (Microsoft, USA). Critical p-value was set to 0.05.
Results: 1077 TKI resistant CML patients were analyzed, among them were 41,5% men (n=447) and 58,5% women (n=630), median age – 50 (from 15 to 74). *BCR-ABL1* mutations were found in 30,8% (332/1077) CML pts. We have detected a total of 415 mutations in 332 patients, giving rise for 58 different mutation variants. Mutation associated resistance rate was higher in women to compare with men (72,3% against 63,2%, *p=0,001*). It turned out that F317L and H396R mutations were statistically more frequent in women, meanwhile T315I mutation prevailed in men group (Pearson's χ^2<0,05). It was of a sudden that *BCR-ABL1* mutation distribution significantly varied according the particular CML pts city location throughout the different regions of Russia. Although for the period from 2006 to 2016 there were no detectable changes in mutation frequency spectrum (Pearson's χ^2 is 0,062), the total amount of mutations associated with TKI CML resistance has decreased from 36,6% in 2006-2008 to 24,95% in 2013-2016, but still remained significant. For particular mutations the following dynamics was detected: frequency of imatinib-resistant mutations decreased gradually from 2006 to 2016, while the rate of F317L and F359V mutations underlining resistance to second generation TKI increased in 2013-2016. T315I mutation rate expanded to the maximal level in 2014 and abruptly decreased afterwards. This tendency change may be the consequence of the second generation TKIs and other therapeutic strategies involvement into clinical practice.
Summary/Conclusions: As far as different BCR-ABL1 kinase domain mutations are associated with various types of mutation associated resistance to TKI treatment, the detection of trends in mutation distribution in CML patients receiving TKI treatment is very important for long time treatment strategy decision making and early prognosis of resistance. We believe here that regional difference of mutation profiles should also be considered. Therefore, to enable correct triggering of particular types of TKI for CML treatment it is necessary to obtain data of when, which and where a particular type of BCR-ABL1 mutation is prone to appear in a distinguished cohort of CML pts.

PB1818

IMPACT OF BCR-ABL1 TRANSCRIPT TYPE IN CHRONIC MYELOID LEUKEMIA TREATED FRONTLINE WITH NILOTINIB

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Background: Chronic myeloid leukemia (CML) is driven by different transcript types, but the majority of patients have a e13a2 (b2a2) transcript, a e14a2 (b3a2) transcript or a co-expression of e13a2/e14a2 transcripts. In imatinib-treated patients, the e13a2 transcript has been associated to slower and inferior molecular responses. Few data on the prognostic impact of BCR-ABL1 transcript type in CML patients treated with second generation tyrosine kinase inhibitors (TKIs) are still available.
Aims: To assess the impact of BCR-ABL1 transcript type on molecular response and outcome in newly diagnosed adult CML patients treated frontline with nilotinib (NIL).
Methods: An analysis of 345 CML patients at diagnosis (chronic phase) enrolled within 3 multicentric prospective studies of the GIMEMA CML Working Party (NCT00481052, NCT00769327, NCT01535391) was performed. The initial treatment was NIL 300 mg BID or NIL 400 mg BID. Definitions: major molecular response (MMR), BCR-ABL1^{IS} ratio <0.1%; deep molecular response ($MR^{4.0}$), BCR-ABL1^{IS} ratio <0.01% with >10,000 ABL1 copies; progression, transformation to advanced phases; death, at any time and for any reason. Cumulative incidences of response were estimated under consideration of competing risks (progression, death) and compared by Gray test. Progression-free survival (PFS) and overall survival (OS) were estimated using the Kaplan-Meier method and compared by log-rank test.
Results: Patients expressing rare transcripts (e1a2 or e19a2; n=7) and patients with unknown transcript type (n=10) were excluded: 328 patients were evaluable, 38% with e13a2 transcript, 53% with e14a2 transcript and 9% expressing both transcripts. No significant differences in age, gender, Sokal or EUTOS long-term survival score distribution, presence of clonal chromosomal abnormalities in Ph+ cells, or NIL dose were observed. The median follow-up was 60 months (range 24-82 months). The response rates and the survival probabilities were uniformly lower in patients with e13a2 transcript (N=124) compared to patients with e14a2 transcript (N=174), but the differences were not significant: MMR by 12 months, 66% *vs* 72%, p=0.244; $MR^{4.0}$ by 36 months, 56% *vs* 66%, p=0.067; estimated cumulative incidence of MMR, 82% *vs* 88%, p=0.135; estimated cumulative incidence of $MR^{4.0}$, 60% *vs* 69%, p=0.101; estimated PFS, 88% *vs* 93%, p=0.547; estimated OS, 89% *vs* 94%, p=0.436. The responses and the survival probabilities of patients co-expressing the e13a2 and the e14a2 transcripts (N=30) were similar to or even better than the ones of e14a2 patients. Grouping together the patients with e14a2 transcript alone and the patients with co-expression of both transcripts (N=174+30=204), and comparing them to patients with e13a2 transcript alone (N=124), the response differences were significant (cumulative incidence of MMR and $MR^{4.0}$, p=0.050 and p=0.036, respectively), but no outcome differences emerged (PFS and OS, p=0.340 and p=0.276, respectively).
Summary/Conclusions: Lower molecular response rates in patients with e13a2 transcript were observed, but the differences were small and mostly not significant. No outcome differences were detected. Further studies in larger patient cohorts are required in order to clarify whether including the transcript type in the calculation of the baseline risk scores may improve prognostic stratification, and whether NIL or other second generation TKIs should be preferred as first-line therapy in patients aiming at treatment-free remission.

PB1819

IMATINIB (IM) 400MG IN PATIENTS WITH CML1ST CP RESULTS IN A HIGHER MOLECULAR RESPONSE RATE AT 6 MONTHS COMPARED TO IM/ HYDROXYUREA. FINAL RESULTS OF THE CML2004 STUDY. NCT 02480608

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Background: Imatinib (IM) monotherapy remains an acceptable option to treat newly diagnosed patients with chronic myeloid leukemia (CML) in the chronic phase (CP). Hydroxyurea (HU) is effective in controlling elevated white blood cell counts and has been widely used to treat CML prior to the era of tyrosine kinase inhibitors (TKIs). The combinations of IM and HU have been tested *in vitro* and showed a additive suppression of CML CFU-GM cells. Combinations of IM and cytotoxic agents such as cytarabine have been tested also in vivo, but no data are available for the combination of IM and HU in CML.
Aims: The East German Study Group conducted a phase I study to identify the dose of HU in combination with standard dose IM (400mg daily) that would result in mild myelosuppression (white blood cell count 3,000-4,000/mL). Starting dose of HU was 500mg daily which was increased by 500mg every 3 weeks to a maximum of 3,000 mg daily. According to protocol, 500mg HU was identified as the starting dose for the randomized phase II study which tested the combination *vs* standard dose IM, with the rate of major molecular response (MMR) at 18 months as the primary endpoint.
Methods: Starting in 2002, 20 adult patients with newly diagnosed CP-CML were included in the phase I study. Additional 93 patients were enrolled in the phase II of the study, 5 of whom were excluded. With ratio 2:1 in phase II, 88 patients were randomized to the IM/HU (n=59) and IM (n=29) arm, respectively.

Three patients (2 IM/HU, 1IM) were lost to follow-up. As prospectively designed, all available IM/HU patients (n=77) were analyzed together. According to the study protocol, patients from the CML IV study were to be added to obtain equal numbers for analysis. To arrive at a total of 77 IM patients, from study IV another 49 patients were selected by propensity score matching. The median age of the 154 patients was 55 years (range 18 – 82). The ELTS prognostic score was available for 141 patients and was high in 8 (5.7%), intermediate in 35 (24.8%) and low in 98 (69.5%), with no significant differences between treatment groups.

Results: The 5-year overall survival (OS) / progression-free survival (PFS) probabilities were 90.4 and 86.7% in the IM/HU and twice 84.9% in the IM arm (p=not significant). With IM/HU, the probabilities of complete cytogenetic response (CCR) at 6, 12, and 18 months were 54.3, 84.0, and 93.7%. In the IM arm, the corresponding numbers were 70.4, 84.9, and 83.3% (p=not significant). Primary endpoint was MMR rate at 18 months. There was no significant difference between IM/HU (65.8%) and IM (66.0%). At 6 months, MMR rates were 21.6 (IM/HU) *vs* 41.1% (p=0.0383) and at 12 months 41.9 (IM/HU) *vs* 58.9% (not significant). Time to event analyses of OS and PFS did not result in significant differences; neither did group comparisons between the probabilities of CCR and MMR. The median HU dose was 500mg (range 152-3000); the median IM dose was 400 mg (range 145-617mg) in both arms. The gross numbers of adverse events in general or of adverse events of grade 4 were not different between the two arms, but cumulative incidences showed an earlier occurrence in the IM/HU than in the IM arm (p= 0.0343, Gray test)

Summary/Conclusions: Compared to Imatinib only, the combination of Imatinib and HU resulted in a lower MMR rate at 6 months but a similar MMR rate at 18 months. Furthermore, IM/HU was associated with more early adverse events. There was no indication of a beneficial effect in the treatment of CML patients in 1st chronic phase using the combination of IM with HU.

PB1820

A MULTICENTER, OBSERVATIONAL, AMBISPECTIVE STUDY EVALUATING EFFICACY AND SAFETY OF GENERIC IMATINIB COMPARED TO GLEEVEC IN CHRONIC MYELOGENOUS LEUKEMIA IN CHRONIC PHASE - 3 MONTHS RESPONSE ANALYSIS

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Background: The efficacy of branded imatinib (Gleevec) in the first-line treatment of chronic myeloid leukemia (CML) has been demonstrated in several clinical studies. However, there are few consistent data in the literature on the efficacy and adverse effects of generic formulations of imatinib. In Brazil, CML patients have been treated in the national public health system with generic imatinib since June 2013.

Aims: The present study aims to evaluate the efficacy and safety of generic imatinib in the treatment of CML in comparison with the reference drug (Gleevec) in the first three months of imatinib treatment.

Methods: This is a multicenter, observational, ambispective, cohort-type study. The study was initiated in January 2015 with the intended participation of 17 Brazilian centers. In the prospective group, were selected chronic phase CML patients who started their first-line treatment with generic imatinib between January 2015 and October 2016, whereas retrospective group was treated with Gleevecbetween January 2010 and December 2011. All patients started imatinib less than six months from diagnosis. Study data were collected and managed using REDCap electronic data capture tools. Demographic data were collected at diagnosis: age, gender, Sokal, Hasford, EUTOS score, comorbidities, cytogenetics, BCR-ABL transcript type. The definition of the responses followed the European Leukemia Net 2013 criteria. Adverse events were assessed based on the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0.3, 2010. Statistical analysis: SPSS version 21.0 was used applying the chi-square and t-test, when adequate. All analysis considered p-value <0.05 as significant.

Results: Ten centers have registered 177 patients in the retrospective group and 68 patients in the prospective group so far. For this preliminary analysis, response data from 132 patients were available (47 from prospective and 85 from the retrospective groups). The median age of patients was 54 years in the prospective group and 46 years in the retrospective group (P=0.12). Sokal score in prospective and retrospective groups, respectively: low risk 42%/52%; intermediate risk 42%/31% and high risk 45%/67% (P=0.48). There was no difference between the groups concerning gender, Hasford, EUTOS scores, ECOG, blood cell counts at diagnosis and before starting imatinib and BCR-ABL transcripts. Regarding responses, there was no difference in the hematological, complete cytogenetic responses and rate of BCR-ABL transcripts >10% at three months. However, there was a higher rate of failure at three months according to the ELN 2013 criteria in the prospective group (14.9% *versus* 4.7% Gleevec group, P=0.04). There was no significant difference in grade 3 and 4 hematological and non-hematological toxicity, but there was one early death in the prospective group (acute peripheral arterial occlusion and renal failure). Four patients discontinued imatinib: one from Gleevec group (resistance) and three from the generic group due to intolerance (1) and resistance (2).

Summary/Conclusions: According to ELN-2013 criteria, there was a higher rate of failure in the prospective group (generic Imatinib) at three months, but no difference in toxicity. The register is ongoing; the confirmation of this data and the impact in prognosis will be evaluated in the long-term follow-up, after increasing the number of patients.

PB1821

COMPLEX ADDITIONAL CHROMOSOME ABERRATIONS IN PH-POSITIVE CELLS IMPACT ON CHRONIC MYELOID LEUKEMIA PATIENTS' SURVIVAL IN THE ERA OF TYROSINE KINASE INHIBITORS

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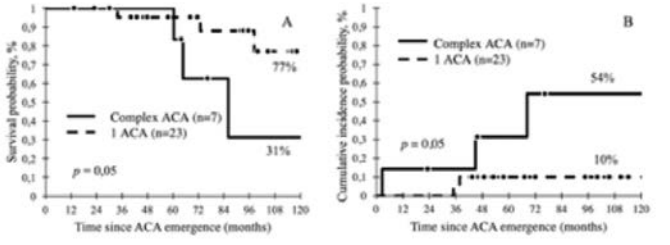
Background: Additional chromosomal aberrations (ACA) as marker of clonal evolution in chronic myeloid leukemia (CML) patients were previously noted in association with resistance to therapy. The presents of ACA have been associated with a worse prognosis for survival in the pre-TKI era. The ACA classification proposed earlier was based only on its frequencies. Whereas ACA's clinical impact had not yet been clearly established.

Aims: The aim of our study was to evaluate the long-term impact of the ACA presence in Ph-positive cells in CML patients on TKI treatment results.

Methods: 30 patients with ACA in Ph-positive cells treated in our center from 2005 to 2015 years were included in this study. Cytogenetic analyses of at least 20 Giemsa-banded bone marrow metaphases were interpreted per ISCN 2013. We analyzed overall survival (OS) and cumulative incidence of CML-related death on TKI treatment. Cox regression was used for multivariate survival analysis, that included next covariates: number of ACA, type of ACA, age, TKI type, CP or AP at diagnosis. OS was estimated by Kaplan-Meier method with log-rank test for comparison. Cumulative incidence of CML-related death was estimated into consideration the presents of competing risks (CML-unrelated death) using Gray's test for comparison between groups.

Results: Median follow-up period in ACA group (n=30) was 51 months (3-124). ACA at diagnosis were detected in 16 (53%) of 30 patients. Chronic phase CML at diagnosis was determined in 23 (77%) patients. Imatinib was used as first-line in 20 (67%) patients, 3 (10%) patients were initially treated with Nilotinib. Accelerated phase was defined in 7 (23%) patients. In that group treatment of 6 patients was started with Imatinib and Dasatinib was given initially for one patient. «Major-route» ACAs (trisomy 8, +der(22)t(9;22)(q34;q11), i(17)(q10), trisomy 19,) were detected in 16 (53%) of 30 patients. Complex aberrations (2 ACA and more) were revealed in 7 (23%) patients, 4 patients from this group had «major-route» ACA. 10-years OS in the whole ACA group was 67%, 10-years cumulative incidence of CML-related death was 23%. Number of ACA (p=0.03, HR=13.2) and age (p=0.03, HR=1.14) had statistical significance influence on survival by regression analysis. 10-years OS was 31% and 77% (p<0.05) in patients with complex ACA and single ACA respectively, 10-years cumulative incidence of CML-related death was 54% for patients with complex aberrations *versus* 10% for single ACA patients (p<0.05) (Figure 1).



Overall survival (A) and cumulative incidence probability of «death due to CML» (B) in CML patients with complex ACA (n=30)

Figure 1.

Summary/Conclusions: Our results showed that TKI treated CML patients with complex ACAs have a higher risk of progression and death in comparison with single-ACA patients.

PB1822

BCR/ABL1 TRANSCRIPT E13A2 IS ASSOCIATED WITH HIGHER CUMULATIVE PROBABILITY OF LOSS OF MAJOR MOLECULAR RESPONSE IN CML PATIENTS TREATED WITH NILOTINIB AS THE 2-ND LINE THERAPY

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Background: Several types of transcripts can be produced during chromosomal translocation, which lead to formation of the BCR/ABL fusion gene in patients with chronic myeloid leukemia (CML). Previous results of a few large studies showed that patients with CML in chronic phase (CP) with e13a2 transcript have inferior responses to frontline imatinib therapy compared to patients with the e14a2 transcript.
Aims: To investigate the prognostic significance of e13a2 and b14a2 BCR/ABL1 transcripts in CML patients switched to nilotinib after suboptimal response or failure on frontline imatinib.
Methods: CP-CML patients (N=143) who did not achieve complete cytogenetic response (CCR) after imatinib therapy (600 or 800 mg once daily) and were switched to nilotinib 400 mg twice daily, were enrolled in present study (55 patients with e13a2 transcript and 88 patients – with e14a2 transcript). The median of imatinib treatment before switching to nilotinib was 44 months (range 1-137). A qualitative RT-PCR for BCR/ABL1 transcript was performed at diagnosis. The patients who achieved CCR but did not have major molecular response (MMR) as well as patients with rare BCR/ABL1 transcripts and coexpression were excluded from the analysis. Probability of overall survival (OS), progression-free survival (PFS) and event-free survival (EFS) were calculated using Kaplan-Meier method. Event in EFS was defined as death of a patient on treatment for any reason, progression of disease, or loss of CCR or MMR. Differences between groups were assessed using log-rank, χ2-tests and Mann-Whitney U-tests. Cumulative probability of CCR, MMR, MR4,0 (BCR/ABL<0,01%) and loss of CCR and MMR was assessed using Kaplan-Meier method.
Results: The median follow up was 23 (range 4 – 82) months. The groups with both of the BCR/ABL1 main transcripts were comparable for the disease phase, Sokal risk score and the proportion of patients with additional chromosomal abnormalities in Ph-positive cells. No correlation of transcript type with age or sex was observed. Transcript e13a2 was associated with higher WBC (120x109/L *vs* 95.3x109/L, p=0.02) and lower baseline percentage of eosinophils (p=0.041). No differences were found in other differential counts of peripheral blood, hemoglobin concentration, or spleen size.
The time to CCR, MMR and MR4,0 and rate of CCR (52% and 52%), MMR (38% and 33%) and MR4 (23% and 22%) were comparable in patients with e13a2 and e14a2 transcripts respectively. Estimated probability of CCR, MMR and MR4,0 also did not differ in both groups. The rate of optimal response, primary and secondary resistance to nilotinib therapy was comparable in both groups. Whereas there were no differences in the estimated probability of CCR loss in both groups, but rate and cumulative incidence of MMR loss was significant higher (69% *vs* 11%, p=0.037) in patients with e13a2 transcript. No difference between groups was observed with regard to PFS, EFS and OS.
Summary/Conclusions: Analisys of 143 CML patients treated with nilotinib as the 2-nd line therapy suggests that patients with e13a2 transcript have less stable therapy response and demonstrate higher cumulative incidence of MMR loss (molecular relapse). But outcome differences were nor observed. Further analysis of a larger number of events and longer observation is required.

PB1823

ANALYSIS OF GENERIC IMATINIB EFFICACY IN CHRONIC MYELOID LEUKEMIA TREATMENT: MORE THAN FOUR YEARS OF EXPERIENCE IN SOUTHERN SERBIA

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Background: Tyrosine kinase inhibitors (TKIs) are the golden standard in the treatment of chronic phase chronic myeloid leukemia (CP-CML) due to their high efficacy and mild toxicity profile. Because of the high price of these drugs, the use of generics is encouraged to reduce health care costs. In the literature, there is still limited data and some concerns about the effectiveness of generic imatinib (GI), although there is a growing number of countries in which it is used instead of original imatinib (OI).
Aims: The objective of this study was to evaluate efficacy and safety of GI in newly diagnosed CP-CML patients treated with frontline GI and in patients switched from OI to GI.
Methods: Cohort of 101 adult patients with CP-CML was analysed, treated with TKIs in our institution during period from August 2012 to February 2017. First group consisted of 53 patients treated with GI (Anzovip). According to European LeukemiaNet (ELN) 2013 guidelines, rate of optimum therapeutic response was analysed, as well as rate of treatment failure at 6 and 12 months. The second group consisted of 48 patients switched from OI to GI, in which the rate of achieved therapeutic response at the time of switching and the rate of maintenance of CCyR, MMR and MR4 after a minimum of 12 months under therapy with GI were both analysed. In order to investigate safety of GI, in both groups rate of hematological and non-hematological adverse effects (AEs), all grades according CTCAE criteria, were analysed.
Results: Analysis of the optimal response by ELN criteria in the group with GI showed that at 6 months 33/53 (62.3%) patients achieved CCyR, BCR-ABL<1% was in 27/52 (51.9%) patients, while 15/52 (28.8%) of patients achieved MMR. At 12 months of therapy, 35/49 (71.4%) of analysed patients achieved CCyR, and 25/49 (48.9%) achieved MMR. ELN criteria for treatment failure at 6 months fulfilled 12/53 (22.6%) patients, while at 12 months ELN criteria satisfied 13/49 (26.5%) of analysed patients. After 18 months of therapy with GI the rate of CCyR was 35/46 (76.1%) and MMR was 28/45 (62.1%) and showed trend of increase. During the median follow-up period of 23.8 months 3 patients have progressed to blast phase and total of 7 patients died. In the second group, in time of switching from OI to GI, the rates of achieved CCyR, MMR and MR4 were 82.5%, 65.8% and 49% of patients respectively. The rate of maintenance of previously achieved CCyR was 95%, of MMR 88% and of MR4 72% in the course of the median duration of GI exposure of 37.8 months. When comparing first and second group respectively, the rates of patients which have been switched to 2nd generation of TKI because of the failure or intolerance to imatinib were 27.8% *vs* 24.8%, and 60.5% *vs* 64.5% of them achieved secondary optimal therapeutic response (CCyR plus MMR), while 25% *vs* 20% of them have been sent to BMT. Group switched from OI to GI had not significantly different non-hematological AEs of all grades compared to GI group (21.7% *vs*.24.2%, p=0.991). Furthermore, the rate of grade 3-4 hematological AEs were similar in both groups (13% *vs* 15%, p=0.952).
Summary/Conclusions: Results of this study with extended follow-up of more than four years are further evidence of that the generic imatinib is at least non-inferior to the original imatinib regarding efficacy both when used initially or as a subsequent replacement for branded imatinib.

PB1824

ACHIEVING OPTIMAL RESPONSE AT 12 MONTHS IS ASSOCIATED WITH A BETTER HEALTH-RELATED QUALITY OF LIFE IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA: A PROSPECTIVE, LONGITUDINAL, SINGLE CENTER STUDY

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Background: Health-related quality-of-life (HRQoL) profile is now recognized as an important component in the management of Chronic myeloid leukemia (CML).
Aims: To explore the HRQoL profiles of patients with CML in the chronic phase (CP) who were treated with front-line imatinib or nilotinib, in order to assess the relationship between early response and HRQoL outcomes.
Methods: A prospective, longitudinal, single center study was conducted to assess the response to treatment with imatinib or nilotinib and the HRQoL profile of patients who were newly diagnosed with CML-CP enrolled into ENESTchina study. Responses based on molecular and cytogenetic outcomes were measured according to the European LeukemiaNet recommendations, and patient-reported HRQoL profile was measured by the SF-36 health survey.
Results: Fifty-nine patients were randomly assigned to receive imatinib (n=31) or nilotinib (n=28). In multivariate analysis, the use of nilotinib was identified as an independent factor affecting the achievement of optimal response at 6 months (OR=3.9, 95% CI, 1.0-14.9; P =0.043) and 12 months (OR=5.6, 95% CI, 1.7-17.9; P =0.004). With a median follow-up of 60 months, the probabilities of failure-free survival (all P values <0.001) and progression-free survival (all P values <0.05) at 5 years were significantly higher in patients who achieved optimal response at 3, 6, or 12 months than those who achieved non-optimal response (warning or failure), and overall survival rate at 5 years was significantly higher in those who achieved optimal response at 12 months (P =0.047). Achieving optimal response at 12 months was associated with better role limitations due to physical health problems (P =0.0019) and role limitations due to emotional problems (P =0.0110) and was the sole factor associated with significantly improving physical component summary over time (P =0.0160). In addition, achieving optimal response at 6 months had a tendency of high physical functioning (P =0.0674), social functioning (P =0.0571), and role limitations due to emotional problems (P =0.0916) scores. Age <40 years, female gender, and higher education level were also associated with better HRQoL subscales.
Summary/Conclusions: Achieving optimal response at 12 months was associated not only with longer overall survival and less treatment failure and disease progression, but also better HRQoL in newly diagnosed patients with CML-CP on front-line tyrosine kinase inhibitor.

PB1825

MULTI-COUNTRY RETROSPECTIVE CHART AUDIT STUDY TO EXAMINE DEEP MOLECULAR RESPONSE (MR4.5) ASSOCIATED WITH

SECOND-LINE TYROSINE KINASE INHIBITORS IN CHRONIC PHASE - CHRONIC MYELOGENOUS LEUKEMIA (CML-CP)

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Background: Achieving deep molecular response, ≥4.5-log reduction ($MR^{4.5}$; BCR-ABL1 on the International Scale [IS] ≤0.0032%), is one of the important prerequisites for attempting treatment-free remission. Limited information is available on comparative rates of $MR^{4.5}$ between nilotinib and dasatinib in second-line (2L).
Aims: This study aims to investigate time to achieving $MR^{4.5}$ and major molecular response (MMR; ≥3-log reduction or ≤0.1% in BCR–ABL1 on IS) in CML-CP patients (pts) treated with nilotinib *vs* dasatinib in 2L.
Methods: An online physician panel approach was used to recruit oncologists (N=141) globally to conduct a retrospective medical chart audit. Physicians were instructed to select up to 4 pts who met the following criteria via a random letter generation scheme for the first letter of pt's last name: diagnosed with CML-CP at age ≥18 years, initiated 2L nilotinib or dasatinib between 1/1/11 and 3/1/15, and had ≥12 mos of follow-up data after initiating 1L TKI. Multivariate Cox proportional hazards models accounting for country clustering random effects were used to assess the effect of nilotinib *vs* dasatinib on time to $MR^{4.5}$ and MMR, adjusting for age, gender, Sokal risk score at diagnosis, hydroxyurea use before 1L TKI, 1st *vs* 2nd generation TKI as 1L, and reasons for 1L TKI discontinuation. Adjusted hazard ratios (HR) and 95% confidence intervals (CI) were reported. Adverse events (AEs) were also described.
Results: The study included 236 pts from Australia, Brazil, France, Germany, Italy, and Netherlands treated with nilotinib (N=115[49%]) or dasatinib (N=121[51%]) in 2L. Both groups had a similar mean follow-up of 23 mos, mean age of 57 years, and were 35% female. 8% of 2L nilotinib and 22% of 2L dasatinib pts were treated with the other 2nd generation TKI in 1L (p<0.01). A higher proportion of nilotinib pts had high-risk Sokal score (20.9% *vs* 11.6%, p=0.05) and received prior hydroxyurea (8.7% *vs* 3.3%, p=0.08) *vs* dasatinib. 85% and 11% of 2L nilotinib pts discontinued 1L TKI due to resistance and intolerance, respectively, prior to switching to nilotinib, *vs* 74% and 22% for 2L dasatinib pts (both p<0.05). The univariate Cox model showed that nilotinib had a non-significantly higher rate of achieving $MR^{4.5}$ than dasatinib (32% *vs* 31% at 24 mos for 2L nilotinib and 2L dasatinib pts, respectively, based on the Kaplan Meier estimator; unadjusted HR=1.09, 95% CI [0.87, 1.38], p=0.46); however, after multivariate adjustment, nilotinib reached a significantly higher rate of achieving $MR^{4.5}$ (adjusted HR=1.36, 95% CI [1.07, 1.73], p<0.01) than dasatinib. Among those who achieved $MR^{4.5}$, 45% of nilotinib pts maintained $MR^{4.5}$for ≥1 year *vs* 39% of dasatinib pts (p=0.60). Additionally, high-risk Sokal score (HR=0.31; 95% CI [0.14, 0.72], p<0.01) and resistance to 1L TKI (HR=0.60; 95% CI [0.42, 0.86], p<0.01) were inversely associated with achieving $MR^{4.5}$. There was no significant difference in MMR achievement between 2L TKI groups. Over 3 times more dasatinib pts experienced pleural and pericardial effusion AEs than nilotinib pts (9.9% *vs* 2.6%; p=0.02). One nilotinib pt had ischemic heart disease-related AE *vs* none for the dasatinib group (p=0.49).
Summary/Conclusions: This retrospective chart audit study suggests that 2L nilotinib may be associated with a higher rate of $MR^{4.5}$ than 2L dasatinib in CML-CP. Our results should be taken with caution as this study is susceptible to unmeasured confounding and biases due to its retrospective and observational nature. Rigorous clinical assessment in a prospective setting is needed to conclusively compare rates of patients achieving $MR^{4.5}$.

PB1826

COMPUTATIONALLY INTELLIGENT PREDICTION OF CLINICAL OUTCOME IN CHRONIC MYELOID LEUKEMIA

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Background: Computational intelligence has been applied to a wide range of problems to assist in decision-making, especially artificial neural networks, fuzzy systems and powerful hybrid neuro-fuzzy approaches have already proven their strong potentials in medicine. Despite that, applications in hematology are still scarce.
Aims: In this study we have developed novel ANFIS neuro-fuzzy prognostic models, based on clinical and morphometric diagnostic data, to enable better prediction of complete cytogenetic response (CCgR) for patients with chronic myeloid leukemia.
Methods: This prospective study included a consecutive series of patients with chronic myeloid leukemia (CML) who were started on imatinib therapy. Analysis was performed with CCgR at 6, 12, and 18 months as the outcome variables. A total of 40 patients on imatinib therapy were included in the final analysis. Of these, 25 (62.5%), 29 (72.5%), and 32 (80%), respectively, achieved CCgR at 6, 12, and 18 months after initiation of imatinib. Computationally intelligent neuro-fuzzy models that were developed included EUTOS score on diagnosis and one of the following morphometric parameters: microvascular density, length of the minor axis, area or circularity of the blood vessel. Adaptive neuro-fuzzy systems represent a specific combination of artificial neural networks and fuzzy logic, thus combining the learning ability of artificial neural networks with the knowledge representation capability of fuzzy logic systems. ANFIS (Adaptive Neuro Fuzzy Inference System) consists of five layers of nodes (neurons), each of which performs a particular function on incoming signals as well as a set of parameters pertaining to this node. The basic architecture of ANFIS using hybrid learning algorithm is presented in Figure 1.
Results: All analysed patients have received imatinib mesylate as their first-line therapy for CML. Model predictions (0–1) for any individual patient were interpreted as probability of CCgR at 6, 12 or 18 months. The overall accuracy of the final model was determined by comparing the predicted values with the actual events. A probability cut-off point of 0.50 (50%) was used to classify observations as events or non events, and patents were divided in training, validation and testing groups. Best performing ANFIS model, including EUTOS score and minor axis morphometric parameter was better than a model that includes only EUTOS score and regression model based on the same inputs. Overall model correct classification achieved for EUTOS, two input LR model and two input ANFIS model were respectively 75%, 75% and 77.5%, while areas under curve on ROC graphs were 0.776, 0.829 and 0.875 respectively.

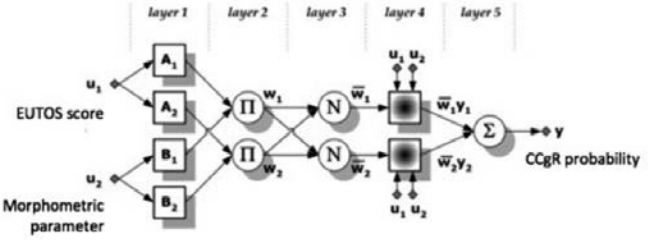

Figure 1.

Summary/Conclusions: The major finding of this study is that ANFIS models using the morphometric parameters, available at diagnosis of chronic phase of the CML, may improve prediction of CCgR at 6, 12 and 18 months on imatinib therapy, in comparison to the EUTOS score being the standard prognostic scoring system and regression models using the same inputs. Using neuro-fuzzy computationally intelligent ANFIS models with morphometric parameters in conjunction with EUTOS score improves prediction of CCgR. Validation on larger groups of patients is needed, but these findings indicate that neuro fuzzy models could aid in individual CML patient risk stratification.

PB1827

A NATIONWIDE OBSERVATIONAL STUDY OF PONATINIB IN CHRONIC MYELOCYTIC LEUKEMIA OUTSIDE CLINICAL TRIALS

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Background: In December 2014 the oral tyrosine kinase inhibitor (TKI), ponatinib was granted an accelerated approval by the FDA based on promising results from the phase II PACE (Ponatinib Ph-ALL and CML evaluation) trial. Yet, nowadays the use of this drug is limited because of safety issues, most notably increased risk of vascular complications. Currently, there is very little real-life information regarding the use of ponatinib outside clinical trials.
Aims: The purpose of the current study is to characterize patients who received ponatinib and to assess the safety profile and efficacy of ponatinib outside clinical trials.
Methods: Data from electronic charts of chronic myeloid leukemia (CML) patients treated with ponatinib were analyzed.
Results: *Patients characteristics*: Between 4.2011 and 1.2017 (69 months) 37 patients with an initial diagnosis of CML in 9 medical centers in Israel received ponatinib. The median age at time of treatment was 43 years (range: 9 to 82) and approximately half of the patients had chronic phase CML (N= 19, 53%). Based on their medical history, 36% (N=12) were at increased risk for vascular complications. *Pre-ponatinib treatments:* Patients received at least one other TKI and most received at-least two different TKI-

based regimens (N=28, 76%). Nine patients (25%) underwent hematopoietic stem cell transplantation (HSCT) prior to ponatinib. The time that lapsed from diagnosis until ponatinib initiation ranged considerably (from 1 to 215 months, median 47 months). *Indications for ponatinib switch:* 26% of patients (N=9) switched to ponatinib because T315I mutation was detected. The remaining switched either because of progressive disease, *i.e.* accelerated (N=5, 14%) or blastic (17%, N=6, 17%) phases, and 14 (39%) because they experienced loss of previous molecular or cytogenetics response. Only 5% (N=2) switched because of unacceptable side effects to previous treatments. *Treatment with Ponatinib:* Patients received ponatinib for a median time of 14 months (range: 1 to 51). The drug started at the recommended dose of 45 mg/day only in 60% (N=22) of patients and in 24% of them (N=9) the dose was reduced during treatment. The median survival time of patients with ponatinib was 38 months (95%CI: 30 to 47 months) (Figure 1). Patients died because of cerebrovascular event (N=1), sepsis (N=2) or graft *vs* host disease that developed shortly after HSCT. *Response assessment*: Response assessment was available for 32 patients (86%). Seventy percent (N=22) achieved molecular response, of which 60% (N=13) achieved at least major molecular response. The median time to maximal response was 7 months (range: 3 to 28 months). *Drug discontinuation:* Twenty four percent (N=9) discontinued ponatinib after a median of 7 months (range: 1 to 18 months) because of disease progression (N=6) or severe adverse effects in two patients (cerebrovascular event and severe pancytopenia).

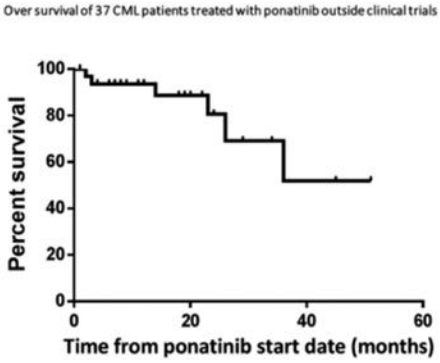

Figure 1.

Summary/Conclusions: In our cohort ponatinib was almost always used in patients who experienced treatment failure to previous TKIs. Still, molecular response was achieved in most patients, even in those with progressive disease in accelerated or blastic phases. The vast majority of patients received reduced doses of ponatinib and although more than one third of patients were at-risk for vascular events, only two patients developed serious life-threatening vascular episodes. In heavily pre-treated patients, ponatinib is effective and safe and can be considered even in patients with cardiovascular risk factors.

PB1828

MOLECULAR RESPONSE TO THERAPY WITH TYROSINE KINASE INHIBITORS IN PATIENTS WITH BCR-ABL1(+) CHRONIC MYELOID LEUKEMIA PRESENTING WITH AN ISOLATED THROMBOCYTOSIS AT THE ONSET

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Background: Generally, chronic myeloid leukemia (CML) and essential thrombocythemia (ET) are characterized by distinctive clinical and laboratory characteristics, including the spectrum of genetic abnormalities - Philadelphia chromosome (Ph) and *BCR-ABL1* fusion transcripts in CML and *JAK2, CALR* or *MPL* gene mutations in ET. Therefore, even in the presence of overlapping features in some cases, the correct diagnosis can be assigned. However, in rare cases Ph chromosome and *BCR-ABL1* fusion transcripts can be found in otherwise typical ET. Due to the low number of reported cases the subsequent course of the disease and the response to tyrosine kinase inhibitors (TKI) in such patients with *BCR-ABL1*-positive thrombocytosis is largely unknown.
Aims: To report the clinical course and response to TKI in patients (pts) with CML presenting with isolated thrombocytosis at the onset.
Methods: In total, 31 pts with Ph(+) and/or *BCR-ABL1*(+) isolated thrombocytosis and a moderate or absent leukocytosis were retrieved from the hospital database. The cohort comprised 17 females and 14 males, at a median age of 47 years (range 23-86). Diagnosis was based on blood and bone marrow morphology and differential, cytogenetics and/or molecular testing according to the WHO criteria (2008). Molecular monitoring was carried out using Xpert BCR-ABL Monitor or Xpert BCR-ABL Ultra tests (Cepheid). In total, follow up data for at least 6 months (mean 65 months) are available for 25 patients treated with TKI as a first-line therapy.
Results: At diagnosis the median leukocyte count was 22 x10^9/l (range 6-36) and platelet count – 1316x10^9/l (range 770-2815). Splenomegaly was found in 5 pts (16.1%). Only one patient was diagnosed in accelerated phase as the remaining presented in chronic phase at diagnosis. Interestingly, 4 pts (12.9%) had a history of an antecedent solid tumor. All patients enrolled in the study were *BCR-ABL1*(+): b3a2 (n=16) or b2a2 (n=15). Karyotypes were available in 23 pts and classical Ph was found in 16 of them (69.6%), while in 5 pts (21.7%) a cryptic translocation was detected as well as a variant Ph in the remaining 2 pts (8.7%). Imatinib was used as a first line therapy in 15 pts and optimal response was achieved in 53.3% (n=8), while 5 were switched to a second line, and 2 - to a third line therapy. First-line treatment with Nilotinib in 10 patients resulted in optimal response in 80% (n=8). In total, major molecular response (MR) was achieved in 80% (n=20), including deep MR in 56% (n=14). One pt was lost of follow up after optimal response was registered. No response was documented in 4 pts (16%) and progression to blast crisis developed in 2 of them. The mean OS was estimated 143 months and the cumulative proportion surviving at 5 years was 91%.
Summary/Conclusions: Interestingly, CML presenting with isolated thrombocytosis at diagnosis in our cohort had high proportion of antecedent malignancies and high incidence of cryptic Ph translocation without any specific correlation with the transcript types. However, the clinical course and molecular response to TKI therapy was similar to the reported in CML in general.
Ackowledgements: Partial support by the National Science Fund.

PB1829

BCR-ABL1 MOLECULAR RESPONSES AT 12-18 MONTHS USING THE QUANTIDEX QPCR BCR-ABL1 IS KIT PREDICT LONG-TERM EVENT-FREE SURVIVAL IN PATIENTS WITH TKI-TREATED CML

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Background: Detection of BCR-ABL1 e13a2 or e14a2 transcripts (major breakpoint, M-BCR) of translocation t(9;22) (also known as the Philadelphia chromosome) is important in CML monitoring tumor burden. The International Scale (IS) was established to standardize reporting relative to a common baseline. As newer TKI therapies create deeper responses, analytical sensitivity has become a critical topic in investigations into TKI discontinuation, where researchers require a clinically validated assay that calls a molecular reduction (MR) of ≥4.5 logs below baseline (*i.e.* MR4.5 or 0.0032%IS).
Aims: To clinically validate the QuantideX qPCR BCR-ABL1 IS Kit and to reaffirm the clinical utility of BCR-ABL1 RT-qPCR monitoring in patients with t(9;22) positive CML, a correlation between molecular response (MR) values and long-term outcome was determined.
Methods: The QuantideX qPCR BCR-ABL IS Kit uses standard TaqMan chemistry to quantitate BCR-ABL1 and the ABL1 reference gene. Associated software reports an international scale BCR-ABL1 value and a log-transformed MR value, with a 3 log-reduction from pre-treatment baseline represented as 0.1%IS or MR3.0. Three laboratories performed BCR-ABL1 testing on banked RNA specimens from 96 chronic phase CML patients from 2 hospitals drawn 12-18 months after starting TKI therapy. Clinical events (TKI therapy change, loss of complete hematologic or cytogenetic response, progression to accelerated phase or blast crisis, kinase domain mutation, or death) were recorded through 36±4 months after starting TKI. Two operators per site also tested serially-diluted reproducibility samples (range MR1.0 to MR4.0) in multiple replicates over 5 days. The 95% LOD for the assay was defined as the median measured%IS value of 4 analogous serially-diluted specimens.
Results: 51 patients had MR<3.0 at 12-18 months post-TKI. Of these 51 patients who did not achieve a major molecular response (MMR), 20 had a subsequent clinical event, 17 had no event, and 14 were lost to follow-up (LFU). 45 patients had MR≥3 at 12-18 months post-TKI. Of these 45 patients who did achieve MMR, 8 had an event, 28 had no event, and 9 were LFU. Kaplan-Meier survival curves demonstrated a 22% prolongation of event-free survival (95% CI 2%>42%) at 3 years between the two MR groups [p=0.028; 58% (95% CI 44%>75%) for MR<3 *vs* 80% (95% CI 68%>93%) for MR≥3)]. Specimens with MR values ranging from MR1 to MR4 showed an average%CV of 2.6%. Day to day agreement was high with MR SD by operator ranging from 0.000 to 0.060. Site to site agreement was high with MR SD by site ranging from 0.000 to 0.069. The 95% LOD for both transcripts (e13a2 & e14a2) was MR4.7 (0.002%IS), allowing sensitive detection of the MR4.5 cutoff that defines "complete molecular response" in ongoing treatment-free remission clinical trials.
Summary/Conclusions: The QuantideX qPCR BCR-ABL IS Kit has excellent reproducibility and analytical sensitivity, and the achievement of MR≥3 (major molecular response) by this assay predicts prolonged event-free survival in TKI-treated CML patients.

PB1830

SHOULD SWITCHING TO SECOND GENERATION TKIS BE A RULE IN PATIENTS WITH CP-CML AFTER 3-6 MONTHS OF IMATINIB TREATMENT: RETROSPECTIVE ANALYSIS OF CML PATIENTS TREATED IN A SINGLE BRAZILIAN CANCER CENTER

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Background: Early molecular response is an important predictor for survival and therapy-free remission in chronic myeloid leukemia (CML). The current guidelines define BCR-ABL1 >10% at 3 months and/or 1-10% at 6 months as warning signs; however, it is not clear if switching imatinib to second generation TKIs in this scenario improves responses and overall survival in patients outside clinical trials.
Aims: To analyze the proportion of patients with major molecular response (MMR) at 12 months according to the molecular response at 3 and 6 months in a cohort of CML population, not enrolled in clinical trials and treated only with imatinib. Also evaluate the incidence of molecular responses log3.0, log4.0 and log4.5 at any time in patients who did not switch to second generation TKIs.
Methods: Retrospective analysis of all 226 patients diagnosed with CML from January 2007 until January 2015 in our hospital. The exclusions criterias were: advanced phases, inclusion in clinical trial, treatment with second-generation TKI in the first 12 months (due to toxicity or failure). The molecular response was evaluated according ELN recommendations: RQ-PCR assessment of BCR-ABL1 levels every 3 months until achievement of MMR, with molecular evaluation every 3-6 months afterward. All samples were analyzed in the same laboratory which was standardized since 2007.
Results: In the first cohort, 150 patients with CML chronic phase were analyzed. Optimal molecular responses by the ELN at 3 and 6 months were predictors of MMR by 12 months (94% *vs* 6%, p<0.0001 at 3m, 89.3% *vs* 10.7%, p<0.0001 at 6m), but there was no overall survival benefit. A second cohort with 119 patients received only imatinib, with a medium follow-up time of 71 months (13-117m). MMR was achieved by 60% of this imatinib-only group after 12 months and by more than 90% after 36 months (Figure 1). Patients with BCR-ABL1 <10% at 3 months and/or <1% had a higher probability of achieving MMR3, MMR4 and MMR4.5 at any time.

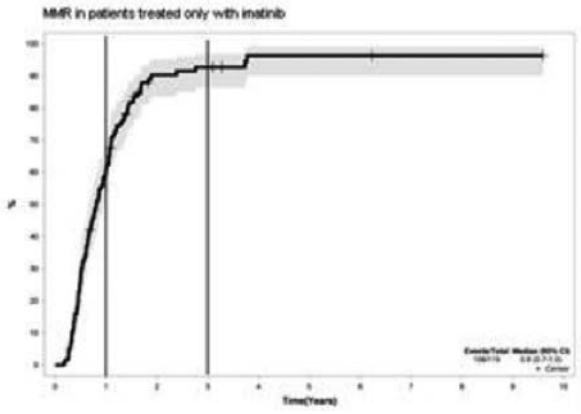

Figure 1.

Summary/Conclusions: Our study shows that around 30% of the patients that do not fail to imatinib at the first year of treatment may be late responders. Not all patients should change therapy, if they have not reached MMR at 12 months. Molecular response at 3 at 6 months might guide the decision to switch TKI, but patient's comorbities, possibility of discontinuation and cost of therapy should also be considered.

PB1831

PREDICTIVE PARAMETERS FOR IMATINIB FAILURE IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background: Development of tyrosine kinase inhibitors (TKI) has significantly changed natural course of chronic myeloid leukemia (CML) and increased 10 year overall survival from 10-20% to 80-90%. Until recently, imatinib was the standard first-line treatment in CML. In 2013, nilotinib and dasatinib were approved as alternative front-line options. However, none of three TKI has been shown to have a clear survival advantage so this raised a debate on treatment selection. The early identification of patients expecting poor outcome is crucial for offering an alternative TKI regimen.
Aims: to analyze predictive parameters for Imatinib response as first-line treatment of CML patients.
Methods: The study was conducted on 168 consecutive patients with chronic phase of Ph+ CML who were diagnosed and treated at single university hospital from December 2000-January 2015. Following data were analyzed in terms of treatment response to Imatinib: demographic characteristics; currently used prognostic scores (Sokal, Hasford, EUTOS); liver and spleen size; laboratory parameters; influence of comorbidities analyzed by three scores (ACE 27, HCI-CI, SCIRS); occurrence of second malignancies; conventional cytogenetic results; therapy, duration of therapy, cytogenetic responses, overall survival (OS) and outcome.
Results: The mean age at diagnosis was 48±14.4 years (range: 18-74) with 87.5% of patients<65 years. The OS at 5 and 10 years was 97% and 91% respectively. Overall response to Imatinib treatment was as the follows: 131 patients (78%) achieved CCyR, 14 patients (8.3%) majorCyR, 4 patients (2.4%) minorCyR, 16 patients (9.5%) had no cytogenetic response, 2 patients (1.2%) had hepatic toxicity verified by liver biopsy in the first six months of Imatinib treatment and 1 patient (0.6%) was lost from follow-up. After achievement of CCyR, 25 patients (19%) had a progression of disease by losing CCyR or development of AP/BP. Median time to progression was 24 months (range 12-102). After the median follow up of 87 months in 61 patients (36.3%), the Imatinib failure was verified. All three prognostic scores (Sokal, Hasford, EUTOS), age, gender, hemoglobin level, leukocyte and platelet count, splenomegaly, eosinophils and basophils in peripheral blood were not found to be statistically significant for the Imatinib failure. Cox regression analysis identified hepatomegaly (p=0.001), leukocytosis≥100x109/L (p=0.001), blood blasts≥1% (p=0.002) and presence of additional cytogenetic aberrations (ACAs) (p=0.002) as a predictors of Imatinib failure. Accordingly, we assigned risk scores based on hazard ratios (HR) to hepatomegaly (HR=4.089; 2 points), leukocytosis ≥100x10^9/L (HR=3.158; 1 point), blasts in peripheral blood ≥1% (HR=2.912; 1 point), and presence of ACAs (HR=11.110; 2 points). A final 3-tiered prognostic model named IMA-FAIL was thus developed, as low (score 0), intermediate (score 1-3), and high risk (score ≥4), according to which imatinib failure had 17% (8/47) of patients in low, 34.9% (30/86) in intermediate and 76.7% (23/30) in high risk group (HR=3.973, 95% CI for HR 2.237-7.053, *p* <0.001). In addition, presence of comorbidities as well occurrence of second malignancy were not predictors for Imatinib failure.
Summary/Conclusions: Hematologists are facing with challenge of making decision which TKI to choose upfront with increasing a chance to achieve best possible response. The new score allows better selection of patients who are suitable for treatment with Imatinib and may guideline the clinical decision for front-line treatment of CML.

PB1832

A MULTICENTRE AUDIT OF SYMPTOMS AND QUALITY OF LIFE IN IRISH CML PATIENTS ON TYROSINE KINASE INHIBITORS

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Background: The development of tyrosine kinase inhibitors (TKIs) over the last 20 years has dramatically improved the outcomes for patients with every stage of chronic myeloid leukaemia (CML). Since the approval of the first TKI, imatinib, in 2001, there are now currently 5 oral TKIs available. Three are approved for frontline use (imatinib, dasatinib and nilotinib) and 2 others (bosutinib and ponatinib) approved for intolerance or failure of prior TKI. Because CML patients need to continue TKI treatment indefinitely, it is necessary to consider not only differences in potency and progression-free survival, but also TKI induced toxicity and quality of life (QOL) when choosing a TKI.
Aims: The aim of this audit was to determine the impact of TKIs on symptom burden and QOL in patients with CML across multiple centres in Ireland, using the MD Anderson Symptom Inventory (MDASI) tool.
Methods: Across 7 centres in Ireland, a total of 87 CML patients currently on TKIs were identified. The mean age was 60yrs with an equal sex distribution (44 male, 43 female). All of these patients were in chronic phase. 79% of patients were in MMR (major molecular remission) at the time of survey. 53 patients were on imatinib, 19 patients on nilotinib, 13 on dasatinib and 2 on bosutinib. Patients from the 7 centres were surveyed at varying time periods between July 2015 and Feb 2017. Patients were contacted by phone. Symptom burden and QOL were assessed using the MD Anderson Symptom Inventory, an extensively validated tool which includes 13 core and 7 CML-specific symptoms, as well as 6 interference items. The questionnaire took on average 5mins to complete and asked patients to rate their symptoms on a scale of 1-10 as experienced over the preceding 24 hours.

Results: Of the 87 patients surveyed, the most commonly prevailing symptoms were fatigue (72.4%), peripheral oedema (48.3%), disturbed sleep (46%), myalgia (43.7%) and dry mouth (39.1%).The least common symptoms were nausea (20.7%) and vomiting (6.9%). Almost half (49.4%) of patients reported at least 1 severe side effect (a score of 7 or more). The most severe side effects were drowsiness (mean score 6.3), myalgia (mean score 6), fatigue, nausea and vomiting (mean score 5.7 each). There was no significant difference in symptom prevalence or severity among the different TKIs. As regards the perceived interference of symptoms on daily functioning, only 29% reported a score of 7 or more in at least 1 of the 6 interference items (*i.e.* general activity, mood, work, relations with others, walking and enjoyment of life), and only 14% reported that their enjoyment of life was severely affected (score of 7 or more). Of note, exactly two thirds of patients reported little or no interference with their enjoyment of life (score of 0-3).
Summary/Conclusions: As demonstrated in this audit, patients with CML on TKIs frequently experience chronic adverse events. Interestingly, CML patients taking second generation TKIs did not appear to have any difference in frequency or severity of symptoms or in QOL compared to patients on imatinib. Despite excellent survival results obtained with TKIs since 2001, an emphasis needs to be placed on symptom burden and QOL. The potential for adverse events with long term therapy may result in dose adjustments, treatment discontinuation, or nonadherence, all of which may negatively affect treatment efficacy. Therefore, assessment of QOL and the symptom burden experienced by patients with CML is useful to facilitate individual treatment decisions and to improve outcome as well as to evaluate the efficacy of emerging therapies.

PB1833

COST-EFFECTIVENESS OF A THERAPEUTIC EDUCATION PROGRAM (TPE) FOR PATIENTS WITH CHRONIC MYELOID LEUKEMIA AND TREATED BY TYROSINE KINASE INHIBITORS

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Aims: Within our cancer centre, an TPE program on ITK in the management of CML has been authorized since 2011. We conducted a pharmacoeconomic study to evaluate the TPE clinical impact on responses to TKI in patients with CML (based on recommendations from European Leukemia Net) and also the costs in terms of use of care.
Methods: Over the 12-month follow-up period, the study population consisted of 2 groups of CML patients monitored in our centre: - Intervention group (n=18) (IG) : Patients who benefited of TPE sessions on TKI between January 2013 and August 2015 - "Matched controls" group (n=18) (CG): Patients who benefited only from the usual care, matched to the "Intervention" group. The method of pairing the 2 groups of patients according to the age at diagnosis, sex, the molecule used in first line and the prognostic risk according to the score of Sokal was used. The main criterion of efficacy was the MMR. The considered costs were: the cost of the TPE program, estimated on the basis of the French health insurance reimbursement per patient and the costs associated with the use of "supplementary" care (examinations, consultations and additional hospitalizations). The point of view was from French health insurance.
Results: Over the 12-month follow-up period, the number of patients in MMR was similar between the 2 groups (9 in IG *versus* 8 in CG). However, the average time to obtain the MMR was significantly shorter in IG (6.9 months±3.8) than in CG (11.3 months±2.1) ($p < 0.05$). The mean duration of MMR maintenance over the 12-month follow-up period was significantly longer in IG (3.2 months±3.5) than in CG (1.5 months±1.9) ($p < 0.05$). Regarding the use of additional care, unexpected hospitalizations were significantly more numerous in CG than in IG (4 *versus* 0). Thus, costs associated with use of additional care were significantly lower in IG (€ 3,566) than in CG (€ 12,709). Thus, € 250 invested (annual allowance per patient) in the TPE saves € 508 in the use of care and reduces the time required to obtain a MMR by 4.4 months.
Summary/Conclusions: Thus, TPE is clinically and economically beneficial in our study population. By increasing the patient capacity to adapt to the treatment through the development of skills and adaptation processes, TPE reduces the costs of seeking care while improving the clinical response to treatment with a faster and more sustainable major molecular response.

PB1834

ROLE OF ALLO-HSCT IN THE TREATMENT OF PATIENTS WITH T315I MUTATION IN THE TKI ERA

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Background: Resistance to tyrosine kinase inhibitors (TKI) in patients with chronic myeloid leukemia (CML) is frequently caused by point mutations in the BCR-ABL kinase domain, including the gatekeeper mutant T315I, which confers a high degree of resistance to all currently approved tyrosine kinase inhibitors except ponatinib. The role of allo-HSCT in such patients is still disputable.
Aims: To evaluate the results of different treatment modalities in CML patients with T315I mutation.
Methods: Retrospective analysis of 53 BCR-ABL^{T315I} –positive CML patients (pts) was done.
Allogeneic bone marrow transplantation (allo-HSCT) was made in 16 pts, 37 pts received only pharmacological therapy (21 pts received TKI as monotherapy or in combination with other drugs other 16 pts received hydroxyurea, interferon- α or chemotherapy). At the time of T315I detection 29 (55%) pts were in CP, 19 (36%) pts had AP and 5 (9%) pts were in BC. Median (Me) age at the time of mutation detected was 47 years (15-76) (38 years in HSCT-group). 2 pts were in BC at the time of HSCT, 5 pts were in AP, 7 pts were in CP≥2. The number of points on EBMT scale: 3-4 points – 12(75%) pts, 5-7 points – 4(25%) pts. 11 (69%) pts received more than 2 lines TKIs before HSCT .In allo-HSCT group 11 (69%) pts had unrelated donors. Conditioning regimen in 13 (81%) pts had reduced intensity, in 3(19%) pts had MAC. Me time to HSCT after T315I detection was 10 months (1-38). Mutation analysis was performed by Sanger sequencing. Overall survival (OS) was estimated by Kaplan-Meier method with log-rank test for comparison between groups. Cox regression was used for multivariate survival analysis that included next covariates: age, phase on the time of mutation detection, performance of allo-HSCT, time to T315I detection from TKI start.
Results: Me follow-up time after T315I detection was 21 months (1-100). 5-years OS in whole group was 42% (Figure 1A). According to multivariate analysis only CML phase at the time of mutation detection significantly affect to survival in whole group. All pts in BC (n=5, 2 in HSCT group and 3 in non-HSCT group) died within first year after T315I indication wherein Me survival time was 1,3 month (Figure 1B). 5-years OS in non-HSCT group (n=37) was 42% with Me survival time 2,8 years. 5-years OS after allo-HSCT (n=16) was 37% with Me survival time 5 months (Figure 1C). All living patients after allo-HSCT are in deep molecular response. There was no significant difference in 5-years OS between TKI (n=21) and non-TKI (n=16) pharmacological therapy (non-HSCT) groups (42% and 47% respectively, p=0,53) (Figure 1D).

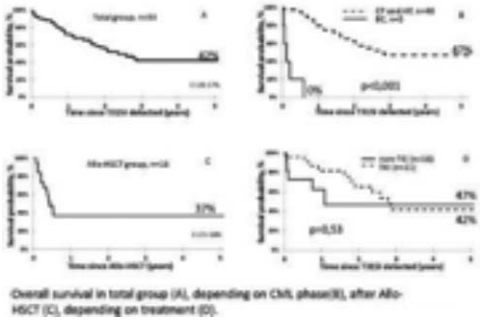

Figure 1.

Summary/Conclusions: Detection of T315I mutation in TKI-resistant patients is extremely unfavorable factor for survival, especially in the advanced phase CML, and it is a great reason for switching to ponatinib or other new potential investigated drugs if possible. Allo-HSCT can be a potential option for this group of patients in case of good selection taking into consideration transplant risk, especially for patients in CP ≥2.

PB1835

THYROID FUNCTIONAL STATUS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA ON TKIS - SINGLE -CENTER RESULTS

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Background: Tyrosine kinas inhibitors (TKI) as target specific compounds profoundly changed the outcome in patients with chronic myeloid leukemia (CML). TKI-induced thyroid dysfunction is now recognized as a common toxicity associated with some TKI. In the previous decade, cases of thyroid dysfunction have been reported in patients treated with different TKIs.
Aims: To evaluate the thyroid functional status in CML patients treated with imatinib and nilotinib.

Methods: This cross-sectional study comprised 85 patients with CML in chronic phase, treated with imatinib and nilotinib, at the Clinic for Hematology, Clinical Centre of Vojvodina, Serbia. Thyroid function was assessed by analyzing the serum FT3, FT4 and TSH levels. Hypothyroidism in relation with TKI therapy was defined as newly diagnosed hypothyroidism (while the patient was already on TKI therapy) requiring hormone substitution therapy or serum fT4 level <11,5 pmol/l and TSH >5.50 mIU/l. Patients with previous medical history of thyroid dysfunction were excluded. The duration of TKI treatment varied from 2 month to 10 years. The dose of imatinib was 400mg daily, while nilotinib was dosed 800mg a day.
Results: From the total number of patients included, 37 (43,53%) were female and 48 (56,47%) were male. Mean age was 56,71 age (range 21-84). The prevalence of hypothyroidism (clinical, and subclinical) was 8,23% (n=7) which is in accordance with the prevalence in general population. Three patients (3,53%) were diagnosed to have subclinical hypothyroidism (defined as normal serum fT4 and TSH >5.50 mIU/l). Hypothyroidism was more common in males (71,5%, p= 0,29, not statically significant). In patients treated with imatinib, 2 (3,4%) had subclinical, while 3 (5,01%) had clinical hypothyroidism. Of the 26 patients treated with nilotinib, subclinical hypothyroidism was detected in 1 (3,85%), as well as clinical hypothyroidism (3,85%). Other thyroid dysfunctions were not detected.
Summary/Conclusions: Hypothyroidism was the only thyroid dysfunction in our cross-sectional study. The prevalence of hypothyroidism in our study group did not differ from general population. Additional study on a larger sample size and evaluation of antibodies is required.

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RESPONSE RATES AND SURVIVAL OF PATIENTS WITH CHRONIC MYELOID LEUKAEMIA INITIALLY TREATED WITH IMATINIB: 11 YEAR EXPERIENCE OF A TEACHING HOSPITAL

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Background: In large trials, patients with chronic myeloid leukaemia (CML) treated with Tyrosine Kinase Inhibitors (TKIs) have relative survival rates of up to 90% that of age-matched controls. Patients achieving complete cytogenetic responses (CCyR) within 2 years of starting Imatinib have survival rates equivalent to the general population. Newer TKIs are associated with faster and deeper treatment responses, but have a more toxic side effect profile as well as being more costly.
Aims: This study looks at the 11 year experience of a single teaching hospital treating a population of almost one million and presents the response and survival data of this unselected population of patients with CML treated with imatinib as initial therapy.
Methods: A retrospective case record review was undertaken on CML patients identified from the regional cytogenetics department. Imatinib was available for routine prescription in the UK from 2003, so a 11-year period from 2003 to 2013 was selected to allow for adequate follow-up.
Results: In total 83 patients were newly diagnosed in this time period. Four patients, treated on SPIRIT2 with dasatinib as initial therapy, have been excluded from the subsequent analysis, leaving 79 patients treated initially with imatinib 400mg daily. The median age at diagnosis was 53 years (range 13-93) with 40 female and 39 male patients. The median follow up was 75 months (range in living patients 29-163 months). Fifteen patients have died (19%). The median age at diagnosis of these was 73 years. Two deaths were transplant-related, both in patients who had failed available TKIs and had mismatched transplants. The only treated patient who died of accelerated disease was intolerant of all TKIs and unfit for transplant. Three patients died of other malignancies (ovarian, bowel and melanoma). Seven patients were transplanted. Of the surviving 5, 2 had sibling transplants early in the TKI era, 2 had MUD transplants after failing imatinib prior to the availability of second line drugs, and one failed to make an adequate response to imatinib then nilotinib and received a sibling transplant. All surviving transplant patients are in a major molecular response (BCRABL:ABL ratio <0.01, MMR). An MMR was achieved by 60/79 (76%) patients. Of the 19 without MMR, 1 is lost to follow-up, and 9 have died, of which only one death was due to accelerated CML in a patient intolerant of all TKIs. Of those 9 patients living not in MMR, 8 have a CCyR. Three are elderly patients in whom we have taken a pragmatic approach, three are related to patient compliance, two to treatment limited by severe side effects and one had TKI interruption to facilitate cancer treatment. Of the sixty patients in MMR, 40 achieved this on standard dose imatinib. Four patients required increased dose of imatinib, 11 were switched to second line TKI and 5 were transplanted. A complete molecular response (BCRABL:ABL ratio <0.000, CMR) was achieved by 10 patients, six on standard dose imatinib.
Summary/Conclusions: This data shows the real life experience of patients treated for CML in the TKI era. At six years follow up, the overall survival was 86% which is remarkably similar to that of the IRIS trial patients. Using an intention to treat analysis in this unselected population, up front imatinib with appropriate escalation of treatment where response is unsatisfactory achieves an MMR rate of 76%. This offers reassurance that where appropriate monitoring is feasible, imatinib remains a good first choice for patients presenting with CML.

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FRONT-LINE NILOTINIB IS A BETTER CHOICE THAN FRONT-LINE IMATINIB FOR CML PATIENTS WITH DELAYED TREATMENT: 11 YEAR FOLLOW-UP

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Background: CML patients in developing world had to wait for the start of TKI treatment, from several months to years. The significant delay in proper treatment with imatinib has had drastic consequences on patient outcomes including survival, CCyR and MMR. Nilotinib was introduced in 2011 as front- and second-line therapy for newly diagnosed as well as patients who waited for TKI treatment for a long time.
Aims: In this study we compared the long-term real life clinical outcomes (OS, CCyR and MMR) of patients receiving front- line imatinib and front-line nilotinib therapy in Bosnia and Herzegovina in the period from 08/2005 to 08/2016, categorized based on delayed start of therapy.
Methods: All newly diagnosed CML patients in CML-CP (n=149) who started their TKI treatment in period from August 2005 to August 2016 were included in this multicentre retrospective cohort study. Patients were categorized as: Group 1 (n=118) consisted of patients who started with front-line imatinib (300 mg, 400 mg or 600 mg twice daily; Glivec or generic imatinib therapy) and Group 2 (n=31) contained patients receiving front-line nilotinib (300 mg twice daily). Patients on imatinib were further categorized by the duration of treatment delay into three subgroups (<5 months, 6-13 months and >13 months) and patients on nilotinib therapy were divided into two subgroups (patients who waited less and more than 6 months on the start of therapy). Nilotinib became available as front or second-line therapy in March 2011. Standard patients' variables were collected and disease progression was established as loss of CCyR and MMR. Survival probabilities were estimated with the Kaplan-Meier method and compared using the log-rank test.
Results: We analyzed 149 patients (median age was 54.5 years; 57% was males) in chronic phase of CML. The median follow-up from time of diagnosis and start of therapy was 45 months and 39 months, respectively (range 3-145 months). Median wait period for therapy in patients who waited less and more than 6 months was 0 months (range 0-6) *vs* 15 months in the waiting group (range 9-63). At 11 years, overall survival for patients on front-line imatinib (Group 1) and front-line nilotinib (Group 2) was 83% and 87%, respectively. According to ITT principle, achievement of CCyR and MMR at 24 months was higher in Group 2 compared to Group 1 (81% *vs* 66% and 74% *vs* 37%, respectively). Rate of death was similar in both studied groups (20/118 *vs* 4/31). When we analysed delayed treatment at 24 months, CCyR for patients who received therapy immediately, who waited 6-13 months and more than 13 months, was 74% *vs* 64% *vs* 40%, respectively. Regarding nilotinib treatment at 24 months, patients on 1st line immediate nilotinib *vs* 1st line delayed nilotinib achieved 83% *vs* 77% for CCyR and 78% *vs* 69% for MMR, respectively.
Summary/Conclusions: Our results after 11 years of follow up suggest that nilotinib demonstrated improved efficacy over imatinib therapy. Achievement of CCyR and MMR at 24 months was higher in patients on front-line nilotinib therapy. Patients who waited for therapy had optimal response regardless the wait period on nilotinib therapy.

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THE INFLUENCE OF AGE ON TREATMENT OUTCOME OF PATIENTS WITH CHRONIC MYELOID LEUKEMIA RECEIVING FRONTLINE IMATINIB

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Background: The tyrosine kinase inhibitor (TKI) imatinib was the first targeted therapy for patients with chronic-phase chronic myeloid leukemia (CP-CML), and its introduction was associated with substantial improvements in response and survival compared to previous therapies. Earlier studies have indicated that the effect of age at diagnosis of CP-CML was minimized in patients treated with imatinib: fewer responses but the same outcome for older patients. However, recently published results from clinical controlled trials indicated that there were differences in clinical outcome depending on age at diagnosis of CP-CML.
Aims: The aim of this study was to evaluate impact of age on the treatment outcome in patients with chronic myeloid leukemia treated with frontline imatinib.
Methods: A newly diagnosed CP-CML patients treated and followed in our institution were surveyed retrospectively from August 2006 to August 2016. According to age, cohort was divided into three groups: young adults (18-45 years) (YA), middle aged adults (46-64 years) (MA) and elderly persons (65 and more years) (EP). Patients' demographics, disease risk scores, duration of imatinib therapy and follow-up, cytogenetic and molecular responses,

adverse event (AEs), the 5-year event-free survival (EFS) and 5-year overall survival (OS) were all evaluated. Clinical features of the patients in different age groups are summarized in Table 1.

Results: The patient cohort consisted of 94 patients with median age of 53.4 years (range 18-78), with a slight predominance of females of 53.2%. There were more patients with intermediate and high Sokal scores in the EP group than in the groups MA and YA (p<0.001). To the contrary of that, most patients with high EUTOS score were observed in the group YA compared to MA and EP groups (p<0.001). The three groups were balanced regarding Euro score. The median duration of imatinib therapy was the longest in MA group (61.4 months *vs* 40.6 months in YA and 38.2 months in EP patients p<0.001). Furthermore, median follow-up duration was also the longest in MA group (64.3 months *vs* 48.5 months in YA and 44.7 months in EP patients p<0.001). The rates of complete cytogenetic response (CCyR) were similar in all three analysed groups (80.6% in YA, 86.5% in MA and 75.9% in EP, p=0.328) while rate of major molecular response was the highest in the MA group (83.3% *vs* 63.3% in YA and 57.1% in EL, p=0.001). The percentages of patients who switched to second-generation TKIs were similar in all three groups (36.7% in YA *vs* 30% in MA *vs* 32.1% in EP, p=0.559). There were the most of non-hematological AEs all grades in EP group (25% *vs* 13.3% in YA and 13.8% in MA, p=0.005). Hematological AEs also were common in EP group but not statistically significant (17.8% *vs* 10% in YA and in 12.1% in MA, p=0.156). The 5-years EFS in the MA group (88% (95%CI 82.1-96.9)) was significantly higher than in YA group (65.3% (95%CI 59.1-78.1)) and in EP group (60.2% (95%CI 49.5-73.7)). The 5-years OS in the EP group (74.7% (95%CI 65.9-89.0)) was significantly lower than in YA group (93.1% (95%CI 87.2-99.5)) and in MA group (90.8% (95%CI 85.8-97.8)). The number of deaths, both CML related or not related, was the largest in the EP group (25% *vs* 13.3% in YA and 13.8% in MA, p<0.001).

Table 1. Clinical features of the patients in different age groups.

	Whole cohort n=94	YA n=30	MA n=36	EP n=28	p
Gender (M/F), n	44/50	14/16	17/19	13/15	0.655
Median age, years (range)	53.4(18-78)	36.2(18-45)	55.9(46-64)	68.2(65-78)	<0.001
Sokal score Low/intermediate/high, %	41/42/17	67/23/10	44/42/14	11/64/28	<0.001
Euro score Low/intermediate/high, %	55/35/7	77/17/7	42/44/11	50/43/7	0.249
EUTOS score Low/high, %	81/19	76/27	83/17	85/14	<0.001
Any comorbidity: Yes/No, n (%)	33(35)/61(65)	3(10)/27(90)	12(33)/24(67)	18(64)/10(36)	<0.001
Median duration of therapy, months (range)	46.7(3-120)	40.6(3-114)	61.4(4-120)	38.2(6-90)	<0.001
Median follow-up, months (range)	52.5(6-120)	48.5(6-116)	64.3(7-120)	44.7(9-93)	<0.001

Summary/Conclusions: Results of this study indicate that age at diagnosis impacts the course of chronic myeloid leukemia treated with imatinib. The best clinical outcomes have middle age patients in terms of the highest rates achieved optimal therapeutic response and longer survival without events and overall survival. The degree of therapeutic responds in the elderly is comparable with that observed in younger patients, but the presence of comorbidity and more frequent occurrence of adverse events were affecting relatively lower overall survival. Although it might be expected that younger patient population has a better clinical outcome than patients middle age, a possible cause of poor outcomes is probably a late diagnosis at an advanced stage of the disease.

Enzymopathies, membranopathies and other anemias

PB1839

CHARACTERIZATION OF HEMATOPOIETIC SAMPLES FROM PYRUVATE KINASE DEFICIENCY PATIENTS

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Background: Pyruvate Kinase Deficiency (PKD) is an autosomal recessive disease caused by mutations in the PKLR gene. PKD produces chronic non-spherocytic hemolytic anemia, which can be fatal during early childhood and may result in lifelong transfusion dependence that in some instances persists despite therapeutic splenectomy. Although not considered a standard-of-care, allogeneic bone marrow transplantation has been curative in a limited number of cases. These findings, and the evidence that PKLR gene mutations are likely the sole etiology of the disorder, make PKD a candidate for gene therapy. Our lab has developed a therapeutic Orphan Drug lentiviral product (EMA: EU/3/14/1330; FDA: DRU-2016-5168) for the treatment of PKD and is working to develop an efficient and safe gene therapy clinical trial for the treatment of PKD.

Aims: In order to improve this new treatment, a more deep knowledge of the disease and its associated pathophysiology is necessary.

Methods: To characterize the hematopoietic profile of this disease, we have standardized flow cytometry protocols to perform both a qualitative and quantitative study of different population subsets. These included subsets of the hematopoietic stem cell compartment, erythroid progenitors, reticulocytes, mature erythrocytes and other mature lineages. Human routine samples consisted of peripheral blood, bone marrow and cord blood from PKD patients. In addition, xenogenic engraftment studies in immnunodeficient (NSG) mice were also performed.

Results: Flow cytometry studies showed a clear imbalance in the erythroid populations. On the other hand, human PKD progenitors were able to engraft into NSG mice demonstrating that the disease does not likely impair hematopoietic stem cell capabilities.

Summary/Conclusions: Pyruvate Kinase Deficiency (PKD) is an autosomal recessive disease caused by mutations in the PKLR gene. Our lab has recently developed a therapeutic Orphan Drug lentiviral product for the treatment of PKD. In order to improve this new treatment, we are also working to deep into the knowledge of the disease and its associated pathophysiology. Flow cytometry studies have shown a clear imbalance in the erythroid populations. Functionally, results in NSG mice we have demonstrated that the disease does not likely impair hematopoietic stem cell capabilities.

PB1840

OSMOTIC GRADIENT EKTACYTOMETRY: A VALUABLE SCREENING TEST FOR HEREDITARY SPHEROCYTOSIS AND OTHER RED BLOOD CELL MEMBRANE DISORDERS

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Background: Red blood cell (RBC) membrane disorders constitute one of the major causes of chronic hereditary hemolytic anemia. Main RBC membrane disorders, namely hereditary spherocytosis (HS), hereditary elliptocytosis (HE) and hereditary stomatocytosis (HSt), alter membrane cohesion, membrane mechanical stability, and RBC volume, respectively. As a consequence, RBC deformability is compromised leading to their premature removal from circulation, manifested as hemolytic anemia. New generation osmotic gradient ektacytometry has become a powerful procedure for measuring red blood cell deformability and therefore for the diagnosis of red blood cell membrane disorders.

Aims: The aim of this study is to evaluate osmotic gradient ektacytometry as an adequate assay to perform screening of membranopathies, focusing on the differential diagnosis between HS and non-spherocytic membrane defects such as HE and dHSt.

Methods: A total of 75 patients with chronic hemolytic anemia oriented as hereditary RBC membrane disorders (hemoglobin disorders discarded and negative Coombs test) were included during a period comprised between January 2015 and August 2016. Normal controls were obtained from blood donors. Osmotic gradient ektacytometry was performed using the osmoscan module of the Laser-assisted Optical Rotational Deformability Cell Analyzer: LoRRca MaxSis (RR Mechatronics). Evaluation of osmoscan parameters

robustness for HS diagnosis was performed using the receiver operating characteristic (ROC) curve analysis. The optimal cut-off was determined as the one with the highest likelihood ratio. Statistical analysis was operated with GraphPad Prism.
Results: Specific patterns of osmoscan LoRRca MaxSis were observed for each individual membranopathy. All HS curves were bell shaped but two different profiles were identified both presenting increased Omin, and decreased EImax and AUC. HE curves showed a characteristic trapezoidal shape with a decreased EImax, Omax and AUC. dHSt curve was bell shaped with a specific decrease in Ohyper and a slight increase in EImin. Reference ranges for each osmoscan parameter were established with 171 healthy subjects and compared with the values of the parameters obtained from the different RBC membrane disorders. ROC curve analysis was performed for HS and each one of the non-HS groups separately. The results determined that EImax was the parameter that better separated HS from normal controls and dHSt, while the Omin was the best to separate HS from HE. The optimal EImax cut-off to differentiate HS from normal controls was <0.5975 (sensitivity 98.46%, specificity 99.42%), while the optimal Omin cut-off to differentiate HS from HE was >159.0 (sensitivity 95.38%, specificity 85.71%). Expressing the results as% of variation in relation to the mean of our normal controls, the best combination of parameters for HS diagnosis would be EImax <-3% and Omin >5.2%. This combination of parameters (EImax <-3% and Omin >5.2%) was used as criteria to classify all the 246 samples included in the present study, and the result showed 62 samples detected as HS and 184 as no-HS. Of the 62 patients identified as HS, 61 were real HS (specificity 98.38%) and 1 was an HE. On the other hand, 4 HS patients were identified as non-HS (sensibility 93.85%).
Summary/Conclusions: We can conclude that, the inclusion of LoRRca osmoscan as a screening test in RBC membrane diagnostic workflow will signify an important advance for the accurate diagnosis of HS patients, as well as for the identification of HE and specially dHSt patients.

PB1841

RARE RED BLOOD CELL ENZYMOPATHIES INDUCED CHRONIC NONSPHEROCYTIC HEMOLYTIC ANEMIA: NEXT GENERATION SEQUENCING BASED MOLECULAR DIAGNOSIS

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Background: Red blood cell enzymopathies are mostly inherited autosomal recessive monogenic disorders. Mutations in the genes encoding red blood cell enzymes could lead to chronic nonspherocytic hemolytic anemia (CNSHA). The clinical manifestations are jaundice, cholelithiasis, splenomegaly, with usually normocytic normochromic hemolytic anemia. Phenotypes vary from having fully compensated hemolysis (without anemia) to severe hemolytic anemia requiring regular transfusions. Definitive diagnosis is difficult when biochemical test results are not consistent/fail to identify defects. Molecular diagnosis by gene-by-gene approach is expensive, time consuming and cumbersome as testing for multiple genes is required.
Aims: Use of targeted resequencing can expedite the molecular diagnosis where the cause for hemolysis remains unexplained after routine laboratory tests.
Methods: Ten patients with clinical and laboratory evidence suggestive of hemolytic anemia were enrolled. Various biochemical and molecular tests were used to exclude Glucose-6-phosphate dehydrogenase (G6PD) deficiency, thalassemias, hemoglobinopathies, autoimmune hemolytic anemia, hereditary spherocytosis and pyruvate kinase deficiency. Common G6PD and PKLR variants were excluded by molecular tests. Family history was negative in all the cases. Libraries were prepared using TruSight One sequencing panel and sequenced on MiSeq™ Sequencing System. MiSeq Reporter™ and VariantStudio™ v2.1 were used for analysis, classification, and reporting of genomic variants.
Results: Two patients with G6PD deficiency, six patients with pyruvate kinase (*PKLR*) deficiency and two patients with Glucose-6-phosphate isomerase (*GPI*) deficiency were found. Unexpected pyruvate kinase defects were found on targeted re-sequencing for six patients. Pyruvate kinase (PK) enzyme activity assay were within normal limits in all these cases. All the mutations were predicted deleterious by PolyPhen/ SIFT/ Provean/ mutpred and Mutationtaster. Mutations were validated in the parents/siblings (where available) to prove the mode of inheritance.
Summary/Conclusions: Unexpected PK deficiency were found after next generation sequencing analysis in the patients where PK enzyme levels were within normal limits. PK deficiency may be missed by conventional testing approaches. Our data demonstrates the clinical utility of next generation sequencing for molecular diagnosis. Timely detection of the cause in our patient is likely to be helpful not just in genetic counselling and future antenatal diagnosis, if required, but therapeutically as well. A splenectomy (performed at appropriate age) can ameliorate the anemia in such patients and can eliminate need for transfusions in those that need them.

PB1842

COMPARISON STUDY OF THE EOSIN-5'-MALEIMIDE BINDING TEST, OSMOTIC FRAGILITY TEST AND CRYOHEMOLYSIS TEST IN THE DIAGNOSIS OF HEREDITARY SPHEROCYTOSIS

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Background: The primary lesion in HS is loss of membran surface area due to defects of the membran protein. Cryohemolysis test and osmotic fragility (OF) test are used for screening. However no test for HS is 100% reliable. The eosin-5'-maleimide (EMA) binding test based on flow cytometry. Eighty percent of the fluorescent-labelled EMA binds to band 3 protein which is lost in HS due to protein 4.1, spectrin and ankyrin deficiency. Thus measurement of the flourescent EMA test detects all the different forms of HS.
Aims: In this study we aimed to evaluate the concordence of EMA binding test with other diagnostic parameters for HS.
Methods: The patients with HS were diagnosed according to clinical findings for hemolytic anemia, splenomegaly and spherocytes in peripheral blood. Hemogram, reticulocyte count, total/direct bilirubin, spherocytes in blood smear (BS), EMA binding test, OF test, and cryohemolysis test were obtained from pateints and control groups. Correlation between EMA, OF and cryohemolysis tests were evaluted.
Results: Twenty-five male, 17 female HS patients aged between 1.0-19.0 years and 38 male, 47 female healthy controls were evaluated. There were no differences between both groups in terms of age and sex (Table 1). The median (range) values of hemoglobin (%), reticulocyte count (%), mean corpuscular volume (fl), MCHC (%) and total bilirubin level were seen in Table 1. Besides MCV values there were differences between groups in terms of these parameters (Table 1). The median MCF of HS patients was significantly lower than that of healthy controls while cryohemolysis and osmotic fragility were higher in HS patients than healthy controls (Table1). There were moderate concordence between cryohemolysis and EMA test (r=-0.355, p<0.001). The sensitivity of EMA was 92.86%, specifity was 82.35%, PPV was%72.22, NPV was%95.89. EMA was superior diagnostic test to osmotic fragility. (sensitivity;%83.33, specifity;%76.47, PPV;%63.64 and NPV;%90.28). The sensitivity of cryohemolysis test was 90.48%, specificity was 94.12%, PPV was%88.37, NPV was%95.24.

Table 1. Comparison of Clinicaland Laboratory Findings in Hereditary Spherocytosis groups and Healthy Controls

	Hereditary Spherocytosis (n =42)	Healthy Controls (n = 85)	*P* Value
Sex, M/F	25/17	38/47	>0.05
Age, median (range), year	8.5 (1.0-19.0)	12.0 (1.0-20.0)	0.416
Hemoglobin, median (range), g/dL	10.8 (5.8-14.7)	14.2 (11-17.2)	<0.001
Reticulocyte, median (%)	6.1 (1.6-38.0)	1.1 (0.1-4.1)	<0.001
MCV, median (range), fL	77.5(73-90)	82(77-94)	0.994
MCHC, median (range), g/dL	33.0(27.0-37.0)	32.0 (27.0-35.0)	<0.001
Total bilirubin, median (range), mg/dL	3.3 (1.0-16.0)	0.9 (0.2-2.9)	<0.001
EMA (%), median (range)	18.38 (8-48)	26.43 (12-45)	<0.001
Cryohemolysis (%), median (range)	37.0 (2.0-83.0)	3.8 (2.0-14.0)	<0.001
OF (%)	62.2	37.8	<0.001

M/F; Male/Female, FC EMA;flowcytometric eosin-5'-maleimide, OF;osmotic fragility, HS; hereditary spherocytosis, MCHC; mean corpuscular hemoglobin concentration, MCV; mean corpuscular volume, BS; bloodsmear

Summary/Conclusions: In this study EMA-FC was more sensivitive and specificity than osmotic fragility. However specificity and PPV of cryohemolysis was higher than other test. Also we showed moderate concordance cryohemoysis and EMA test.
Although high sensitivity and specifity of EMA test there were need to use other tests together with family history of patient , physical examination, evaluation of blood smear and several tests for HS diagnosis.

PB1843

ADVANCES IN DIAGNOSIS OF HEREDITARY HEMOLYTIC ANEMIAS: THERMOGRAVIMETRY COUPLED WITH CHEMOMETRICS

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Background: The differential diagnosis of hereditary hemolytic anemia is generally carried out by applying different diagnostic protocols depending on the specific congenital erythrocyte defects. Thermogravimetric analysis (TGA) coupled with chemometrics has recently been proposed as a rapid and cost effective diagnostic tool for β-thalassemia screening. This model, consisting of Partial Least Square-Discriminant Analysis (PLS-DA), permitted the discrimination of thalassemic patients and healthy individuals, using thermogravimetric curves of blood samples [1].
Aims: In this study, the capability of thermogravimetry in conjuction with a mul-

tivariate statistical analysis was investigated for the screening of hereditary hemolytic anemias due to different erythrocyte defects.
Methods: Whole blood samples collected in K_2EDTA were obtained, after informed consent, from patients suffering from congenital hemolytic anemias and were analyzed using the thermobalance TG7 (Perkin Elmer) without any pretreatment and the resulting curves were compared with those of healthy individuals. Two groups of hereditary hemolytic anemias were considered: the hemoglobinopathies (sickle cells anemia and thalassemia) and the erythrocyte membrane defects (hereditary elliptocytosis and hereditary spherocytosis).
Results: The characteristic profile of the blood sample thermal decomposition and the first derivative (DTG) of the TG curve showed that blood samples from anemic patients were clearly distinguished from those of healthy individuals as a result of different amounts of water and corpuscular fraction. The chemometric approach based on Principal Components Analysis (PCA) allowed a quick identification of differences between healthy and anemic patients in order to point out a model of prediction in patients with heterogeneous congenital hematological disorders.
Summary/Conclusions: The achieved results allow to consider the coupling TGA/Chemometrics as a promising diagnostic approach to provide a high-throughput and sensitive tool to obtain an early detection of hereditary hemolytic anemias using only a few microliters of blood without any pretreatment and with an hour of analysis time.

PB1844

DEVELOPMENT OF A POINT-SCORING SYSTEM FOR EARLY DIAGNOSTIC TESTING IN GAUCHER DISEASE: APPLICATION OF FINDINGS FROM THE GAUCHER EARLIER DIAGNOSIS CONSENSUS DELPHI INITIATIVE

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Background: In the Western hemisphere, Gaucher disease (GD) type 1 is the most common GD phenotype, but the prevalence of GD type 3 is increasing. The spectrum of signs and symptoms of the different GD phenotypes ranges from fatal perinatal to asymptomatic adult disease, and the heterogeneity of its presentation contributes to both misdiagnosis and delays in diagnosis by clinicians unfamiliar with the disease. The Gaucher Earlier Diagnosis Consensus (GED-C) Delphi initiative determined which signs and patient co-variables are regarded by experts in GD as most indicative of GD types 1 or 3 in the early stages.
Aims: From the findings of the GED-C expert consensus, to generate a simple web-based point-scoring system (PSS) suitable for use across clinical specialties, that provides guidance based on patients' presenting signs as to whether GD diagnostic testing is appropriate.
Methods: An anonymous three-round Delphi process, conducted among a global panel of 22 expert physicians, established consensus on which signs and co-variables may be important in early GD type 1 and, separately, in early GD type 3. In round 1, free-text responses provided by the panel were categorized by an independent administrator. This categorization was checked and consolidated into summary factors by the non-voting co-chairs. In round 2, the factors were rated for importance by the panel using a 5-point Likert scale (1 = not important, 3 = important, 5 = extremely important). Any factors assigned an importance score of ≥3 by >75% of respondents were then rated for agreement in round 3, using a 5-point pivoted Likert scale (1 = strongly disagree, 3 = neither agree nor disagree, 5 = strongly agree). Consensus was defined as a score of ≥4 by >67% of respondents. Factors meeting this threshold were classified as major; all other factors were classified as minor. The co-chairs defined value ranges corresponding to mild, moderate or severe forms of five of the major signs of GD (anaemia, hepatomegaly, hyperferritinaemia, splenomegaly and thrombocytopenia). Panel members indicated whether they regarded each range as consistent with a GD diagnosis. This information was used in combination with the classifications of signs and co-variables as major or minor to create a prototype PSS.
Results: There was a 100% response rate in each round. Factors identified as major or minor in GD types 1 or 3 are given in the Table 1. There was 100% agreement that splenomegaly (≥3-fold enlargement) and disturbed oculomotor function (slow horizontal saccades with unimpaired vision) are major signs in GD, and these were assigned a score of 3 in the prototype PSS; other major signs and co-variables were assigned a score of 2. The panel was divided about whether severe anaemia, hepatomegaly, hyperferritinaemia and severe thrombocytopenia were consistent with a GD diagnosis, so these were assigned a score of 1. All minor signs and co-variables were assigned a score of 0.5.
Summary/Conclusions: A prototype PSS to inform GD diagnostic testing has been developed from the GED-C Delphi initiative. The PSS will be validated with retrospective patient data. Total patient scores based on presenting signs and co-variables will be used to determine empirically a minimum threshold score that captures positive tests for GD. Abstract submitted on behalf of the GED-C panel and the EHA Scientific Working Group 'Quality of Life and Symptoms'. Administration of the GED-C initiative was funded by unrestricted educational grants from Shire International GmbH.

Table 1.

	Score	Sign or co-variable	
Major signs and co-variables	3 points	Splenomegaly (≥ 3-fold enlargement) Disturbed oculomotor function (slow horizontal saccades with unimpaired vision)	
	2 points	Thrombocytopenia, mild or moderate (platelet count 50–150 x 10⁹/L) Bone issues, including pain, crises, avascular necrosis and fractures Family history of Gaucher disease Anaemia, mild or moderate (haemoglobin 95–140 g/L) Hyperferritinaemia, mild or moderate (serum ferritin 300–1000 µg/L) Jewish ancestry Disturbed motor function (impairment of primary motor development) Hepatomegaly, mild or moderate (≥ 3-fold enlargement) Myoclonus epilepsy Kyphosis Adult gammopathy – monoclonal or polyclonal	
	1 point	Anaemia, severe (haemoglobin < 95 g/L) Hyperferritinaemia, severe (serum ferritin > 1000 µg/L) Hepatomegaly, severe (> 3-fold enlargement) Thrombocytopenia, severe (platelet count < 50 x 10⁹/L)	
Minor signs and co-variables	0.5 point	Gallstones Bleeding, bruising or coagulopathy Leukopenia Cognitive deficit Low bone mineral density Growth retardation including low body weight Asthenia Cardiovascular calcification Dyslipidaemia	Elevated angiotensin-converting enzyme levels Fatigue Pulmonary infiltrates Age ≤ 18 years Family history of Parkinson disease Blood relative who died of fetal hydrops and/or with diagnosis of neonatal sepsis of uncertain aetiology

PB1845

REGIONAL DISTRIBUTION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN TURKEY AND EVALUATION OF CLINICAL FINDINGS

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Background: Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common inherited enzyme deficiency, that affects more than 400 million people around the world with more than 300 variants. According to data by the World Health Organization which was published in 1989; 7.5% of people in the world have at least one gene G6PD deficiency and this ratio is the highest in sub-Saharan Africa and Southeast Asia (15-26%). This ratio is in the range of 0.5-2.9% in Turkey, as United States and the neighboring countries to Mediterranean Sea. The epidemiological studies about G6PD deficiency in Turkey were mostly regional or limited to a city.
Aims: We aimed to evaluate in terms of regional distribution and clinical features of G6PD deficiency by screening the patients who applied for soldier recruitment.
Methods: The patients who applied for soldier recruitment between January 2011-March 2016, were analyzed retrospectively. Patients, who were diagnosed G6PD deficiency were scanned by using hospital patient information system. The patients' ages, the cities they lived, complaints and the stories of them were questioned. Complete blood count, serum AST, LDH, total and direct bilirubin levels of all the cases in the study were recorded. G6PD levels were measured by quantitative spectrophotometric methods in biochemistry laboratory. The World Health Organization (WHO) is divided G6PD enzyme deficiency into five classes based on enzyme activity levels and clinical findings.
Results: The distribution of the cities where the cases were living, was given on the map in Figure 1. Patients' average age, hemoglobin, and G6PD levels were 26.42±4.62, 14.68±1.51, and 0.86±0.81 respectively. According to clinical history of patients prior to diagnosis, 29 patients (20.7%) were diagnosed after acute hemolytic episodes. Of these patients 23, 4, 2, had hemolytic episodes due to drug, infection, chemical respectively. Subsequently, 78 (54.5%) and 27 (18.9%) of the remaining patients were diagnosed G6PD deficiency after the examinations due to hemolysis after favism and prolonged neonatal jaundice respectively. 6 patients (4.3%) were diagnosed of G6PD deficiency by screening because of family history, but they didn't have any hemolytic episodes before. After the patients evaluated with their clinical history and hemolysis findings; 6 patients (4.3%), who had chronic hemolysis, was considered compatible with Class I variant. 128 cases were considered as Class II variants.
Summary/Conclusions: G6PD enzyme deficiency in Turkey is seen most frequently in the Mediterranean region and the prevalence of G6PD deficiency in Central Anatolia and Aegean regions was seem to be over the Turkey average (2.9%). Nearly half of the patients had hemolytic anemia due to favism. It is followed by hemolysis due to neonatal hyperbilirubinemia and drugs. 128 (91.4%) patients who had severe G6PD deficiency with intermittent hemolysis, were considered as Class II variants.

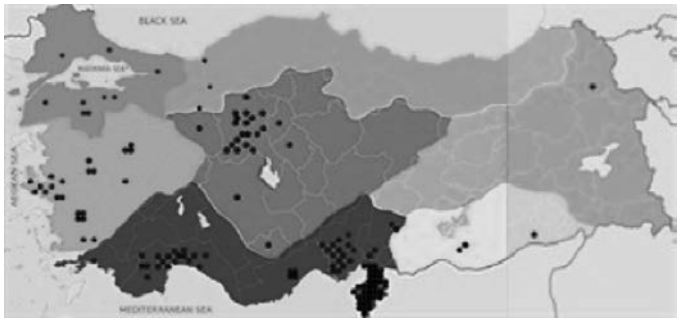

Figure 1.

PB1846

CHARACTHERISTICS AND MANAGEMENT OF AUTOIMMUNE HEMOLYTIC ANEMIA : A SINGLE CENTER STUDY WITH 32 CASES

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Background: Autoimmune hemolytic anemia (AIHA) is characterized by red blood cell destruction mediated with autoantibodies against to RBC antigens. Most common type is warm AIHA which can be either idiopathic or secondary to underlying disorders with immun disturbance. Determining the optimal therapy is a challenge because of insufficient data from prospective controlled trials.
Aims: To evaluate the clinical charactheristics, treatment responses and outcomes of our AIHA patients.
Methods: The clinical data of 32 patients with AIHA diagnosed and treated in our center between 2008 and 2016 were retrospectively analyzed.
Results: Median age at diagnosis of AIHA was 45 years (range:20-74). Male/female ratio was 1/1.3. 24 of 32 patients (75%) had primary AİHA and 8 (25%) had secondary AIHA with underlying disorders as SLE in 2 patients, , mixed connection tissue disease (MCTD) in 2, psoriatic arthritis in 1, chronic lymphocytic leukemia (CLL) in 1, marginal zone lymphoma in 1 and, chronic HCV infection in 1. Median Hemoglobin (Hb) level was 7.4 gr/dl and 5 patients also had thrombocytopenia (<150000) beside hemolytic anemia. Mean LDH level was 544, indirect bilirubin was 2.7, reticulocyte was 11.3%. 18/32 patients (56%) required transfusion. In all patients who required treatment (94%) corticosteroids were the first-line therapy with an initial response rate of 93%. Median steroid duration was 3 months range between 1.5 to 96 months. Relapse was occured in 15 of 30 patients who recieved steroid (50%) with the median time to relapse (TTR) of 12 months (range:5-72 months). 11/30 patients (37%) required second-line therapy; seven had undergone splenectomy, three recieved rituximab, and one recieved danasin. All of the patients who undergone splenectomy had CR in first month and relapse after splenectomy was seen in 5/7 patients (71%) with a median duration of 60 months. Of three patients who were treated with standard dose of Rituximab; two achieved CR and one did not achieve any response. One of two rituximab-responded patients relapsed at 26. and 60. months and re-treated by rituximab; still following with CR for 16 months.
Summary/Conclusions: Although corticosteroids are the first choice of initial treatment of AİHA, most of the patients relapse at follow up. Steroid dependency and intolerance are also challenging. Splenectomy is still a considerable option for second-line therapy because of its high response rates and long remission durations. Rituximab is the other effective second-line therapy option with similar response rates to splenectomy. Until prospective studies will be performed, retrospective data would help the clinicians to choose best treatment algorithm for AIHA.

PB1847

THE IMPACT OF THE REORGANIZATION OF THE PATIENT CARE PROCESS FOR GAUCHER DISEASE IN HEALTH SYSTEM

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Background: Gaucher disease (GD) is a multisystemic disease of lysosomal storage that is caused by deficient activity of the glucocerebrosidase enzyme resulting from a recessive autosomal hereditary mutation in the β-glucocerebrosidase gene. The accumulation of glucocerebrosidase in the lysomes damages the hematological, skeletal, and nervous systems and leads to three varieties of the disease: type 1, which is non-neuropathic, and types 2 and 3, which are neuropathic. In Mexico, the process by which patients with lysosomal disease are cared for was reorganized by the *Clínicas de Referencia Nacional y Grupos de Expertos en Enfermedades Lisosomales* (National Reference Clinics and Expert Groups on Lysosomal Diseases [EGLDs]), who created the *Guías de Práctica Clínica* (Clinical Practice Guidelines) for GD
Aims: This study aimed to evaluate the results obtained for 39 patients diagnosed with type 1GD (25 women and 14 men) through the National Reference Clinics and EGLDs
Methods: The clinical case of 39 patients was analyzed and punctual mutation of the β-glucocerebrosidase gene was determined. The patients were treated with imiglucerase enzyme at 60 UI/Kg every 14 days. The enzymatic activity of the β-glucocerebrosidase and the chitotriosidase was determined. We determinate concentration of hemoglobin and platelets. The degree of hepato-, splenomegaly, bone density and skeletal pain was evaluated.
Results: Four of the 39 patients were found to have been incorrectly diagnosed with GD, the remaining 35 patients completed the treatment goals, which included remission from hepatomegaly, splenomegaly, and skeletal pain. Additionally, increases in the hemoglobin and platelet concentration and bone mineralization were achieved, thereby attaining the patients' therapeutic goals, reducing the therapeutic dose required, and achieving the expected impacts on their health.
Summary/Conclusions: this reorganization of patient care successfully reduced complications, improved care, and optimized the use of resources and costs of GD treatment.

PB1848

NORMOCYTIC ANEMIA IS MORE COMMON THAN MICROCYTIC ANEMIA IN GASTRO-INTESTINAL CANCERS: A LARGE SINGLE CENTRE STUDY

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Background: Microcytic anemia is traditionally associated with GI cancers and led to endoscopic investigations to evaluate for GI cancers.
Aims: We evaluated the haematological profile of a large series (855) of consecutive GI cancer patients at diagnosis in a university hospital.
Methods: This retrospective study analysed the full blood count of 265 colorectal cancer (CRC) patients over one calendar year and 590 patients with esophago-gastric cancers (OGC) over 3 calendar years. WHO guidelines were used to define anemia (Hb <130 g/L in males and <120 g/L in females). Further analysis was done based n severity of anemia (mild>110 g/L, moderate 80-110 g/L and severe<80 g/L), sex, age and tumour location.
Results: Among the 265 CRC patients, 116 (44%) were anemic, of which 72 (27%) were normocytic, 43(16%) were microcytic and 1 was macrocytic. 67/152 (44%)male patients were anemic, of which 42 were normocytic and 24 microcytic. 49/113 (43%) female patients were anemic, of which 30 normocytic and 17 microcytic. Patients above age of 60 had more normocytic anemia (31%) than microcytic anemia(12%), while among those below 60 had more microcytic anemia (30%) than normocytic anemia. Leucocytosis and thrombocytosis were seen only in 14% and 12% of CRC patients.
Among 590 OGC patients, 285 (48%) were anaemic, of which 221(37%) were normocytic, 51(9%) microcytic and 13(2%) macrocytic. 194/390 (50%) male patients were anaemic, of which 148 (38%) were normocytic and 38(10%) microcytic. 91/200 (46%) were anaemic, of which 73(37%) were normocytic and 13 (7%) microcytic. Among 392/590 esophagial cancers, 123 (31%) were normocytic and 25 (6%) were microcytic. Of 198/590 gastric cancers, 98 (49%) were normocytic while 26(13%) were microcytic. Leucocytosis and thrombocytosis was seen only in 16% and 13% of OGC patients.
Summary/Conclusions: There is a higher prevalence of normocytic anemia than microcytic anemia in Gastro-intestinal cancers almost at a ratio of 2:1. Normocytic anemia is more common in elderly patients and those with mild to moderate anemia. The causes may be multifactorial including anemia of chronic disease secondary to malignancy. This highlights the fact that GI cancers must be considered as a cause in normocytic anemia irrespective of iron deficiency and symptoms of GI cancer should be carefully explored and investigations triggered.

Gene therapy, cellular immunotherapy and vaccination

PB1849

DEMONSTRATION OF FUNCTIONAL SIMILARITY OF PROPOSED BIOSIMILAR ABP 798 TO RITUXIMAB

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Background: Proposed biosimilars undergo comprehensive structural and functional characterization before they can be studied in confirmatory clinical trials. ABP 798 is being developed as a biosimilar to rituximab. The originator is approved for treatment of non-Hodgkin's lymphoma, chronic lymphocytic leukemia, severe rheumatoid arthritis, granulomatosis with polyangiitis, and microscopic polyangiitis.
Aims: ABP 798 was compared with rituximab sourced from the European Union (EU). Quality attributes assessed included binding properties (CD20, C1q, FcRn, and Fcγ receptors), antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and induction of apoptosis.
Methods: Binding of ABP 798 and rituximab to the CD20 antigen was characterized using a cell-based CD20 binding assay utilizing the human B-lymphoblastoid, WIL2-S, cell line. A direct binding ELISA was used to assess the binding of the Fc domain of ABP 798 to C1q. Binding of the Fc moiety of ABP 798 and rituximab to FcγRIa, FcγRIIa, FcγRIIb, and FcγRIIIa (158V) were evaluated in AlphaLISA® competitive binding assays. Binding to FcRn was evaluated by an AlphaScreen® competitive binding assay. ADCC activity was evaluated in a functional cell-based assay, with CD20-expressing WIL2-S cells used as target cells and NK92-M1 cells, stably transfected with human CD16 (FcγRIIIa [158V]), used as effector cells. CDC activity was evaluated in a functional cell-based assay using a CD20 expressing human B-lymphoblastoid WIL2-S cell line and baby rabbit complement. Induction of apoptosis was assessed by measuring activation of caspase 3/7 in SU-DHL-4 cells, a CD20-expressing human B cell lymphoma cell line.
Results: Relative binding (%) was comparable between ABP 798 and rituximab (Table 1).

Table 1.

Assay	ABP 798	Rituximab
CD20	91 – 105	82 – 105
C1q	85 – 100	85 – 89
FcRn	89 – 104	92 – 115
FcγRIa	94 – 98	87 – 95
FcγRIIa	96 – 102	96 – 106
FcγRIIb	96 – 108	93 – 109
FcγRIIIa (158V)	88 – 100	67 – 97

The dose response profiles and relative activity for ADCC and CDC were similar (mean ADCC relative activity: ABP 798, 88%; rituximab, 86%; mean CDC relative potency: ABP 798, 103%; rituximab, 104%). The dose response profile for induction of caspase 3/7 was comparable between ABP 798 and rituximab.
Summary/Conclusions: The results presented here suggest that ABP 798 is similar to rituximab sourced in the EU in terms of biological activity across the range of tested functions. These results provide a firm foundation for further clinical development of ABP 798.

PB1850

DELAYED EFFECT OF G-CSF ON THE CYTOKINE SECRETION THROUGH G-CSF MOBILIZATION OF PERIPHERAL BLOOD STEM CELLS IN CHILDREN WITH CEREBRAL PALSY

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Background: Granulocyte colony-stimulating factor (G-CSF) has been widely used to mobilize peripheral blood stem cells. In addition, it also has been tried to reveal the regenerative potential in various neurodegenerative diseases.
Aims: We investigated the short-term and delayed effects of infused G-CSF for peripheral blood stem cell (PBSC) mobilization on the various cytokine secretions in children with cerebral palsy (CP).
Methods: G-CSF (10μg/kg/dose) was administered subcutaneously for 4 days to the children with CP. In first group, blood levels of G-CSF, interleukin (IL)-6, IL-10, insulin-like growth factor (IGF-1), vascular endothelial growth factor (VEGF), and brain derived neurotrophic factor (BDNF) as well as mobilized total nucleated cell (TNC)/CD34+ cell counts in peripheral blood were compared between just before G-CSF injection (D+0) and 1 day after 4 days of G-CSF injections (D+5). In second group, cytokine levels were compared between D+0 and 1 month after 4 days of G-CSF injection (D+30). Cytokine levels were measured by enzyme-linked immunosorbent assay.
Results: Baseline levels of G-CSF were significantly increased (p=0.000) and IGF-1 decreased (p=0.011) at D+5 after 4 days of G-CSF administration compared to control group. In contrast, other cytokine levels including IL-6, IL-10, VEGF, and BDNF did not show any significant changes between before and after G-CSF administration. CD34+ cell counts (p=0.000) as well as TNC counts (p=0.000) were significantly increased from D+0 to D+5 in children who received G-CSF compared to children received placebo. Regarding delayed effect of G-CSF administration, G-CSF levels were significantly increased from baseline to D+30 (p=0.000), along with the increase IL-10 (p=0.035) and VEGF levels (p=0.011) and the decrease of IGF-1levels (p=0.014).
Summary/Conclusions: G-CSF which administered to mobilize PBSCs could induce the delayed effects on the levels of G-CSF itself as well as of other cytokines which could affect on the neuroregenerative potential. Further studies would be warranted to reveal the mechanism and clinical significances of these delayed effect of G-CSF or mobilized PBSCs.

PB1851

MYD88 IN PRAME GENE ACTIVATION

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Background: PRAME is the most frequently expressed non-X-chromosomal cancer-testis gene in solid and hematological cancer. It is important, because PRAME often has a bad prognostic significance. In early studies was found that PRAME frequently coexpressed in translocation-harboring (like t(8;21), t(15;17) and t(9;22)) haematological diseases. Authors supposed that chimeric genes are activators of PRAME expression. But in large cases with normal karyotype PRAME is also expressed. Another reason for PRAME expression is promoter demethylation. But demethylating agents cannot activate PRAME expression in hematological cells taken from healthy donor. So presence of chimeric genes and methylation status only are not enough to explain why PRAME can be expressed in high level. Wadelin *et al.* found that PRAME expression level was increased in cell during lipopolysaccharide-treatment conditions. Role of MYD88 in this process still be unknown.
Aims: To check if MYD88 participates in activating PRAME expression in leukemia cell lines.
Methods: Three cell lines were used for incubation with anti-PRAME antibody: chronic myeloid leukemia cell line K562 with high PRAME expression level (645%), acute monocytic leukemia cell line THP-1 with intermediate PRAME expression level (2,92% relative to ABL) and acute myeloid leukemia cell line NOMO-1 with low PRAME expression level (0,46%). All cell lines were incubated in RPMI 1640 with addition of LPS in final concentration 10 ng/ml. After 1 and 4 hour of incubation total RNA was extracted and PRAME and MYD88 expression levels were measured.
Results: After 1 and 4 hours of experiment in K562 cell line PRAME expression level was increased in 2,7 and 7 fold under control, respectively, and MYD88 expression level increased in 1,1 and 2,5 fold under control. In THP-1 line PRAME expression level was increased in 20 and 25 fold, respectively, and MYD88 expression level was increased in 5,5 and 6,5 fold. In cell line NOMO-1 PRAME expression level was increased in 10 fold after 1 hour and in 14 fold after 4 hours, and MYD88 expression level was increased in 2,4 and 3,2 fold after 1 and 4 hours of experiment, respectively. Strong correlation between MYD88 and PRAME expression levels was observed (Pearson correlation coefficient 0,98).
Summary/Conclusions: We conclude that LPS after binding with TLRs initiates activating signal to PRAME gene via MYD88.

Hematopoiesis, stem cells and microenvironment

PB1852

PD-1 IS HIGHLY EXPRESSED ON MEMORY T-CELL SUBSETS RESIDING IN BONE MARROW BUT NOT IN PE-RIPHERAL BLOOD IN HEALTHY INDIVIDUALS

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Background: Recently memory T lymphocytes were shown to be a highly heterogeneous cell compartment comprising different phenotypes, functional activities, gene expression profiles and survival capacities. Phenotypically due to the differentiation stage and functional activities memory CD8+ T cells can be divided into stem cell memory (Tscm), central memory (Tcm), terminal memory (Ttm), effector memory (Tem) and terminal effector (Tte) and reside in bone marrow (BM) as long-lived persistent T cells [Mahnke YD *et al.*, 2013]. Programmed cell death protein 1 (PD-1) is well known as a negative immune regulator of T cells that has detrimental effects on anti-viral, anti-tumor immunity, mediates tissue tolerance to protect against immune-mediated tissue damage. Currently anti-PD1 immunotherapies are among the most effective anti-cancer immunotherapies available. PD1 pathway blockade is a key pathogenetic mechanism [Boussiotis VA *et al.*, 2014]. Understanding the influence of PD-1 pathway on memory T cell homeostasis in BM might be critical for improving treatment of patients with cancers and hematological malignancies, but is still not well understood.
Aims: To evaluate PD-1 expression on distinct memory T cell subsets in BM and PB of healthy donors.
Methods: The first portion of BM and a sample of PB were obtained from healthy donors (n=10, m=6, f=4) with age 37.5 (22-53) years old.. Numbers of white blood cells (WBC) in BM and PB samples were evaluated by Sysmex XE-2100 hematology analyzer. 1*10^6 of WBC (excluded nucleated red blood cell) from BM and PB were stained using "lyse-wash-stain" standard protocol. The CD8-APC-Cy7, CCR7-PE-Cy7, CD28-PE, CD45R0-FITS, PD1-APC antibodies were used for cell staining and 7-AAD was used for to discriminate dead cells during flow cytometry.
Results: PD1 expression by T memory cell subsets is shown in the Table 1 (median with interquartile range). The percentage of PD1+ cells within Tcm CD8+ subset was 34,2%±8,033% in BM *versus* 10,4%±1,23% in PB. Similar trend was observed in other subsets: Tscm, Tem, Ttm, Tte. Median of PD1+ CD8+ cells were 3,8%±1,015%, 22,7%±6,39%, 42,7%±7,86%, 21,9%±4,047% and 2,6%±0,41%, 6,6%±2,59%, 12,7%±1,25%, 8,9%±0,825% in BM and in PB respectively.

Table 1.

PD1 expression (median with IQR)	Tnv+Tscm $CD45R0^{-}$ $CCR7^{+}CD28^{+}$	Tcm $CD45R0^{+}$ $CCR7^{+}CD28^{+}$	Ttm $CD45R0^{+}$ $CCR7^{-}CD28^{+}$	Tem $CD45R0^{+}$ $CCR7^{-}CD28^{-}$	Tte $CD45R0^{+}CCR7^{-}$ $CD28^{-}$
BM	3,8 %±1,015% ns	34,2%±8,033% p<0,01	42,7%±7,86% p<0,01	22,7%±6,39% p<0,01	21,9%±4,047% p<0,01
PB	2,6 %±0,41%	10,4%±1,23%	12,7%±1,25%	6,6%±2,59%	8,9%±0,825%

Summary/Conclusions: We found higher frequencies of PD-1 expressing memory BM T cells comparing to PB. This might point to the important roles of PD-1 in regulation of memory T cells homeostasis in BM. In physiological conditions PD-1 is thought to neutralize self-reactive naive T cells that in its turn leads to restraining T cells excessive activation and blockade the development of autoimmunity in BM. On the other hand low expression of PD1 on T cells in PB can be explained by needs the opportunity for prompt reactivity with pathogens that also provide normal «robust control» and prevent developing of a disease.

PB1853

BONE MARROW STROMAL CELLS MAY HAVE GENETIC ABERRATIONS AND ARE CAPABLE TO GAIN THEM IN A CULTURE

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Background: Stromal microenvironment posses a key role in the regulation of both normal hematopoiesis and its reconstitution after hematopoietic stem cell transplantation (HSCT). Recent data supports the idea that bone marrow stromal cells (BMSC) also have genetic aberrations and may tightly involved in the pathogenesis of HSCT complications. These findings justify the need for more detailed study of genetic aberrations in BMSC.
Aims: The **aim** of this study was to evaluate genetic aberrations in BMSC and check the ability to gain them in coculture system.
Methods: The interaction of BMSC with hematopoietic tumor cell lines bearing specific genetic aberrations (BCR-ABL fusion transcript for K-562 and JAK2 V617F mutation for Uke-1 cell line) was investigated in stromal cells harvested from 17 patients and 8 healthy donors. We performed cultivation of BMSC monolayer and tumor cells suspension using semipermeable membrane plates inserts with different pore size (0,4 μm and 3,0 μm) in order to exclude direct cell-to-cell contact. We looked also for existing specific genetic aberrations (point mutations and fusion transcripts) in BMSC of patients with the respective aberration in their leukemic clone. For this purpose we used both karyotyping (7 patients) and RQ-PCR method. BMSC were examined by flow cytometry to evaluate the possible contamination with cells of hematopoietic lineage.
Results: We investigated the BMSC karyotype in seven patients and only one case led us to a remarkable finding. The clonal chromosomal rearrangement t(1;7) was detected in 25% of BMSC metaphases. Interestingly, this aberration was never detected in patient's leukemic cells.
We also examined BMSC from leukemia patients bearing recurrent genetic abnormalities and in one case the leukemia-specific marker was detected by RQ-PCR - we observed expression of ETV6-RUNX1 gene (≈0,02%) in BMSC by patient with t(12;21) acute lymphoblastic leukemia. At the moment of BMSC culture initiation ETV6-RUNX1 expression in patient's bone marrow was detected at high level (ETV6-RUNX1/ABL*100=321%). Before carrying out RNA extraction BMSC were harvested after the second passage and no contamination with CD45+/CD34+ cells by flow cytometry was observed (50,000 events collected from the sample). When BMSCs and Uke-1 cell line were cocultured by using of 3,0 μm pore culture dishes inserts, the BMSC population gained the Jak2V617F mutation (allele burden ≈ 30,39%). We reproduced similar experiments with the K-562 cell line and got similar results - CD45+ cells were also detected in BMSC population (≈ 30%). Moreover we detected CD45+ non-cellular particles by flow cytometry analysis. Implying K-562 cells are not likely to cross the semipermeable membrane (3,0 μm pores *versus* 20,0 μm cells as measured during microscopy). Besides BCR-ABL gene expression in BMSC was detected by RQ-PCR (BCR-ABL/ABL*100=19%). We repeated same test with 0,4 μm pore inserts and without them in order to check implication of cell-to-cell interaction. We didn't obtain any similar results with smaller pores, but the fusion transcript was detected in CD45- BMSC population when these two cell populations weren't devided. Both findings point out at possible horizontal gene transfer mediated by membrane vesicles larger than 0,4 μm and direct whole cell fusion.
Summary/Conclusions: Our data stands for the existence of horizontal gene transfer between leukemic clone and BMSC. This process seems to be mediated by membrane vesicles larger than 0,4 μm in size, though cell fusion can also take place. We also confirmed the fact BMSCs can bear clonal genetic rearrangements which are not specific to tumor cell populations. These findings show tight interaction between tumor and microenvironment cells and can partly explain nature of PCR-based MRD persistence in complete remission.

PB1854

CIRCULATING ENDOTHELIAL PROGENITORS CELLS AND METABOLIC FACTORS IN CHILDHOOD CANCER SURVIVORS

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Background: Circulating Endothelial Progenitor Cells (CEPCs) play a significant role in the maintenance of vascular integrity,balancing the anti-coagulation mechanisms and modulating the immune system by regulating the leukocyte trafficking, as well as controlling the vascular tone. Additionally, it is well-established, that patients who underwent chemotherapy have increased incidence of hypertension and obesity. Nevertheless, numerous studies have shown a negative correlation between CEPCs and obesity, underlining poor vascular repair.
Aims: The study of CEPCs in children who received chemotherapy for Acute Lymphoblastic Leukemia (ALL) and solid tumors (ST) and the investigation of their levels in correlation with patients Body Mass Index (BMI) and blood pressure (BP) regarding the time following treatment.
Methods: Peripheral blood from children with ALL (n=77), ST (n=81) and children without malignancies as control group (n=71) were studied. Four colour flow cytometry was performed to determine the subpopulations CD34+CD 45negdimCD133+, CD34+CD45negdimVEGFR2+ and CD34+CD45negdim-CD133+VEGFR2+ of CEPCs. The BMI of the patients was calculated and the BMI percentile was established specific by the age and gender. Normal weight defined with BMI percentile over 5th and bellow 85th percentile, and overweight/obesity over 85th percentile. The systolic blood pressure (BP) was measured and the percentile was calculated specified by the age, gender and height. Normal BP was defined BP over 5th and bellow 90th percentile and hypertension with a BP over 90th percentile. The post treatment period of time was divided in three groups under or equal of 1 year, 1 to 3 years, and equal and over 3 years. The statistical analysis was conducted using t-test (Holm-Sidak) and 2way ANOVA (Tukey's multiple comparisons test).

Results: The mean values of CEPCs subpopulation CD34+CD45negdimVEGFR2+ estimated in ALL, ST and Controls were 0.00360 (SE=0.00072),0.00613 (SE=0.00146) and 0.002953 (SE=0.0004) respectively. The mean percentage of CD34+CD45negdimCD133+VEGFR2+ in ALL, ST and Controls was 0.00331 (SE=0.00072), 0.00499 (SE=0.00113) and 0.002663 (SE=0.00037). The correlation of CEPCs showed statistical significant difference of CD34+CD45negdimVEGFR2+ between the ST and controls (Mean Diff 0.003174, 95CI of diff 7.716e-005 to 0.006272). In ALL the levels of CD34+CD45negdimVEGFR2+ the 1st year after treatment completion were 0.00458(SE=0.0026), during 1-3years 0.0031(SE=0.00066) and >3 years 0.003423(SE 0.00081). The levels of CD34+CD45negdimCD133+VEGFR2+ during the 1st year after chemotherapy have a mean value 0.0045(SE=0.0026), 1-3 years 0.0027(SE=0.00063) and >3years 0.0031(SE=0.00081). In the ST group the mean value of CD34+CD45negdimVEGFR2+ the 1st year after treatment was 0.0114 (SE=0.0048),1-3years 0.0047(SE=0.0013) and >3 years 0.0036(SE=0.0008). Whereas the percentage of CD34+CD45negdim CD133+VEGFR2+ the 1st year after chemotherapy was 0.0092 (SE=0.0037),1-3 years 0.0034(SE=0.00097)and>3 years 0.00336(SE=0.00085).Statistical significant results were calculated for the levels of CD34+CD45negdimVEGFR2+ in ST group between the groups <1 year and over years' post treatment(Mean Diff 0.007747, 95 CI of diff 0.0002441 to 0.01525). The study of body weight in ALL and ST groups in relation with CEPCs showed no statistical significant difference, although a negative trend between obesity and CEPCs was found in the ALL group and a positive one in the ST group. The same trend also appeared in BP between ALL and ST regarding the CEPCs, with hypertensive patients in ALL group having higher levels of CEPCs than the ST hypertensive individuals.

Summary/Conclusions: The higher levels of CEPCs were estimated in ALL and ST just after treatment completion with a gradual decrease as time passes. The highest percentages of CEPCs were evaluated in ALL patients with normal weight and blood pressure in contrast with the solid tumor group. Further invstigation is necessary to highlight the importance of these data.

PB1855

HEMATOLOGICAL PARAMETERS IN NATIVE HIGHLANDERS OF LADAKH AGED 4-19 YEARS

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Background: High altitude (HA) has always intrigued physiologists because of the remarkable ability of man to adapt to the hostile environment. Hematological changes associated with HA exposure is believed to be driven by hypobaric hypoxia of HA. Majority of the studies on HA physiology and hematological adaptation have focused on the hematological adaptation in lowlanders visiting HA or have compared the hematological profile of native highlanders from Andes and Tibet with those of the neighboring lowlanders. These studies have mostly been directed towards adult population with no or little reference to children and adolescent age groups. Moreover these studies have been done mostly on the highlanders of Andes and Tibet with no data on Indian highlanders.

Aims: We aimed at assessing hematological parameters in native highlanders in the age group of 4- 19 yrs and compare the same with Indian lowland population as well as native highlanders around the globe.

Methods: A total of 390 native highlanders of Ladakh in the age group of 4-19 yrs with no history of travel to lowland were taken for the study. A written informed consent was taken from the parents of all the subjects before starting interviewing them for the laboratory investigations. After taking antiseptic precautions, blood samples were drawn from the ante-cubital vein and complete hemogram including red blood cell indices were measured. The study subjects were stratified into five age groups (less than 5y, 5-8y, 8-10y, 10-12y, 12-15y and children more than 15y). Appropriate statistical analysis was done to compare the hematological parameters between the stratified age groups as well as between boys and girls.

Results: A total of 197 girls and 193 boys were included in the study. The mean age of the subjects was 128±80 (mean±2SD) months. The mean hematocrit value increased with age (38.68±2.51% in <5 yrs age group to 43.84±2.04% in >15 yrs age group). Similarly the mean corpuscular volume (MCV) also showed a rising trend with age (79.07±3.36 fL in <5 yrs age to 85.66± 2.72 fL in >15 yrs age). In contrast to the rising values of hematocrit and MCV we found that the mean corpuscular haemoglobin concentration (MCHC) decreased with age from 36.91±2.85% at <5 yrs of age to 33.78±2.31% at >15 yrs of age. The variations among the age groups are significant for hematocrit, mean corpuscular volume (MCV) and MCHC ($p<0.01$). On comparison of hematological parameters between boys and girls we found that the mean hemoglobin concentration in girls (13.99±0.29 g/dL) was significantly lower than boys (15.43±0.28g/dL). The same findings were replicated in the mean RBC count (4.79±0.08 in girls v/s 5.07 ±0.08 in boys) and mean hematocrit (39.49±0.82% in girls v/ 41.57±0.82% in boys). The mean MCHC in boys (37.23±0.93%) was significantly higher than those in girls (35.69±0.94%). The mean platelet count in boys was significantly higher than in girls ($p=0.0003$) (Figure 1).

Figure 1.

Summary/Conclusions: The hematological adaptation of Ladakhi kids is different as compared to other native highlanders. There is also a significant difference in the hematological response to hypobaric hypoxia with growing age and between boys and girls.

PB1856

AGE VARIATION OF B-CELL PRECURSORS IN BONE MARROW: NORMAL VALUES AS A REFERENCE FOR MDS IN BRAZIL

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Background: Decrease of bone marrow (BM) B-cell precursors (BCP) is an important diagnostic feature in myelodysplastic syndromes (MDS). Moreover, their number is associated with patients' overall survival. However, BCPs vary with age in normal BM.

Aims: In a multicenter study from the Brazilian Group of Flow Cytometry we analyzed the variation of BCPs in normal BM according to age, antibody combinations used for quantification and reproducibility after a centralized reanalysis. We set up a reference pattern of normal values for evaluation of patients with a suspected MDS.

Methods: In a retrospective study including 10 centers we retrieved analyses of BM donors and cases examined for elucidation of transitory reactive cytopenias presenting a normal BM immunophenotyping. BCPs were enumerated as CD19/CD34/CD45/CD10 cells (panel 1) or CD19/CD34/CD45 cells (panel 2), among the total nucleated cells and as percentage among CD34+ cells. Statistics: multiple regression to analyse the dependence of BCS from the variables analysed.

Results: 134 cases were included. Panel 1 was applied in 106 cases (all centers) and panel 2 was used in 28 cases (3 centers). Age range: 10 months to 89 years. In the same age range, values for panel 2 were lower than those for panel 1. In a multiple regression,**%** BCP/total cells=-0.389 "log age" (years) – 0.313 (for panel 2)+correction factor for labs +1.873. The correction factor for labs was 0 to -0.40. Age explained alone 49.6% of the variance of% BCPs/total cells, while "laboratory" explained 5.2% and panel used explained only 0.8%. Age explained only 24.9% of the variance of BCPs/CD34+ cells.

Table 1.

	% total CD34+ cells	BCPs/total cells	BCPs/CD34+ cells
0-6 years (n=10)	3.05% (1.5 - 5.1)	2.8% (0.35-3.8)	62.1% (22.8-62.6)
7-18 years (n=19)	1.43% (0.25 – 3.2)	0.4% (0.02-1.8)	41.5% (3.1-64.5)
19-55 years (n=70)	0.84 (0.07-2.76)	0.13% (0.02-0.8)	20.8% (2.6-60.4)
>56 years (n=35)	0.71% (0.06-2.48)	0.08% (0.02-0.68)	12.9% (1.3-55.2)

Summary/Conclusions: in a normal population BM B-cell precursors varied mainly with age, but were also dependent on technical peculiarities of operators and equipments. Analysis by phenotype and as percentage of total cells was more accurate and less susceptible to variation.

PB1857

PERIOSTIN/BIGH3 RATIO AS A PROGNOSTIC MARKER OF IDIOPATHIC THROMBOCYTOPENIA AFTER ALLOGENIC HEMATOPOIETIC CELL TRANSPLANTATION FOR THE PATIENTS WITH ACUTE MYELOGENOUS LEUKEMIA

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Background: Disrupted hematopoiesis is life-threatening complication of allogeneic hematopoetic cell transplantation (allo-HCT). The interactions of haematopoietic stem/ progenitor cells (HSPCs) and bone marrow (BM) microenvironment, niche(s), control the homeostasis of BM. TGF-b induced gene 3 (BIGH3), one of BM extracellular matrix (ECM) which is produced by niche cells maintain the homeostasis and regeneration of BM.
Aims: We analyzed the relationship between the idiopathic thrombocytopenia after allo-HCT and the BM expression of periostin as the only paralogue of BIGH3.
Methods: We reviewed twenty patients who transplanted with matched sibling donor for acute myelogenous leukemia at Kyungpook National University Hospital from January 2010 to August 2015. BM biopsy specimens at the time of day 28, day 90, day 180, day 365 since allo-HCT were decalcified and stained with primary antibody of BIGJ3 and periostin. Expression of periostin in BM slides were reviewed by pathologist as follows; normal (0), minimal staining around blood vessels; (+1), sparse staining and/or focally staining; (+3), diffuse and strong staining; (+2), between (0) and (+3).
Results: The median age at transplant was 38.5 years (range, 17-68 years) and male was 13 patients (65%). Twelve patients (60%) were in CR1 (complete remission), 8 (40%) in CR2. Thirteen patients (65%) received myeloablative conditioning regimen. The median dose of CD34+ cell was 3.87×10^6/kg (range, $1.6\sim7.67\times10^6$/kg). All patients achieved the neutrophil engraftment with a median time of 13 days (range 9-24days). The median time of platelet engraftment was 15.5 days (range, 13-77days). Idioathic thrombocytopenia developed as follows; 13 patients at day 28, 16 at day 90, 6 at day 180, and 3 at day 365. There was no significant difference between idiopathic thrombocytopenia and the expression of BIGH3 or Periostin (p=0.128) However, BM with thrombocytopenia manifested the low periostin/BIGH3 ratio (p=0.007). Acute GVHD was observed in 12 patients (60%) and chronic GVHD developed in 13 patients (65%). The developemnet of thrombocytopenia dose not differ according to acute and chronic GVHD (p=0.847) (Figure 1).

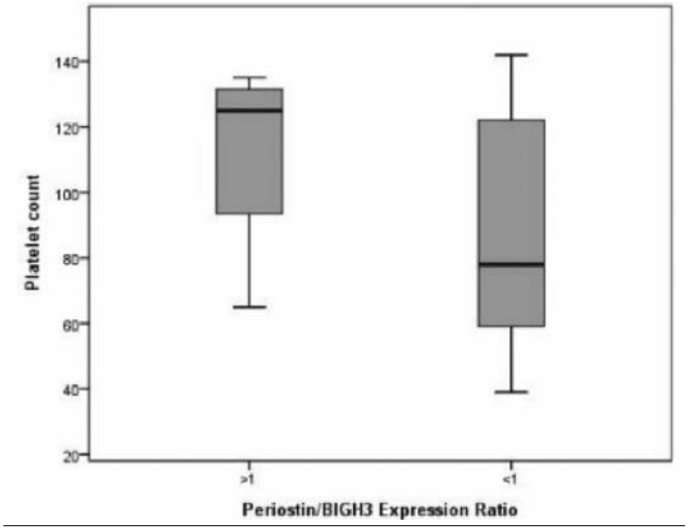

Figure 1.

Summary/Conclusions: The periostin/BIGH3 might represent the status of BM niche during the homeostasis and regeneration of hematopoiesis. High periostin/BIGH3 ratio could predict the recovery of the idiopathic thrombocytopenia.

PB1858

ASSOCIATION WITH OMENN SYNDROME AND CYSTINURIA: CASE REPORT

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Background: Omenn syndrome is one type of combined immunodeficiency, characterized with hepatosplenomegaly, lymphadenopathy, recurrent infections and has an autosomal recessive pattern of inheritance. T lymphocyte count can be normal in peripheral blood but their functions are impaired. B lymphocyte count is very low to none. Cystinuria is renal reabsorption defect of dibasic amino acids, inherited autosomal recessive. Because cysteine solubility is lower than other amino acids cysteine stones are formed in kidneys. In the literature, no association was found between Omenn Syndrome and cysteine stones hence, our case is very interesting.
Methods: 5 months old girl applied to the Pediatric Immunology Department of Erciyes University Children Hospital with skin eruption. There was no family history for immune deficiency and no consanguineous marriage between mother and father. Patient had one sibling who is healthy. Patient was not performed with BCG or other live vaccines. In her physical examination, we observed exfoliative erythroderma and hepatomegaly. In laboratory examination, leukocyte count 6540/mm^3, absolute neutrophil count 2270/mm^3, absolute lymphocyte count 1560/mm^3, absolute eosinophil count 2220/mm^3, serum IgG level 171mg/dl, IgA level 5,81mg/dl, IgM level 24,5mg/dl, IgE level 1270 mg/dl were found. T lymphocyte count 1092/mm^3, B lymphocyte count 6/mm^3, NK count 332/mm^3 were found respectively. Blood sample of patient was sent to Erasmus for genetic analysis. The patient had no full-match family donor. Hence, haploidentical bone marrow transplantation from her father was planned. In preparation for bone marrow transplantation, bilateral kidney stones were showed in abdominal CT. Cystinuria was detected in urine and thought to be bilateral cysteine Stone. Percutaneous nephrolithotomy operation was performed, then the patient was given scholl solution. Stone analysis revealed to be cysteine stone.
Results: Association with two different diseases inherited autosomal recessive is very interesting. Challenging incident that can be caused by a reason or it can be only coincidence? In Omenn Syndrome is known to be sequencing alteration of cysteine and tyrosine amino acids. Perhaps, cysteine stones took form as a result of this alteration.

PB1859

LABEL-FREE IMAGING BY AUTO-FLUORESCENCE PERMITS IDENTIFICATION OF ERYTHROID PRECURSORS IN BONE MARROW AND DETECTS CHANGES OF SOLUBILITY OF HEMOGLOBIN IN ERYTHROCYTES

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Background: In the fluorescence lifetime imaging (FLIM) technique, the image contrast is created by determining the delay of the fluorescence photon emission at each pixel of the image and transforming it in pseudo-colors. This delay, also called lifetime depends on the type of molecules and their physicochemical characteristics.
Aims: We investigated the utility of this technique for the characterization of erythropoietic cell line and changes in the solubility of hemoglobin.
Methods: we used unstained BM smears of 24 normal BM and 8 megaloblastic anemia patients and unstained peripheral blood smears of 10 patients with sickle cell anemia. Images were captured by a confocal microscope with a HPM-100-40-Hybrid detector and excitation at 405 nm (diode laser,80 MHz). In order to create equivalent images of the cytological smears, pseudo-colors were attributed to different lifetime ranges. Images were compared with May-Grünwald-Giemsa (MGG) stained smears.
Results: FLIM created highly contrasted images, where different cell types could be easily recognized by their similarity with MGG images. Erythrocytes exhibited the shortest lifetimes (210.4±42.1 ps). Normal shaped erythrocytes in smears of sickle cell patients showed similar values (214.6±3.1 ps), whereas crenated erythrocytes as well as drepanocytes revealed significantly elevated values (314.2±66.7 ps and 312.5±67.0 ps respectively). Regarding erythropoiesis, the cytoplasm of erythroblasts showed significantly shorter lifetimes (623.5±271.2 ps) than that of myeloblasts (835.9±198.4 ps) and the same was the case when comparing the nuclei (erythroblasts: 895.4±262.8 *versus* myeloblasts: 1166.4±287.9 ps). The same differences could be found in megaloblastic anemias. There were no significant differences between the FLIM values of the different cell types between normal hemopoiesis and megaloblastic anemia.
Summary/Conclusions: The FLIM technique is easily applicable on unstained routine smears and revealed images of good quality permitting cell identification. It allowed also to distinguish between erythroid and myeloid precursors cells and indicates the major physico-chemical changes during the process of falcization.

PB1860

TWO HEMATOLOGICAL MALIGNANCIES, SIMULTANEOUS OR CONSECUTIVE OCCURRENCE. EXPERIENCE OF A CENTER

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Background: Numerous reports of coexistence or consecutive occurrence of hematological malignancies are found in the literature.
Aims: : this study reports cases of patients with two hematological malignancies treated in a single center.
Methods: Retrospective study of patients with two malignancies occurring simultaneously or consecutively in patients in a hematology department during a 15 years period.
Results: Thirteen (13) cases were identified (5 women, 8 men). Their demographic characteristics, diagnoses, treatment and overall survival are shown

on Table 1.There are three deaths because of refractory diseases. Five patients needed treatment for the first disease and nine patients needed treatment for the second disease. Four patients had treatment for both diseases.

Table 1.

no	gender	DOB	1st diagnosis	AGE	stage	Treatment	2nd diagnosis	AGE	Stage	Treatment	Karyotype	OS	Cause of death
1	F	1947	CLL	63	I	none	MM	67	smoldering	none	46XX	6	alive
2	F	1968	CLL	39	I	none	HL	46	IIA	ABV	46XX	9	Refractory lymphoma
3	F	1935	CLL	59	I	none	HL	69	IIIB	CHOP/A BVD		10	alive
4	M	1929	MDS	71	RA	EPO/GCSF	SMZL	83	III	Rituximab	46XY del 11	16	alive
5	M	1963	CML	39	CP	Imatinib,nilotinib,dasatinib	MGUS	52		None	complex		Acute leukemia
6	M	1931	DLBCL	59	IV	CHOP	ET	68		Hydroxyurea/Anagrelide		25	alive
7	M	1944	CLL	66	0	none	HCL	69		Pentostatin		6	alive
8	M	1951	CML	56	CP	imatinib	MGUS	64		none	46XY, 46XY, -Y	9	alive
9	M	1947	CLL	67	IV		Plasmablastic Lymphoma	67	IIIB	Bortezomib, dexamethazone	46XY	0.5	Refractory Lymphoma
10	M	1950	CMML	64		none	HL	64		ABVD.Nivolumab	46XY	2	alive
11	F	1932	SMZL	78		none	Acute Leukemia	82		supportive	46XX, del 5, t(15:17)	8	alive
12	F	1947	PV	65		hydroxyurea	FL	69	IE	R CHOP		5	alive
13	M	1942	FL	73	IV	none	MDS, 5q-	74			46XY, del5	2	alive

CLL:Chronic Lymphocytic Leukemia, MDS: myelodysplastic syndrome, CML: Chronic Myeloid Leukemia, PV: Polycythemia Vera, DLBCL: Diffuse Large B cell lymphoma, ET: essential thrombocytosis, CMML: Chronic Myelomonocytic Leukemia, FL: follicular lymphoma, HL: Hodgkin Lymphoma, SMZL: splenic marginal zone lymphoma, HCL: hairy cell leukemia, MGUS: monoclonal gammopathy of undermined significance

Summary/Conclusions: occurrence of two malignancies in the same patient can be a challenge for the hematologist. Findings of the second disease can be attributed to the fist disease or considering them to be results of treatment. Follow up and initiation of treatment in those patients can be more complex than usual. . As far as origin is concerned there are conflicting reports in the literature supporting a common or different cells of origin. Recording of these cases and biobanking can be of great interest for understanding mechanisms of hematologic neoplasms.

Hodgkin lymphoma - Clinical

PB1861

B SYMPTOMS AND ELEVATED ESR AS PREDICTORS OF OVERALL SURVIVAL IN HODGKIN LYMPHOMA. A 20 YEAR FOLLOW UP MULTICENTER ANALYSIS.

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Background: The prognosis of Hodgkin lymphoma (HL) has improved significantly with the implementation of a risk-adapted treatment that combines chemo and radiotherapy. Altough this approach has led to the greatest advance in disease response, the benefit in terms of overall survival (OS) has been jeopardized by long term toxicity.
The identification of risk factors is crucial to assign each patient to a well defined risk group and prevent under or overtreatment, minimizing the risk of relapse and long term toxicity.
Aims: To analyze the risk factors associated with survival in HL treated with an ABVD based regimen that restricted radiotherapy only to bulky disease.
Methods: We retrospectively analyzed HL patients diagnosed in 4 centers in Tarragona area (Catalonia, Spain), between 1995 and 2015, treated uniformly according to a local protocol.
Patients were assigned into 4 groups: G1: favorable early stage: ABVDx6 cycles, G2: Bulky early stage without other risk factors: ABVDx6+IFRDT. G3: unfavorable early stage (B symptoms) and advanced stage without bulky disease: ABVDx8, G4: Bulky advanced stage: AVBDx8+IFRDT
Results: A total of 183 patients were analyzed with a median follow up of 82 months [range 1-244]. Male/female ratio was 1,29. Median age was 36 years [range 16-82]. Complete response was achieved in 160 patients (87,4%). The estimated OS at 20 years for the whole group was 62.7%. Kaplan–Meier method and log rank test were used for survival analysis. Cox proportional hazard model was used for univariate analysis to identify predictive factors for OS. Factors with significance ($p < 0.05$) were considered for multivariate Cox regression. In univariate analysis, worse OS was found in patients with increased LDH, non-NS subtype, albumin <3,5 g/dL, B symptoms, HIV+, advance stage and ESR >50 mm (log rank *p=0,012; p=0,049; p=0,024; p=0,002; p=0,005; p=0,004* and *p=0,001* respectively). The multivariate Cox regression analysis identified B symptoms and ESR >50 mm as independent prognostic factors for OS (*p=0,002*; *p=0,006* respectively). These variables allowed us to identify 3 patient groups: low (no risk factors), intermediate (either B symtoms or ESR>50 mm) and high risk (both risk factors), with significant differences in OS. Estimation for OS was uniformly analyzed at 216 months (18 years), which is the shortest follow up period for patients in the low risk group. Patients in the low, intermediate and high risk groups had an estimated OS of 85.7%, 65% and 40.1% (*p<0.001*) (Figure 1).

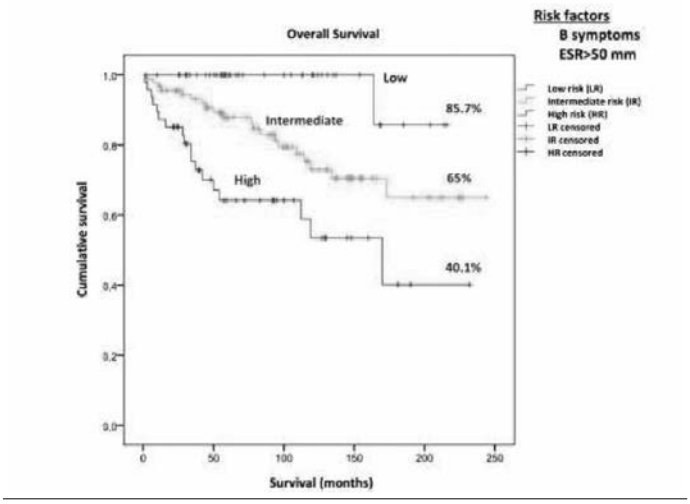

Figure 1.

Summary/Conclusions: B symptoms and ESR>50mm are independently associated with OS. The combination of these factors can stratify patients in low, intermediate and high risk groups with significant differences in OS, regardless their clinical stage.

PB1862

ADVANCED HODGKIN LYMPHOMA PATIENTS WITHOUT LARGE TUMOR MASS – A NEW PROGNOSTIC SCORE IDENTIFIES PATIENTS WITH FAVORABLE OUTCOME

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Background: ABVD and escalated BEACOPP are still the standard of care in patients with advanced Hodgkin Lymphoma (HL). The use of escalated BEACOPP gives better disease control but it is associated with more acute and late toxic effects. The identification of patients who require more or less aggressive initial approach remains the main goal for many investigators in the field of HL.
Aims: The aim of this study was to identify among patients with diagnosed advanced HL without large tumor mass, the subgroup which should not be considered for more aggressive approach than ABVD.
Methods: A retrospective study was performed on 149 patients classical HL, diagnosed in the period June 1997-December 2011. All the patients were in clinicil stage III or IV and didn't have any tumor lesion of 5 cm or more in its longest diameter. The standard of initial care was 6-8 cycles of ABVD followed by radiotherapy. Prognostic relevance of age more than 45 years, gender, CS IV, presence of B symptoms, IPS score, ESR≥50 mm/h, Hgb <10.5 g/dL, WBC≥15,000mm³ and lymphopenia (lymphocytes <600/mm³ or <8% of WBC count) were examined.
Results: The median age of analysed patients was 37 (range 17-80). The median follow up was 98 months. For the whole group 5-year event free survival (EFS) was 63.1% and 5-year overall survival (OS) was 80.6%. In univariate analysis, worse OS was found in patients older than 45 years (5-year OS 66.7% *vs* 87.8%), patients with CS IV (5-year OS 70.2% *vs* 87.0%), B symptoms (5-year OS 77.1% *vs* 93.4%), IPS≥3 (5-year OS 71.8% *vs* 90.1%), ESR≥50 mm/h (5-year OS 75.0% *vs* 89.5%), lymphopenia (5-year OS 65.6% *vs* 84.6%) (log rank; p=0.001, p=0.006, p=0.040, p=0.003, p=0.007, p=0.010, respectively), while gender, anaemia and leukocytosis didn't influence OS (log rank; p=0.303, p=0.714, p=0.522, respectively). Worse EFS was found in patients with CS IV (5-year EFS 50.9% *vs* 70.7%, kog rank p=0.002), IPS≥3 (5-year EFS 53.8% *vs*.73.2%, (log rank; p=0.006) and lymphopenia (5-year EFS 50.0% *vs* 66.7%, kog rank p=0.025), while age, gender, B symptoms, ESR≥50 mm/h, anemia and leukocytosis didn't influence EFS (log rank; p=0.0.078, p=0.437, p=0.068, p=0.151, p=0.384, p=0.158, respectively). The multivariate Cox regression analysis identified age more than 45 years, ESR≥50 mm/h and lymphopenia as independent prognostic factors for OS, while only IPS was identified as an independent factor for EFS. Afterwards, we performed survival analysis with aggregate scores of identified negative prognostic factors for OS for each patient. Since there was no difference in OS in intergroup analysis, groups with 2 and 3 negative prognostic factors were merged. Finally, we developed prognostic model for identifying patients at low (0 factors), intermediate (1 factor) and high risk (2-3 factors) for poor outcome (p=0.000). According to this model, in the examined group 34 (22.8%) patients had low, 64 (43.0%) intermediate and 51 (34.2%) high risk for poor outcome, with 5-years OS of 100%, 84.3% and 60.8%, respectively.
Summary/Conclusions: According to the score which we developed, ABVD is very effective in the subgroup of advanced HL patients without large tumor mass and without identified risk factors.

PB1863

TREATMENT ESCALATION IN CASE OF POSITIVE PET 2 AND IMPACT OF EARLY PET IN EXTENSIVE STAGE HODGKIN LYMPHOMA

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Background: ABVD therapy has been for a long time the reference to advanced stage hodgkin lymphoma (LH). More recently, the emergence of more sustained dose / intensity regimens like BEACOPP has improved the progression-free survival of patients, in particular due to better initial control of the disease. The choice between these two regimens of treatment is always controversial in particular because of an immediate and delayed toxicity potentially increased after BEACOPP. With the use of PET-scanner, escalation and de-escalation protocols based on the PET response were studied. Recent studies have looked at the impact of the PET response to adapt the treatment: de-escalation after BEACOPP in case of a good response, escalation with more aggressive regimens after ABVD in positive PET.
Aims: We report here our experiment of escalation in case of positive PET after 2 cycles of ABVD (PET 2) in patients with advanced Hodgkin lymphoma.
Methods: Among the 102 patients with Hodgkin lymphoma treated between 2008 and 2016, 50 patients had advanced disease (Stage III or IV of Ann Arbor). The majority of patients were treated on front line by ABVD (47 patients), 2 by BEACOPP and 1 by VABEM. All patients underwent PET evaluation at diagnosis and after 2 cycles of treatment. The analysis of the metabolic response was carried out according to the Deauville criteria.
Results: The median age of the patients was 48 years (min-max: 19-85). 20 patients (40%) had an unfavorable prognosis, 24 (48%) had an intermediate prognosis. 11 patients (22%) were refractory to the ABVD protocol and had an escalation of treatment. The median PFS was 66 months (47-85). The median overall survival was not achieved; OS at 60 months was 65%. We found no difference in survival between patients with negative PET and those with positive PET with escalation of treatment. The study of PET 2 response, its impact on survival, as well as escalation of treatment will be presented to the EHA with update of follow-up.
Summary/Conclusions: This study evaluated the value of escalating treatment in patients with advanced PET 2 in patients with advanced Hodgkin lymphoma treated in first-line by ABVD. This management aims to reduce the toxicity of intensive treatments. The aim of our study is also to identify the higher risk patients for whom more intensive treatment could be used as first-line treatment.

PB1864

THE PROGNOSTIC IMPACT OF 18F-FDG PET/CT IN LYMPHOMA PATIENTS AFTER STANDARD CHEMOTHERAPY

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Background: The lymphomas are a heterogeneous group of malignant diseases. The exact diagnosis, precise staging and follow up is very important for treatment and prognosis of these patients (pts). Accurate pretreatment evaluation and response assessment are critical to the optimal management of lymphoma pts. Differentiation of post-therapeutic residual tissue from active lymphoma is unsatisfactory when using only morphological imaging approaches. Positron emission tomography/computed tomography (PET/CT) is the most sensitive and specific imaging technique for monitoring therapy response currently available for lymphoma pts after standard chemotherapy and determining which pts would benefit from additional treatment.
Aims: The aim of the study was to assess the clinical value of 18F-FDG PET/CT for staging and response evaluation in lymphoma pts with Hodgkin's disease (HD) and non-Hodgkin's lymphoma (NHL).
Methods: Two hundred and twenty six pts with biopsy proven lymphoma – (HD n=92 and NHL n= 134), aged 18-76, were retrospectively reviewed. These pts were examined 4-6 weeks after the completion of the standard chemotherapy by 18F-FDG PET/CT, according to the accepted protocol. PET/CT was used to assess response in FDG-avid histologies using 5-point scale, both for interim analysis and treatment end assessment. The Lugano classification has proved extremely useful in the standardization of treatment response. A score 1, 2, 3 is considered to represent complete metabolic response; score of 4, 5 – partial, no response or progressive disease.
Results: By applying PET/CT results two pts' groups were formed: 1.group (n=153 pts) with negative PET/CT results (Deauville score 1-3) and 2.group (n=73 pts) with PET/CT positive results (partial metabolic response or progressive disease). Using Deauville criteria complete response was observed in 95 patients (70.9%) NHL and 58 (63%) HD pts. These pts were in continuous complete remission. Partial response, stable or progressive disease (Deauville score 4-5) were detected in 39 (29.1%) and 34 (37%) NHL and HD pts, respectively. One hypermetabolic lesions and disseminated nodal or extranodal involvement were detected in 15 and 24 NHL pts as well in 12 and 22 HD pts. The pts with one hypermetabolic lesions were considered for radiotherapy, while pts with more than one nodal or extranodal lesions after completion of standard chemotherapy were considered for high dose chemotherapy±autologous stem cell transplantation (ASCT).
Summary/Conclusions: 18F-FDG PET was useful in HD and NHL pts after standard chemotherapy not only for determination of those who need additional therapy, but for the choice of the further management: radiotherapy, chemotherapy, or ASCT. A negative PET/CT study after the completion of therapy is an excellent predictor of good prognosis.

PB1865

BCL-2 AND CD30 EXPRESSION IN HODGKIN AND REED-STERNBERG CELLS OF CLASSICAL HODGKIN'S LYMPHOMA AS A POORER PROGNOSIS CRITERIA

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Background: There are a lot of prognosis criteria for risk stratification of Hodgkin's lymphoma (HL). The most applicable is the IPS-7, however this score is ignoring a tumor cells phenotype. There are data about dependence survival and antigenic profile of Reed-Sternberg (RS) cells. To determine the clinical significance of bcl-2 and CD30 expression in RS cells of classical HL, we have correlated it's expression with available IPS criteria and failure-free survival (FFS) after treatment by ABVD (adriamicyn, bleomicyn, vinblastine, dacarbazine).
Aims: To determine predictive possibility proapoptotic protein bcl-2 and CD30 antigen on RS cells aggregating with criteria IPS.

Methods: In study were included 85 previously untreated patients, presented with classical HL between 2002 and January 2016. This retrospective study did not require approval by the Local ethical committee. Inclusion criteria were: a histologically confirmed diagnosis of classical HL, the presence of a fixed in paraffin before treatment a lymph node sample or other diseased tissue, the minimum follow-up was not less than 18 months.
Results: In the study population (n=85) identified 30 (35%) histological samples bcl-2+, and 55 biopsies (65%), bcl-2. Group bcl-2+ patients had a lower response rate after ABVD chemotherapy - only 24 (28%) patients achieved CR or better result, as compared with 49 patients (57.6%) of the bcl-2 group. Three-year event-free survival (EFS) in bcl-2+ patients had lower 82% *vs* 96% than in bcl-2 group (p=0.018). Multivariate analysis using the Cox proportional hazard model with the inclusion of bcl-2+; CD30 +; bcl-2+/ CD30 +, age 45 and older, B-symptoms, III-IV stage, anemia, decreased serum albumin, increased LDH, leukocytosis revealed that the expression of bcl-2 on RS cells was an independent factor of poor prognosis. 3 year EFS was 52% *vs* 90% in bcl-2 population (p=0.022; RR=1,4). The greater relative risk was observed in a population with double expression of bcl-2 and CD30, where the 3-year EFS was 47% (p=0.012; RR=1,6).
Summary/Conclusions: The expression of bcl-2 on HRS cells can be an independent prognostic factor, co-expression of bcl-2 and CD30 can be viewed as a more powerful factor of poor prognosis than bcl-2+ cells.

PB1866

SURVIVAL ANALYSIS OF PATIENTS WITH CLASSICAL HODGKIN'S LYMPHOMA TREATED WITH ABVD: RESULTS FROM TWO REFERRAL CENTERS IN MEXICO CITY.

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Background: Classical Hodgkin´s lymphoma (cHL) is a neoplastic disease with a favorable prognosis since 85% of patients can be considered cured with current treatment strategies. Combined chemotherapy with Adriamycin, Bleomycin, Vinblastine and Dacarbazine (ABVD) has been the standard therapy for over 20 years. Epidemiological information and the regimen´s results as first-line therapy in Mexico are limited.
Aims: The aim of this study was to conduct a survival analysis in adult patients from two referral centers in Mexico City.
Methods: This is a retrospective analysis of all patients with cHL treated at the Instituto Nacional de Cancerología and the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, between 2009 and 2013. The study was approved by the local Ethics Committee.
Results: We included a total of 193 patients with a *de novo* diagnosis and initially treated with ABVD: 60.6% of cases were male, with a median age of 36 years (17-81 years), 71.5% were diagnosed in late clinical stages (CS). The most frequent histopathological subtypes were: nodular sclerosis and mixed cellularity (46.6% and 40.9%; respectively). The observed overall response rate (RR) was 85.7% [Complete response (CR) was 78.2%]. The RR was 90% in early CS *vs* 83.8% in late CS (CR rate was 84% *vs* 75.8%; respectively, *p*=0.23). Univariate analysis by logistic regression in the early CS group revealed that having a Lymphocyte:Monocyte ratio <1 presents an unfavorable tendency to achieve CR [OR 0.150 (95%CI 0.018-1.274; *p*=0.082)]. In the group in late CS, we found that the lymphocyte percentage tended to favor CR [OR 1.048 (95%CI 0.994-1.105; *p*=0.081)] and the opposite was observed in terms of the absolute monocyte count [OR 0.999 (95%CI 0.998-1.000; *p*=0.082)]. Median follow-up was 35 months (0-96 months), 10.9% of cases had died at last follow-up, and median overall survival (OS) of the entire cohort had not been reached at the time of analysis (5-year OS, 87.1%). However, at the time of this analysis, the group of patients in complete remission had a greater OS than the group that did not achieve CR (*p*=0.0001). With Cox multivariate analysis of OS according to CS, we detected that in the group in early CS, none of the analyzed factors were significant while in the late CS group, age >45 years was an independent risk factor [HR 6.9 (95%CI 1.80-26.60; *p*=0.005)] and achieving CR had a protective effect [HR 0.02 (95%CI 0.004-0.108; *p*=0.0001)].
Summary/Conclusions: Although OS medians had not been reached at the time of analysis, it is noteworthy that CR (84%) in early CS is lower than that reported in the literature and no related prognostic factor has been identified. The role of lymphocytes and monocytes may prove to be significant in larger series with a longer follow-up.

PB1867

OUTCOME OF PD-1 BLOCKADE IN PATIENTS WITH RELAPSED HODGKIN LYMPHOMA AND ACTIVE GRAFT-*VERSUS*-HOST DISEASE

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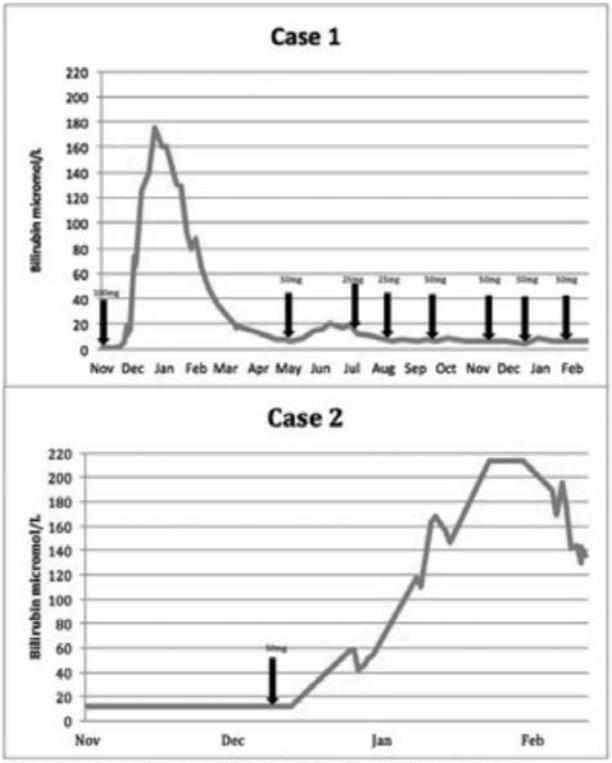
Background: Efficacy of PD-1 (programmed death-1) inhibitors in relapsed/refractory Hodgkin lymphoma (HL) has been established, but their role in relapse after allogeneic stem cell transplant (alloSCT) remains controversial due to the perceived risk of exacerbating graft-*versus*-host disease (GVHD). The literature is largely limited to case reports in patients with no or quiescent GVHD.
Aims: To describe the outcome of PD-1 inhibitor therapy and subsequent management in patients with concomitant biopsy proven active GVHD and progressive HL after alloSCT.
Methods: We describe the treatment and management of two patients in our centre.
Results: Case 1 had both extensive bony, lung and nodal HL with active skin, pleuropericardial and liver GVHD 6 months after donor leucocyte infusion (DLI) and immunosuppression withdrawal and 24 months after sibling alloHSCT. Fifty% of the standard pembrolizumab dose (100mg) produced a PET partial response after 5 weeks but with concomitant biopsy proven, severe exacerbation of liver GHVD. The latter was managed with prednisolone, everolimus, ursodeoxycholic acid (UDCA) and subsequently tacrolimus with gradual but substantial improvement in liver function over the next 5 months (Figure 1) in the absence of further PD-1 blockade, but with progression of lymphoma. Pembrolizumab 50mg was then given with lymphoma response but again a significant (but less severe) flare of liver GVHD occurred. Subsequent 25mg doses failed to prevent lymphoma progression. Reintroduction of 50mg doses approximately each 6 weeks for 4 doses with prophylactic everolimus, low dose prednisolone and ruxolitinib, has resulted in ongoing substantial but incomplete PET responses with associated stable liver GHVD. Case 2 had progressive mediastinal and pulmonary HL despite DLI-induced extensive liver and skin chronic GVHD 38 months post sibling alloSCT. Initial therapy consisted of optimisation of liver GVHD with 8 weeks of UDCA and prednisolone with improvement in liver indices (Figure 1). Pembrolizumab 50mg was then given, together with sirolimus and ruxolitinib as GVHD 'prophylaxis', resulting 5 weeks later in complete metabolic remission on PET. Concomitantly liver GVHD was aggravated (See Figure 1) together with pancytopenia and marrow hypoplasia attributed to an immune-mediated phenomenon. Despite addition of tacrolimus and increased steroids, he remains with severe liver dysfunction and pancytopenia 10 weeks after the single dose of PD1 inhibitor therapy.



Bilirubin level as a marker of liver GVHD, with relationship to pembrolizumab dosing. Arrows denote time-points and doses of pembrolizumab given.

Figure 1.

Summary/Conclusions: PD-1 inhibitors can exert powerful graft *vs* HL effects even in patients with progression in the context of active GVHD, but at the expense of substantial GVHD exacerbation. Further exploration of approaches such as individualised dose titration according to response and GVHD activity and prophylactic therapy with non-calcineurin based immunosuppression which may not mitigate the anti-lymphoma effect will help evaluate whether durable responses with tolerable toxicity is possible in this context.

PB1868

PROGNOSTIC VALUE OF THE RED CELL DISTRIBUTION WIDTH IN PATIENTS WITH CLASSIC HODGKIN LYMPHOMA

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Background: The current gold standard for risk stratification in Hodgkin lymphoma (HL) is the International Prognostic Score. There are certain molecular and immunohistochemical prognostic markers in patients with HL, but their cost and technical constraints make such an application in routine impractical and expensive. Therefore, prognostic models for classic HL (cHL) that are inexpensive, simple, and easy to perform and interpret are needed.
The red blood cell distribution width (RDW) is associated with short- and long-term outcomes of various malignancies. The prognostic value of the RDW in cHL remains unknown.
Aims: The aim of this study was to analyze the prognostic significance of RDW in cHL patients.
Methods: We retrospectively analyzed data from 54 cHL patients diagnosed from 2005 to 2016 at the University Hospital Center Osijek, Osijek, Croatia. We evaluated disease outcome, overall survival (OS) and event-free survival (EFS), and demographic, clinical and laboratory factors affecting outcome. Univariate analysis and Cox regression analysis were used.
Results: The median age of patients was 36 years, 29 were men (54%). Higher RDW levels (%) were found in patients with advanced Ann Arbor clinical stage (15.34 ± 2.28 *vs* 13.12 ± 1.3, $P < 0.001$) and in those with poor response to therapy (15.65 ± 3.37 (progression) *vs* 16.68 ± 2.09 (partial remission), 13.95±1.82 (complete remission), $P = 0.008$). Patients with RDW values of >14.5% (cutoff value calculated by receiver-operating characteristic) had a significantly worse two-year EFS (62.4% *vs* 90.4%, P=0.009) but did not differ significantly in terms of OS (P=0.2). Univariate analysis revealed that a high RDW (>14.5) was correlated with poor EFS (P=0.019). Multivariate Cox regression analysis showed that RDW >14.5% was an independent prognostic factor for EFS (hazard ratio [HR] 3.801, 95% confidence interval [CI] 1-14.45, P=0.05). The RDW allowed further borderline statistically significant risk stratification in patients who were considered to be at low risk on the basis of an International Prognostic Score less than 4 (P=0.053).
Summary/Conclusions: High baseline RDW is an independent prognostic marker of poor outcome in patients with cHL. RDW ratio is asimple, inexpensive, and independent prognostic factor for EFS that may improve the ability to identify high-risk patients with cHL. It could be an easily available and inexpensive marker for the risk stratification in patients with cHL.

Indolent Non-Hodgkin lymphoma – Clinical

PB1869

HIGH FREQUENCY OF SECONDARY MALIGNANCIES IN PATIENTS WITH LARGE GRANULAR LYMPHOCYTIC LEUKEMIA: A SINGLE INSTITUTIONAL EXPERIENCE

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Background: Large granular lymphocyte (LGL) disorders represent a spectrum of aberrant T-cell or natural killer cell lymphocytic proliferations. LGLL is classically associated with autoimmune conditions and bone marrow (BM) failure disorders. SM has been reported in association with LGLL in about 10%.
Aims: The aim of this study is to evaluate the impact of SM on the clinical course of LGLL
Methods: This is a retrospective study of LGLL patients evaluated at Moffitt Cancer Center between January 1995 and May 2016. The diagnostic clinicopathological criteria consisted of LGL count > 0.5 k/μL with T-cell receptor gene rearrangement. Lower absolute number of clonal circulating LGLs with characteristic immunophenotype associated with BM involvement, cytopenias, splenomegaly and/or associated symptoms were also diagnostic. Patients with myelodysplastic syndrome were excluded. Survival analysis was performed using the Kaplan-Meier method with log-rank test. Chi-square and T-test were used to analyze association among various variables. Significant P-value was considered < 0.05.
Results: Out of 668 screened patients with LGL expansions in peripheral blood, 261 met criteria for LGLL. Secondary malignancies were present in 44% (116/261) of LGLL patients, of which 38% were hematological and 80% arose prior to onset of LGLL. Most common solid secondary malignancy included skin cancer (14%), prostate cancer (12%), and breast cancer (12%), while most common hematological secondary malignancy consisted of non-Hodgkin lymphoma (17%) and chronic leukemia (14%). 5-year overall survival (OS) for all LGLL patients was 75% and 10-year OS 63%. There was a statistically significant difference in 5-year OS between LGLL patients with a secondary malignancy compared to without (p = 0.049), but no difference between both groups in median OS or 10-year OS. Patients diagnosed with a secondary malignancy prior to LGLL had worse 5-year OS (p = 0.031) and 10-year OS (p = 0.05) compared to all other LGLL patients.
Summary/Conclusions: This study showed that the frequency of a secondary malignancy is higher than previously described, especially with onset prior to diagnosis of LGLL. Even though median age of LGLL is around 60 years, it appears that age itself cannot explain this phenomenon. Our results suggest that having a secondary malignancy is a poor prognostic factor in LGLL patients.

PB1870

BENDAMUSTINE AND RITUXIMAB COMBINATION THERAPY WITH SUBSEQUENT RITUXIMAB SUPPORTING THERAPY IN RUSSIAN SUBJECTS WITH RELAPSED OR REFRACTORY INDOLENT B-CELL NON-HODGKIN LYMPHOMAS

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Background: Combination of bendamustine and rituximab has been established in many international guidelines as treatment for patients with indolent B-cell non-Hodgkin lymphoma (iNHL).
Aims: Objectives of this study were to evaluate the effectiveness, safety, and tolerability of bendamustine/rituximab combination followed by rituximab maintenance therapy for relapsed or refractory (R/R) iNHL patients in the Russian Federation.
Methods: Adult subjects (≥18 yr), diagnosed with R/R iNHL according to local diagnostic standards, and were enrolled in this prospective observational study. Intravenous therapy was administered in 2 stages (Figure 1): a combination therapy stage followed by a rituximab supporting therapy stage for subjects who achieved complete response (CR) or partial response (PR) during the combination therapy stage. Overall response rate (ORR) was assessed after

3 (Evaluation 1) and 6–8 (Evaluation 2) 28-day cycles. Data from the full analysis set (FAS) were used for the primary analysis and the per-protocol (PP) set for a subgroup analysis. Safety/tolerability was a secondary endpoint and was assessed in the safety analysis set (SAF). Response assessments used the LOCF method for substitution of missing data; overall survival (OS) and progression-free survival (PFS) were calculated using Kaplan–Meier estimates, safety/tolerability was assessed by adverse event (AE) frequency and described using descriptive statistics.

Results: Of the 102 subjects enrolled between June 2012 and October 2015, 83 subjects (52M/31F; median age 59 yr [range: 27–84]) with various NHL histology; subjects with mantle cell lymphoma [n=4], diffuse large B-cell lymphoma [n=2], and follicular lymphoma transformation [n=1] were excluded from the PP population due to deviation from the iNHL inclusion criteria. Most study subjects were heavily pretreated with a median number of 2 prior lines of therapy before entering the study (range: 1–6). At Evaluation 2, ORR in the FAS was high (n=58; 69.9%) with 35 (42.2%) subjects achieving CR (confirmed, n=20 [24.1%]; unconfirmed, n=15 [18.1%]) and 23 (27.7%) achieving PR; ORR (defined as [CR+CR unconfirmed +PR]) in the PP population was 70.8% (Table 1). For FAS patients, at follow up (17 mo) neither median OS nor PFS had been reached; 2-year OS was 88.9% (95% CI: 79.7–99.0%) and 2-year PFS was 87.9% (95% CI: 80.7–95.7%). In the SAF, 31 of 96 subjects (32.3%) reported ≥1 AE. Decreased neutrophil count, decreased white blood cell count, and infections were the most commonly reported AEs and serious AEs. Twelve deaths occurred: 5 due to disease progression (n=2) or relapse (n=3), 5 were not related to lymphoma or occurred during remission, 1 cause of death was unknown, and 1 subject died from hyperthermia and respiratory failure, which was the only death in the study considered related to combination therapy.

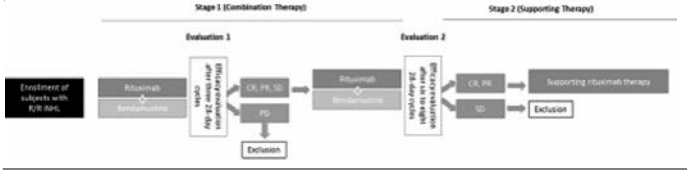

Figure 1.

Table 1.

	FAS Population (n=81)		PP Population (n=72)	
	Evaluation 1	Evaluation 2	Evaluation 1	Evaluation 2
ORR, n (%)	56 (67.5)	58 (69.9)	49 (68.1)	51 (70.8)
CR/CRu	24 (28.9)	35 (42.2)	22 (30.6)	33 (45.8)
PR	32 (38.6)	23 (27.7)	27 (37.5)	18 (25.0)

Summary/Conclusions: Bendamustine plus rituximab therapy followed by rituximab maintenance therapy was generally well tolerated and demonstrated clinical effectiveness in Russian R/R patients with iNHLs. Although a number of subjects with aggressive lymphomas were included in the FAS, the ORR rate was not considerably different from the PP population (ORR: 69.9% [FAS] *vs* 70.8% [PP]).

PB1871

PROGNOSTIC VALUE OF G8 SCREENING TOOL IN PATIENTS WITH NDOLENT B-CELL LYMPHOPROLIFERATIVE NEOPLASMS – A SINGLE CENTRE EXPERIENCE

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Background: Indolent B-cell lymphoproliferative neoplasms (B-LPN) are malignant diseases of advanced age. The most common among them, follicular lymphoma (FL), marginal zone lymphoma (MZL) and chronic lymphocytic leukemia (CLL) together represent about 40% of all B-LPN. However, as indolent B-LPN are most often the slow-growing diseases, an approach "watch and wait" is often recommended. But, when treatment is necessary, the advanced patients' age indicate the need for geriatric assessment (GA) in aim to indentify functional, cognitive, social, nutritional and psychological parameters of the general health status. G8 screening tool (G8) is recognized as fast, easy to perform and sensitive test for selecting the group of fit elderly patients capable for curative treatment approach. Although incorporated in routine oncological practice, GA and G8 are not widely evaluated in hematological practice, so far.
Aims: To evaluate the impact of G8 screening tool on clinical outcome and survival of elderly patients with indolent B-LPN s'> The most common among them, follicular lymphoma (FL), marginal zone lymphoma (MZL) and chronic lymphocytic leukemia (CLL) together represent about 40% of all B-LPN. However, as indolent B-LPN are most often the slow-growing diseases, an approach "watch and wait" is often recommended. But, when treatment is necessary, the advanced patients' age indicate the need for geriatric assessment (GA) in aim to indentify functional, cognitive, social, nutritional and psychological parameters of the general health status. G8 screening tool (G8) is recognized as fast, easy to perform and sensitive test for selecting the group of fit elderly patients capable for curative treatment approach. Although incorporated in routine oncological practice, GA and G8 are not widely evaluated in hematological practice, so far.
Methods: Total of 89 consecutive elderly patients (45males and 44 females with median age at diagnosis 74,6 years, range 65-88 years) with indolent B-LPN (24 with FL, 26 with MZL and 39 with CLL) who fulfilled criteria for treatment initiation were included in study. Patients were treated with antracycline, fludarabine or alkylated agents based chemotherapy regimens +/- monoclonal anti-CD20 antibody. Validity of G8 was compared with standard relevant clinical and laboratory parameters.
MZL) and chronic lymphocytic leukemia (CLL) together represent about 40% of all B-LPN. However, as indolent B-LPN are most often the slow-growing diseases, an approach "watch and wait" is often recommended. But, when treatment is necessary, the advanced patients' age indicate the need for geriatric assessment (GA) in aim to indentify functional, cognitive, social, nutritional and psychological parameters of the general health status. G8 screening tool (G8) is recognized as fast, easy to perform and sensitive test for selecting the group of fit elderly patients capable for curative treatment approach. Although incorporated in routine oncological practice, GA and G8 are not widely evaluated in hematological practice, so far.
Results: For all 89 patients median overall survival (OS) was 77 months, and disease free survival (DFS) in 58 (77.3%) patients achieving remission was 25 months. Among laboratory paremeters, hemoglobin, platelet, neutrophil and monocyte count, as well as C-reactive protein, beta-2 microglobulin didn't influence CR rate, OS and DFS. Elevated lactate dehydrogense was foud significante for CR rate, and low albumin level (<40g/L) for predicting OS. Among clinical parameters age, sex, presence of "B" symptoms, splenomegaly (>13cm), bulky disease (>10cm), extranodal (EN) disease, as well Charlson comorbidity index (CCI; ≤3/>3), ECOG performance status (PS; <2/≥2) and G8 screening tool (>14/≤14) were evaluated. EN and G8 were found significant for CR rate: and EN, B symptoms, ECOG PS and G8 for OS. Multiplarameter analysis singled out G8 as most sensitive prognostic parameter for both CR (P=0.005;HR 1,343, 95%CI:0.214-2.552) and OS (p=0.018; HR 11,262, 95%CI:1.503-4.400)
Summary/Conclusions: According to our experience, the implementation of G8 is good prognostic parameter. Its incorporation into standard hematological indices may help in improving the optimal treatment approach decision in elderly patients.

PB1872

A PROSPECTIVE PHASE 2 TRIAL EVALUATING MONOTHERAPY WITH OFATUMUMAB FOR RELAPSED/REFRACTORY SPLENIC B-CELL MARGINAL ZONE LYMPHOMA (MORE TRIAL): SAFETY ANALYSIS RESULTS

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Background: Due to the lack of prospective clinical trials, treatment guidelines for splenic marginal zone lymphoma (SMZL) are mainly based on single-center expertise. Treatment options for progressive disease include splenectomy, chemo-immunotherapy, or anti-viral therapy in HCV-positive cases. As SMZL cells strongly express CD20 molecule, rituximab has been used in patients unfit for chemotherapy or splenectomy with high response rates. Ofatumumab is a fully humanized, high-affinity anti-CD20 monoclonal antibody able to induce a more potent complement-dependent cytotoxicity if compared to rituximab. We designed this multicenter, open-label, single-arm phase 2 trial addressing activity and safety of ofatumumab monotherapy in patients with relapsed/refractory (R/R) SMZL.
Aims: The primary objective is the activity of ofatumumab in terms of complete response (CR) rate. Secondary objectives aim at evaluating the safety and tolerability and exploratory endpoints investigate biological features potentially related with response to ofatumumab.
Methods: All patients provided written informed consent. Key eligibility criteria include R/R disease after ≤2 prior line(s) of chemotherapy or immunochemotherapy (including single-agent rituximab). Patients are treated with ofatumumab (1st dose: 300 mg, 2nd-8th doses: 1000 mg) up to 8 weekly doses. Response assessment is scheduled 3 months after the last dose. Sample size was defined assuming a P0 of 45% CR, and a P1 of 65% CR. Per Simon optimal Two-Stage design (type I error=0.05, power=80%), 43 patients should be recruited. A safety analysis was planned after the enrollment of the first 10 patients. With an expected rate of adverse events (AEs) of 13%, if less than 3 AEs leading to withdrawal from treatment are reported, the accrual will

continue to the planned 15 patients (interim analysis). Here we present safety analysis results.
Results: Ten patients (6 males, 4 females; median age: 69.5 years, 9 ≥65 years, 1 <65 years) were analyzed for safety. Eight patients were previously treated with rituximab. 26 adverse events (AEs) occurred in 7 patients, with only 5 grade 3-4 AEs. Ten AEs were drug-related, 30% were of grade 3 (Table 1). Three SAEs occurred: hypersensitivity, n=2, both related, and dyspnea, n=1, unrelated to study drug. No AEs leading to treatment withdrawal was reported and no patients died on study. Hematological and biochemical abnormalities included: neutropenia (any grade 6 cases, grade 3-4: 4), thrombocytopenia (grade 1-2: 3 cases), lymphopenia (grade 1-2: 2 cases), leukopenia (grade 1-2: 5 cases), 1 case of GGT increase (grade 3, at baseline grade 2), 6 cases of ALP increase (all grade 1-2), 1 case each of AST, ALT and bilirubin increase (all grade 1). Preliminary response assessment in these 10 patients documented 5 CR, 4 Partial Responses (PR) and one patient with progressive disease (PD) at the end of treatment.

Table 1: List of AEs.

Drug-related AEs	N of events (any grade/grade 3-4)	Non-drug related AEs	N of events (any grade/grade 3-4)
Neutropenia	2 (2)	Neutropenia	1 (1)
Flushing	2 (0)	Anemia	1 (1)
Hypersensitivity	3 (1)	Neoplasm of nasopharynx	1 (1)
Throat irritation	1 (0)	Fatigue	2 (0)
Post herpetic neuralgia	1 (0)	Dyspnea, orthopnea	2 (0)
Hypertension	1 (0)	Pleural effusion, pulmonary failure	3 (0)
		Bone pain	2 (0)
		Constipation	1 (0)
		Oral herpes	1 (0)
		Pelvic pain	1 (0)
		Atrial fibrillation	1 (0)

Summary/Conclusions: Ofatumumab is safe and generally well-tolerated even in elderly patients with R/R SMZL. No cases of unexpected adverse drug reactions were documented. In a series of patients largely pre-treated with rituximab, ofatumumab resulted in a 90% overall response rate, 50% being CR. Complete results of the interim analysis will be presented at meeting.

PB1873

TREATMENT PATTERNS AND TREATMENT RESPONSE IN PATIENTS WITH FOLLICULAR LYMPHOMA IN ROUTINE CLINICAL CARE – A UNITED STATES ELECTRONIC MEDICAL RECORD DATABASE STUDY

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Background: FL represents 70% of all indolent non-Hodgkin lymphomas, and it is widely recognized that FL is a heterogeneous disease, with patients presenting with differing amounts of tumor burden and prognostic indicators. The NCCN guideline recommends using rituximab as a single agent or in combination with other chemotherapies as first-line therapy (1LT) or second-line therapy (2LT). No recommendations are provided beyond 2LT.
Aims: To evaluate treatment patterns and associated response in patients with newly diagnosed FL in routine care in the US.
Methods: Newly diagnosed FL patients aged ≥18 years were selected from Humedica, a large, national US EMR database, between 01/01/08 and 07/31/15 if they had ≥1 inpatient record or ≥2 outpatient records with FL diagnosis codes. The date of the first FL record was the index date. Patients were followed from index until end of continuous activity, progression to diffuse large B-cell lymphoma (DLBCL), death, or end of study period (09/30/15) and were evaluated for FL treatment patterns and treatment response. Possible remission was defined as no additional chemotherapy and no supportive care use or receipt of supportive care <30 days after end of line of therapy (LOT) for <30 days. Lack of remission was defined as receipt of supportive care <30 days after end of LOT for >30 days. Progression was defined as initiation of another LOT, transition to DLBCL, or evidence of supportive care >30 days after end of LOT.
Results: Of the 3,756 patients selected into the study, 1,346 (35.8%) initiated 1LT, and median (interquartile range [IQR]) time to therapy was 1.3 (0.5–5.9) months. Overall, treatment regimens were mainly rituximab-based. In 1LT, more patients initiated combination chemotherapy (61.4%) *vs* single-agent chemotherapy (38.6%). Bendamustine+rituximab (26.9%) and R-CHOP (15.1%) were the most common combination regimens, and rituximab (33.1%) was the most common single agent. Median (IQR) duration of 1LT was 4.3 (1.7–10.4) months. At the end of 1LT, 54.7% (n=736) had evidence of remission, 25.5% (n=344) progressed, and 1.6% (n=22) had no evidence of remission. Among patients with progression after 1LT, 201 initiated 2LT; 34.3% received a single agent, and 65.7% received combination chemotherapy. 2LT regimens were similar to 1LT, with rituximab (18.9%) remaining the top single agent, while bendamustine+rituximab (25.9%) and R-CHOP (6.0%) remained the top combinations. Median (IQR) duration of 2LT was 3.6 (1.4–6.1) months. Of patients receiving 2LT, 41.3% (n=83) had evidence of remission, 35.4% (n=71) progressed, and 1.5% (n=3) had no evidence of remission. 45 patients who progressed after 2LT received third-line therapy (3LT); 35.6% received a single agent, while 64.4% received combination chemotherapy. In 3LT, rituximab (11.1%) was the most common single agent; bendamustine+rituximab (20.0%) and cyclophosphamide+rituximab+vincristine (8.9%) were the most common combinations. Median (IQR) duration of 3LT was 2.8 (1.4–4.7) months. Following 3LT, 26.7% (n=12) had evidence of remission, 39.9% (n=18) progressed, and 4.4% (n=2) had no evidence of remission.
Summary/Conclusions: FL treatment in routine clinical care aligns with treatment guidelines in 1LT and 2LT, with most patients receiving rituximab-based combination chemotherapy. Similar regimens were used in the 3LT setting. As expected, the rates of remission decreased with subsequent LOTs.

PB1874

PET-CT AND BONE MARROW BIOPSY IN STAGING FOLICULAR LYMPHOMA IN A SINGLE INSTITUTION

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Background: Follicular lymphoma (FL) is an indolent lymphoid B neoplasm corresponding to 20-25% of non-Hodgkin lymphomas (NHL). Bone marrow biopsy (BMB) is part of standard work-up in indolent NHL since up to 40-70% of cases have bone marrow infiltration. This fact is important, so advance stage is one factor considered in the FLIPI-1 and FLIPI-2 prognostic index. Positron emission tomography/computed tomography (PET-CT) is a noninvasive technique that shows high sensitivity of detecting nodal and extranodal lymphoma involvement. specially in aggressive subtypes. Some studies have described a high sensitivity (62-100%) and specificity (96-100%) in the detection of bone marrow involvement in agressive NHL. However, its role in low-grade indolent lymphoma such as folicular lymphoma remains controversial.
Aims: To analyze retrospectively the diagnostic accuracy of PET-CT in comparision with BMB in the initial staging of new FL in a single centre in daily practice.
Methods: One hundred and thirty-six patients with de novo FL have been diagnosed in our institution from June 2005 to October 2016. Of them, 64 who underwent both BMB and PET-CT before treatment were evaluated. The BMB was evaluated by hemato-pathologist and the interpretation of PET-CT images was interpreted by a nuclear radiologist. Positive BMB was defined as the presence of CD20 +,CD10+ y Bcl-2+ lymphoid infiltration. No molecular biology techniques were done in the bone marrow tissue. PET-CT bone marrow involvement was defined as an elevated FDG uptake in the bone marrow than those in liver or mediastinum.
Results: Thirty-five male and 29 female were included. The median age at diagnosis: 58 years (range 23-84). Thirty-four patients had grade 1-2 FL and 30 grade 3a FL. Bone marrow involvement was diagnosed in 33 of 64 patients (51.1%) by BMB. Out of the 17 patients with positive PET-CT, 4 had negative BMB. Out of 33 patients with positive BMB, 13 had a positive PET-CT (Table 1). The sensitivity and specificity of PET-CT was 39% and 87%, respectively. The positive predictive value and negative predictive value was 76.5% and 57%, respectively.

Table 1. Detection of BMO involvement: BMB and PET-CT results.

	BMB –	BMB +	TOTAL
PET -	27 (87%)	20 (61%)	47 (73%)
PET +	4 (13%)	13 (39%)	17 (27%)
TOTAL	31 (100%)	33 (100%)	64 (100%)

Summary/Conclusions: Our study shows a very low sensitivity of PET-CT in the daily practice. These results contrast with those reported in some recent studies in agressive lymphoma. However, the high positive predictive value raises the question about the uselfulness of BMB in these PET-CT positive cases. In our opinion, with the current data, BMB should be performed in indolent NHL patients.

PB1875

SURVIVAL OUTCOMES AFTER FIRST-LINE THERAPY IN FOLLICULAR LYMPHOMA USING A UNITED STATES ELECTRONIC MEDICAL RECORD-BASED COHORT

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maceutical Company Limited, Cambridge, [2]Xcenda LLC, Palm Harbor, United States

Background: FL is a heterogeneous disease, and clinical presentation is highly variable. The Follicular Lymphoma International Prognostic Index (FLIPI-2) identifies prognostic factors at diagnosis but does not predict in whom and when to initiate first-line therapy (1LT).[1] Recommended therapies for 1LT vary by stage, symptomatology, and tumor burden but include monotherapy with rituximab (R) or in combination with other chemotherapies. Survival of FL patients in the R era has greatly improved, but few studies have evaluated survival outcomes in patients seen in routine clinical care.
Aims: This study aimed to evaluate survival outcomes in a US population of newly diagnosed FL patients seen in routine clinical care.
Methods: A retrospective study was conducted in which the presence of ≥1 inpatient record or ≥2 outpatient records with FL diagnosis codes were used to identified newly diagnosed FL patients from Humedica, a large US EMR database, between 01/01/08 and 07/31/15. The study index date was the first FL record. Patients who subsequently initiated 1LT for FL were followed from the date of treatment initiation until death, loss to follow-up, or end of study (09/30/15) for the evaluation of the survival outcomes. Median progression-free survival (PFS) (defined as initiation of second-line therapy, evidence of supportive care >30 days after the end of a line of therapy, or death), median overall survival (OS), and PFS and OS rates at 2 years following initiation of 1LT were evaluated using Kaplan-Meier analyses.
Results: 1,346 newly diagnosed FL patients who initiated 1LT met the patient selection criteria. 47.7% were male, and the mean age was 65.4 years (SD: 12.7). At baseline, 16.6% of patients had a Charlson Comorbidity Index of ≥2, and the most common comorbidities were diabetes (14.5%) and chronic pulmonary disease (11.2%). 1LT consisted of both monotherapy (38.6%) and combination therapy (61.4%). For monotherapy, R was the predominant agent used (85.8%); for combination therapy, bendamustine+R (43.8%) and R-CHOP (24.6%) were the most common. Kaplan-Meier analysis revealed that the 2-year OS and PFS rates (from initiation of 1LT) were 86.9% and 64.6%, respectively. Median OS was not reached, and median PFS was 48.1 months (95% confidence interval: 39.4, 58.4).
Summary/Conclusions: The 2-year OS and PFS rates in this newly diagnosed FL patient cohort who received 1LT (the majority of which was R-based) were consistent with expectations in a post-R era. Future analysis will explore the differences clinical characteristics and survival outcomes for patients who received R monotherapy and various R-combination therapies.

Reference
Federico M, *et al*. *J Clin Oncol*. 2009;27(27):4555-4562.

PB1876

Abstract withdrawn.

PB1877

RITUXIMAB MAINTENANCE AFTER R.BENDAMUSTINE FOR PATIENTS WITH UNTREATED FOLLICULAR LYMPHOMA : A REAL LIFE STUDY IN SOUTHERN ITALY ON BEHALF OF RETE EMATOLOGICA PUGLIESE
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Background: Results from phase 3 "StiL" and BRIGHT trials demonstrated the effectiveness of the combination Bendamustine-Rituximab {BR} compared to R-CHOP/R-CVP regimens as frontline treatment for advanced Follicular Lymphoma {FL}, emphasizing BR as a standard strategy in this subset of patients. However, only a few studies investigated the efficacy and safety of R maintenance after frontline BR, indicating a global beneficial effect of R administration in term of PFS but not OS, in keeping with a good toxicity profile even over two years of tratment
Aims: In this study, we evaluated the role of maintenance therapy with R after induction with BR in previously untreated FL, and compared its efficacy and safety profile with recent publicly available results of "StiL" trial.
Methods: FL patients [pts] were treated with a maximum of 6 cycles of B-R [Bendamustine 90 mg/m2 8days 1+2), Rituximab 375 mg/m2] every 28 days followed by 2 additional cycles of Rituximab monthly. Patients showing complete response [CR] or partial response [PR} were then allowed to receive R maintenance [375 mg/m2] administered every two months. To date 118 pts (65 women and 53 men) with FL have been recorded. Median age was 61 years (range 28-86); 15 (13%), 41 (35%), 62 (52%) pts had respectively stage II, III and IV; median number of nodal areas was 4, bone marrow involvement was found in 56 (47%) pts, and median FLIPI was 3.
Results: Among the 118 pts, 94 were evaluable for response and safety. The overall response rate [ORR] was 89.2% with 83 pts achieving a remission after BR therapy. The CR rate was 84.4%, 7 pts had partial response, 5 pts (6.1%) had stable disease, whereas 3 (3.5%) showed no response to BR and had a progressive fatal disease. All of the pts achieving remission received the full planned 2 years Rituximab maintenance treatment and, among them, 24 pts (28.9%) were administered with R over the first two years. Primary adverse events recorded were of grade 3 anf 4 in 25% of cases. Infectious (grade 3-4) and neutropenia (grade 3) were the most common adverse event, no additional unexpected toxicities were observed, whereas no occurrence of secondary malignancy was registered so far.
Summary/Conclusions: Our data, compared with recent reports about the role of Rituximab maintenance, support the efficacy of BR as backbone treatment of choice in previously untreated advanced FL. These results, moreover, are in line with those of other studies indicating that Rituximab standard maintenance and also over 2 years for FL appears safe and well tolerated, with no additional toxicities.

PB1878

Abstract withdrawn.

PB1879

ROLE OF F-18 FDG-PET/CT IN DETECTING LYMPHOMATOUS BONE MARROW INVOLVEMENT IN THE INITIAL STAGING OF PATIENTS WITH LYMPHOMA
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Background: The role of F-18 FDG-PET/CT for the assessment of bone marrow involvement in the staging of newly diagnosed patients with lymphoma was reviewed in the Recommendations of Lugano Classification. They conclude that if a PET/CT is performed, a bone marrow biopsy is no longer indicated for a routine staging of Hodgkin lymphoma (HL) and most difusse large cell lymphoma (DLBCL). Data are insufficient in follicular lymphoma (FL) and bone marrow biopsy is always recommended.
Aims: We study the value of F-18 FDG-PET/CT for the detection of bone marrow involvement in the initial staging of patients with lymphoma.
Methods: Newly diagnosed patients with HL, DLBCL and FL who underwent F-18 FDG PET/CT and bone marrow biopsy for initial staging between January 2007 and June 2016 were included. We analyze sensitivity, specificity and concordance of PET/CT compared with bone marrow biopsy. In discordant cases, we review if there was any difference in the staging.
Results: 161 patients were included,69 DLBCL(38 male,31 female, median age 59 years),44 HL(24male,20female, me32y),48 FL (23male,25female, me55y). Four of the 44 patients with HL had bone marrow infiltration in bone marrow biopsy (BMB+) and PET/CT detected bone marrow involvement in all of these patients. PET/CT was positive in bone marrow (BMPET+) in 7 of the 40 patients without bone marrow infiltration in bone marrow biopsy (BMB-), these patients had bone marrow lesions on locations other than iliac crest. Six of the 7 patients were in advanced stage regardless of bone marrow involvement and a patient had sternal involvement by contiguity. Seven of the 69 patients with DLBCL had BMB+, 6 patients with DLBCL and 1 patient DLBCL and FL. PET/TC had detected bone marrow involvement in all of them. Sixty-two patients of 69 DLCL did not have bone marrow infiltration by biopsy(BMB-), but nine of them had BMPET+. Seven of the 9 patients were in stage IV because of extranodal involvement of other organs. One patient had primary bone involvement of jaw and another patient had vertebral involvement by contiguity. Fourteen patients of 48 patients with FL had BMB+. Of these 14 patients with bone marrow involvement by biopsy, 5 patients had BMPET- and PET/CT could not detect another extranodal involvement in three of these five patients. Of the 34 patients without bone marrow infiltration by biopsy BMO-, 8 patients had PET-TAC+, and 6/8 could be classified in stage IV regardless of bone marrow involvement (Table 1).

Table 1.

	n	BMB+		BMB-				
		PET+	PET-	PET+	PET-	Sensitivity	Specificity	Concordance
HL	44	4	0	7	33	100%	82,5%	84%
DLBCL	69	7	0	9	53	100%	85%	87%
FL	48	9	5	8	26	64,2%	86,6%	72,9%

Summary/Conclusions: Our series confirms that PET/CT is useful to detect bone marrow involvement in the initial staging of Hodgkin Lymphoma and DLBCL. We can avoid bone marrow biopsy in these hystological variants of lymphoma. In follicular lymphoma, PET/CT did not detect more than one third of patients with bone marrow infiltration by biopsy. These results support the histological assesment of bone marrow in the initial staging of follicular lymphoma.

PB1880

PREDICTIVE FACTORS FOR INFECTIOUS ADVERSE EVENTS IN PATIENTS WITH B-CELL NON-HODGKIN LYMPHOMA TREATED WITH BENDAMUSTINE-RITUXIMAB (R)±R MAINTENANCE. RESULTS OF A RETROSPECTIVE ANALYSIS

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Background: The combination of bendamustine (B) and rituximab (R) is an effective and well tolerated treatment for B-cell malignancies. However, previous reports have shown a higher incidence of lymphopenia and secondary infectious complications in patients treated with BR than in patients treated with other chemoimmunotherapy regimens.
Aims: We performed a retrospective analysis at our institution in patients treated with BR with or without R maintenance, with the aim of determining the incidence of the infectious adverse events (AEs) and of identifying potential predictors factors.
Methods: We collected data from 65 patients with B-cell non-Hodgkin lymphoma (NHL) who received at least two cycles of BR±R maintenance between 2010 and 2016 at our institution. The AEs – including neutropenia (N), neutropenic fever (NF), lymphopenia, infections episodes and the occurrence of second tumors - were recorded according to the CTCAE v4.0 grade score. We compared the risk factors of patients who developed infections and those who did not. Univariate analysis with Fisher's exact test was used to evaluate the potential risk factors.
Results: The median age at the first treatment cycle was 66 years (range 36-89), 33 patients (50%) were ≥65 years, 27 (41%) were male, 53 (82%) had advanced disease and 37 (60%) had bone marrow involvement. Thirty (46%) patients had follicular lymphoma, 17 (26%) mantle cell lymphoma, 11 (17%) marginal lymphoma, 5 (7%) diffuse large B-cell lymphoma and 4% other indolent lymphomas. Thirty two patients (49%) received BR as first line treatment, 51% as second line and above. Bendamustine was administered either at the dosage of 70 or 90 mg/sqm iv on days 1, 2 and R was administered at a dose of 375 mg/sqm iv or sc, on day 1. Therapy was administered every 4 weeks up to 6 courses. Twenty nine patients (46%) received R maintenance every 8-12 weeks for two years. The mean number of cycles administered was 5 (range 2-6), 13 patients (20%) discontinued treatment due to toxicity: 8/13 for non-hematologic toxicity. Primary or secondary G-CSF prophylaxis was administered to 25 patients (38%), while the prophylaxis with trimetropin-sulfametoxazole against Pneumocystis jiroveci pneumonia was given to all patients. Twenty two patients (34%) had at least one infection. Bacterial pneumonia was identified in 6/22 patients, varicella zoster virus infection in 4/22, cytomegalovirus reactivation in 2/22 and other infections in 10 patients. At univariate analysis, the infectious AEs were associated only with lymphopenia during the second cycle (p=0.043) and with neutropenia during the second, third and fourth cycle (p=0.026; p=0.003, p=0.018, respectively). No correlation with age, line of treatment and G-CSF administration was documented. Other AEs were: grade 3/4 neutropenia (49%), neutropenic fever (3%), grade 3/4 lymphopenia (80%). We reported also a 5% incidence of second tumors after treatment (lung cancer in 2 patients and prostate cancer in 1).
Summary/Conclusions: In our analysis, BR±R maintenance confirms a toxicity profile similar to that reported in previous experiences. According to our results, an early lymphopenia and neutropenia (after two cycles) are predictive factors for infections AEs and for premature treatment discontinuation. Twenty% of patients discontinued treatment mostly because of the early withdrawal due to infectious complications. These data raise the question on the role of antibacterial, antiviral and primary G-CSF prophylaxis in all patients treated with BR.

PB1881

CAUSES OF DEATH OF FOLLICULAR LYMPHOMAS. MONOCENTRIC AND RETROSPECTIVE STUDY WITH A LONG PERIOD OF OBSERVATION

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Background: Follicular lymphomas are usually defined as incurable diseases with a natural history characterized by continuous relapses.
Aims: This study was launched to evaluate after a long observation period the causes of death during follow-up.
Methods: All patients with histologically confirmed diagnosis of follicular lymphomas grade I-II or IIIa were selected from our data base starting from January 2000 untill December 2004 in such a way to have more than 10 years of observation for alive patients. We considered all patients with this diagnosis regardless to treatment and considering also patients followed with watch and wait. Patients were followed with ambulatory evaluation and for those lost to follow-up consulting the regional cancer registry.
Results: One hundred and fourthy-six patients were diagnosed and treated at our Institution. The median age at diagnosis was 61 years (range 30-87). Stage I-II in 47 patients, III-IV in 86. Bone marrow biopsy was positive in 87 patients, FLIPI 0-1 in 40, FLIPI 2 in 48, FLIPI 3 in 40 and FLIPI 4 in 18 patients. According to treatment 98 patients were treated with antracycline containing regimens, 34 with fludarabine containing regimens and 14 were observed or treated with radiotherapy. Rituximab was used in 98 patients, as sequential treatment in 74 or chemotherapy combined in 24; 48 patients did not use rituximab. The median observation period for alive patients was 13,4 years (range 11-15 years) and 8 years (range 0,09-15 years) for dead patients. Sixty-five patients dead during this long period of observation and the causes were: 35 due to lymphoma progression (54%), 16 second neoplasms (25%), 12 other disease (18%), 1 car accident and 1 unknown. The overall survival with a median period of observation of 127 months (range 2-196) was 71%. In univariate analysis the best overall survival was statistically associated with low FLIPI score, the use of Rituximab and the obtainment of complete remission. In multivariate analysis FLIPI 0-1 and the obtainment of complete remission manteined the significance. Exactly the same results were observed if we considered the cause specific mortality.
Summary/Conclusions: In conclusion this retrospective monocentric study confirms that after a long follow-up period about half patients died of lymphoma and the other half died for complications related to therapy or to lack of immunological control (second neoplasm or other diseases). Follicular lymphoma confirms to be a good prognosis lymphoproliferative disorders and in the long observation period of patients clinicians must have maintained a careful evaluation of concomitant pathologies.

PB1882

CLINICAL CHARACTARISTICS AND PROGNOSIS OF PATIENTS WITH INDOLENT NON-HODGKIN LYMPHOMA AND RISK OF TRANSFORMATION TO AGGRESSIVE LYMPHOMA: A SINGLE JORDANIAN CENTER EXPERIENCE

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Background: Indolent Non Hodgkin Lymphomas (INHL) are slow growing lymphomas that usually arise from B-cells. They are characterized by slow appearance and progression of symptoms compared to aggressive non Hodgkin lymphoma (NHL) namely Diffuse large B-cell Lymphoma (DLBL). Small percentage of INHL might transform to aggressive NHL.
Aims: We aim to describe the clinical characteristics, prognosis and risk of transformation to aggressive lymphoma in patients with INHL in North Jordan as a model for other Middle East countries in which such data is lacking.
Methods: All patients diagnosed with INHL between Jan 2003 to Jan 2017 were retrospectively reviewed. Clinical and laboratory data at time of diagnosis including gender, age, lactate dehydrogenase level (LDH), pathological subtypes, CT and PET/CT scans were studied. Extranodal involvement was confirmed either by histopathological studies or CT and PET/CT scan. Transformation to aggressive lymphoma was confirmed by histopathological studies. Patients were followed and overall survival rate was calculated. Mean survival times were calculated using Kaplan-Meier method.
Results: Among 265 patients diagnosed with NHL, only 88 patients (33.20%) confirmed to have INHL. 54 patients (61.4%) were males and 34 patients (38.6%) were females. Their ages at diagnosis ranged from (29-83) years with a mean (SD) of 59.26 (12.39). Among these patients, 45 patients (51.1%) had small lymphocytic lymphoma / chronic lymphocytic leukemia (CLL), 20 patients (22.7%) had follicular lymphoma (FL), 15 patients (17%) had marginal zone lymphoma (MZL), 6 patients (6.8%) had mantel cell lymphoma (MCL) and 2 patients (.78%) had unspecified INHL. Mean age of MZL (53.2 years) and FL (55.3 years) were significantly lower than mean age of MCL (58 years) and CLL (62.77 years). 22 patients (23.9%) had extra nodal Involvement. There was significant association between INHL subtypes and extra nodal sites involvement. (P-value=.001). 60% of patients with MZL, 50% of patients with MCL, 20% of patients with FL and 8.9% of patients with CLL had extranodal sites involvement. 11 patients (12.5%) from all INHL had transformed to Diffuse Large B-cell Lymphoma (DLBL). There was significant association between INHL subtypes and transformation to DLBL, (P-value=.02). 7 from 20 patients with FL (35%) and 4 from 45 patients with CLL (8.9%) had transformed to DLBL. Mean LDH level (886.1 U/L) in patients with transformation to DLBL was significantly higher than mean LDH level (490.7U/L) in other patients, (P-value=.0004). There was no significant association between mean age and mean albumin level with risk of transformation to DLBL. The overall survival rate was 56.8%. 10 years and 5 years survival rates were 47% and 60% respectively. Mean survival time in patients with MCL (31.8 months) was significantly lower than mean survival time in patients with follicular (85.48 months) , MZL (90.6 months) and CLL(103.6 months) patients , (p-value=.oooo4). There was no significant difference in survival rate between patients who transformed and patients who didn't transform to DLBL.
Summary/Conclusions: Prevalence of INHL among patients with NHL in North Jordan is 33.2%. The most common INHL subtypes in our patients were

CLL (51.1%) and FL (20.7%). These findings are significantly different from Saudi Arabia and Western Countries in which FL is the most common subtype. FL and CLL are associated with higher risk of transformation to DLBL. High LDH level is considered a risk factor for transformation to DLBL in our patients. MCL is associated with significantly lower mean survival time than other INHL subtypes.

PB1883

OCULAR ADNEXAL LOW GRADE LYMPHOMA TREATMENT OUTCOMES AND LONG TERM FOLLOW UP: A SINGLE CENTRE EXPERIENCE

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Background: Ocular adnexal lymphoma (OAL) accounts for 1-2% of Non-Hodgkin Lymphomas (NHL) and 8% of all extra-nodal sites. The majority of cases, >95%, are of B cell origin and 80% are low grade lymphomas. Secondary ocular involvement occurs in approximately 2.4-5.3% of patients with advanced systemic NHL. Marginal zone lymphoma or mucosa-associated lymphoid tissue (MALT) lymphoma is reported in approximately 50% of patients. Current treatment options for low grade OAL include radiotherapy and chemotherapy. Chlamydia Psittaci DNA has been reported in up to 80% of tumor biopsies from patients with OAL suggesting a possible value of anti-Chlamydia Psittaci antibiotic therapy.
Aims: To report a single centre's experience in the outcomes of patients diagnosed with OAL over a 13 year period.
Methods: A Retrospective cohort of patients with low grade OAL treated in a single Centre between 2003 and 2016 was analyzed. Chemotherapy was the first choice of therapy until 2008, afterwards radiotherapy became the first line treatment for OAL.
Results: A total of 20 patients with OAL were identified. 60% (12/20) of patients were females with a median age of 61.5 years (range 45-85 years). 80%(16/20) had unilateral disease at presentation. MALT lymphomas comprised 75%(15/20), Follicular NHL 15% and CLL/SLL 10%. Only 10% (2/20) had a prior diagnosis of NHL. At presentation 20% (4/20) had evidence of systemic involvement: 19% (3/16) had bone marrow involvement and 1 patient had small volume lymphadenopathy on CT scan. 45% (9/20) were treated with first line chemotherapy, single agent Chlorambucil in 78% (7/9) and 2 patients received Fludarabine based chemotherapy, 30% (6/20) received first line radiotherapy, 24Gy in 12 fractions in 67% (4/6), and 25% (6/20) were managed under observation. In the chemotherapy group 55% (5/9) experienced 1 relapse (3/5 local recurrence and 2/5 extra-ocular relapse), 3 patients experienced ≥2 relapses, 2 patients had disease transformation to high grade and 1 patient subsequently died as a consequence of their disease. 33% (2/6) patients treated with radiotherapy experienced disease recurrence, mainly extra-ocular and 50% (3/6) suffered complications following radiotherapy in the form of dry eyes and cataract. Median follow up was 9.5 years (range 1-14 years). Overall survival was 95% (19/20) with an event free survival of 65% (13/20) (Table 1).

Table 1. Summary of the management modalities of ocular adnezal low grade non-Hodgkin lymphoma.

	Chemotherapy	Radiotherapy	Observation	Total
Number of patients	9	6	5	20
Follow up median, range years	11 (9-14)	2.5 (2-5)	2 (1-9)	9.5 (1-14)
Relapsed	5 (55%)	2 (33%)	0	7 (35%)
Event Free Survival	4 (45%)	4 (67%)	5 (100%)	13 (65%)
Complications	2 (22%)	3 (50 %)	0	5 (25%)
Overall Survival	8 (89%)	6 (100%)	5 (100%)	19 (95%)

Summary/Conclusions: The majority of patients in our cohort had favorable outcomes. Currently there is no national guideline for the management of OAL in the UK. Several treatment options exist including chemotherapy, radiotherapy, immunotherapy, observation or more recently the use of eradication treatment for Chlamydia Psittaci. Factors to consider when choosing a treatment include a patient's co-morbidities, risk of visual impairment, need for systemic therapy, histological diagnosis and anticipated side effects. As treatments are so effective the long term consequences and possible late effects need to be acknowledged and avoided if at all possible. Observation is an acceptable approach in asymptomatic patients when there is no immediate risk of visual impairment. Radiotherapy is an effective first line treatment in symptomatic and localized OAL, the exact dose of radiotherapy to achieve disease control with minimal long term side effects is yet to be determined. Reviews with larger number of patients are needed to inform a practical approach to the management of OAL.

PB1884

AGE AS A POTENTIAL NOVEL PROGNOSTIC INDICATOR IN PRIMARY CUTANEOUS B-CELL LYMPHOMA

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Background: Primary Cutaneous B-Cell Lymphoma (PCBCL) comprises a rare group of cutaneous Non-Hodgkin's lymphomas (NHLs) with an estimated annual incidence of 2.5 per 1,000,000 persons. They usually present with papules or nodules on the head, trunk, and/or extremities. The International Society for Cutaneous Lymphoma (ISCL) and the European Organization for Research and Treatment of Cancer (EORTC) developed a new way to classify PCBCL into three different subtypes. Indolent subtypes include Primary Cutaneous Marginal Zone Lymphoma (PCMZL) and Primary Cutaneous Follicular Center Lymphoma (PCFCL). Primary Cutaneous Diffuse Large B-Cell Lymphoma (PCDLBCL) is an aggressive subtype with a fatality rate of 50%. The Cutaneous Lymphoma International Prognostic Index (CLIPI) can risk stratify indolent subtypes, but criteria do not include age. Here we present our single institutional analysis of clinicopathological features and outcomes of patients with PCBCL.
Aims: To analyze clinical and laboratory characteristics such as age, lesion characteristics, hematological parameters, and treatment modalities in order to determine their impact on progression free survival (PFS) in PCBCL.
Methods: This is a retrospective study of patients evaluated at Moffitt Cancer Center between January 1990 and December 2016. Patients were identified using our PCBCL database and diagnosis was verified by independent hematopathologists and dermatopathologists. Staging was determined according to ISCL/EORTC recommendations. Demographics, lymphoma subtype, stage, disease course, and CLIPI scores were collected. Kruskal Wallis ANOVA and Fisher's Exact tests were used to compare differences among the four subtypes for continuous and categorical variables, respectively. Kaplan Meier curves were produced to estimate PFS for different strata, and differences among the strata were tested using the log-rank test.
Results: We identified 37 patients who met diagnostic criteria for PCBCL (35% PCFCL, 40.5% PCMZL, 13.5% PCDLBCL, and 11% indolent, unspecified). Male:female ratio was 2.4:1. 51% of patients were ≥60 years old (yo) and 49% were <60 yo. 54% had stage T1 disease, 27% T2, and 19% T3. Median PFS for patients <60 was 1.1 years, but was not reached for those ≥60. Mean follow-up time was 2.6 years for all patients. Log rank test showed a statistically significant difference in PFS between the two age groups (p=0.01). This was consistent when comparing PFS by age in both high (PCDLBCL) and low grade (indolent) subtypes. PFS according to stage in indolent subtypes showed a marginally statistically significant difference (p<0.06). Stratification of patients according to CLIPI did not show a significant difference in PFS among indolent subtypes.
Summary/Conclusions: We found that age is a highly statistically significant prognostic parameter in PCBCL, as patients ≥60 years had a longer PFS compared to younger patients, even after adjusting for stage and CLIPI. This is an interesting finding as most NHL studies demonstrated a negative impact of advanced age on PFS. Our results suggested that age is a possible novel prognostic indicator in patients with PCBCL, however validation on a larger sample set is needed.

PB1885

EPIDEMIOLOGY, CHARACTERIZATION AND THERAPEUTIC MANAGEMENT OF MARGINAL ZONE LYMPHOMA: A SINGLE-CENTER EXPERIENCE

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Background: Marginal zone lymphomas are a group of relatively uncommon lymphomas whose cells are derived from B lymphocytes of the "marginal zone" of the secondary lymphoid follicles.
Aims: The objective of this study is to review our series evaluating the epidemiology, clinical presentation, morphological, immunohistochemical and molecular characterization and therapeutic management in a tertiary hospital.
Methods: We evaluated a total of 56 patients diagnosed between May 2008 and February 2017. We collected the epidemiological and clinical data, including location, clinical stage, FLIPI and associated risk, antigenic stimulus, symptomatology at diagnosis (ECOG, constitutional syndrome, B symptoms) and response to treatment. We reviewed the levels of LDH, beta2microglobulin, ESR, peripheral blood (PB) immunophenotype and studied the morphological, immunohistochemical and molecular characteristics (MALT1 translocation and

immunoglobulin heavy chain rearrangement (CDR2 / CDR3 of IgH)) in PB, bone marrow and affected organs. All diagnoses were classified according to WHO (2016 revision). In addition, we performed an autoimmunity test in most patients.
Results: Among the 56 patients, 26 were men (46.4%) and 30 women. The median age at diagnosis was 64 years (37-92). The most frequent subtype was the splenic marginal zone lymphoma (17 patients, 30.4%), followed by MALT: 10 pulmonary (17.9%), 10 gastric (17.9%), 5 cutaneous (8.9%), 5 ORL (8.9%), 2 (3.6%), 1 hepatic, 1 thyroid and 1 lacrimal gland (1.8%) and nodal marginal zone lymphoma (3 patients, 5.4%). Five of them presented with multifocal disease (8.9%). Fifty percent (28) had a clinical stage III / IV and 32 patients (57.1%) had a low risk at diagnosis (FLIPI 0-1). We found an antigenic stimulus in 11 patients (Helicobacter pylori, Sjögren's syndrome, Hashimoto's thyroiditis). The molecular study of MALT1 was performed in 25 patients and 3 presented the translocation (12%). Six of seventeen cases (35.3%) showed IgH rearrangements. Antinuclear antibodies were positive in 15 of 32 patients (46.9%). A total of 46 patients (82.1%) required a treatment, of whom 35 achieved a complete remission (CR) (76.1%) and 10 partial remission (PR) (21.7%) after the first line of treatment. Among these, 17 received immunochemotherapy (37%), 10 immunotherapy (21.7%), 8 surgery (17.4%), 7 antibiotics (15.2%) and 4 radiotherapy (8.7%). We observed 7 relapses (16.7%) and 3 progressions (7.1%), achieving a CR in 7 (70%) and PR in 3 (30%) after rescue treatment. There was just one case of high grade transformation (1.8%), who was the only patient decesed in the series (1.8%), with a median follow-up of 70 months.
Summary/Conclusions: Marginal zone lymphoma is an indolent lymphoma, with a good prognosis and very good response to current therapy. It is sometimes associated with autoimmune phenomena and infectious agents. It is essential a correct staging and characterization to optimize its therapeutic management and outcome.

PB1886

HAIRY CELL LEUKEMIA AND B-RAF MUTATIONS
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Background: Hairy cell leukemia(HCL) is a B cell lymphoproliferative disorder, presenting with splenomegaly, hepatomegaly and bone marrow infiltration. HCL accounts for%4,5 of non-Hodgkin lymphomas, more commonly seen in man. Diagnosis is based on the examination of peripheral blood smear, flow cytometry and the bone marrow aspiration-biopsy. Recently, B-RAF^{V600E} mutation was demonstrated in%100 of Tiacci HCL case series.
Aims: Aim of our study is to investigate the frequency of B-RAF^{V600E}mutation and other rare mutations of B-RAF (B-RAF^{G464E}, B-RAF^{G466E}, B-RAF^{G469V}) and their relation with clinical data and treatment responses.
Methods: Charts of 13 patients diagnosed with HCL were analyzed retrospectively. Patients' clinical paremeters were evaluated. HCL variant type patients were excluded. Paraffin blocks of spleen or bone marrow tissues are obtained from the pathology archives. One thin section (10 micron) of bone marrow or three sections of spleen are cut and DNA extracted by spin column technique using DNA extraction kit. (QIAamp DNA FFPE Tissue Kit, Qiagen) After spectrophotometric measurement of DNA; common and uncommon mutations of B-RAF were investigated. (Qiagen PyroMark Q24 system, Therascreen BRAF Pyrokit 24, V1 (1/2) kit) Mutation and clinical data analysis were conducted using the SPSS 15.0 software. The study was approved by the local ethics board of Dokuz Eylul University.
Results: Male/female ratio was 9/4. Median age at diagnosis was 48(37-59). Median follow-up time was 59 (3-96) months. At the time of diagnosis, 46,2% (n=6) of patients were asymptomatic. All of the patients had splenomegaly (n=13), five patients had hepatomegaly and two had intraabdominal lymhadenopathy. Approximately half of the patients (%46,2) diagnosed with splenectomy. Only one patient was pancytopenic at diagnosis. Four patients were anemic (Hemoglobin<10 gr/dL), six were thrombocytopenic (Platelets<150000/µl). Leucopenia was seen in 84,6% (n=11) of the patients. Monocytopenia commonly seen in HCL was detected in 61,5%. One of the patients was diagnosed and treated due to Mantle cell lymphoma (MCL) a year ago and found in remission for both MCL and HCL; one was diagnosed Kaposi carcoma just before the diagnosis of HCL and lost in follow-up. Twelve patients were hospitalized and treated with one cycle of cladribin (0,1 mg/kg/day IV for 7 days). One of these patients received SC IFNα at a dose of 4,5 mIU/day prior to cladribin therapy. Treatment responses could be evaluated in eleven patients and all of the patients gained CR. Survival analysis couldn't be done because non of the patients had progressed or died. B-RAF^{V600E} mutation was positive in 10(%76,9) patients. Three (%23,1) of the patients had B-RAF 464-469 codon mutations (One B-RAF^{G464E}, one B-RAF^{G466E}, one B-RAF^{G469E}) Two patients were positive for both mutations. No relation could be determined between clinical findings and mutation state.
Summary/Conclusions: B-Raf mutations are variable and common mutations in HCL patients. B-RAF^{V600E} mutation testing can be used as a supportive test for the diagnosis of HCL due to high incidence of mutation. Also it can be used as an indicator for patient selection that are appropriate for target therapies.

PB1887

BENDAMUSTINE-RITUXIMAB IN PATIENTS WITH RELAPSED FOLLICULAR LYMPHOMA PREVIOUSLY EXPOSED TO RITUXIMAB. EXPERIENCE IN SEVEN HOSPITALS OF THE SPANISH GELTAMO GROUP
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Background: Follicular lymphoma (FL) is characterized by a course of relapses and increasingly shorter responses to the consecutive treatments. In first relapse after immunochemotherapy, in patients who are not considered refractory to rituximab, there is no standard treatment. In Spain, bendamustine in association with rituximab (BR) has not been approved for this indication. Nevertheless this combination has shown high efficacy and excellent tolerance in patients previously treated with and without rituximab.
Aims: To evaluate the efficacy and safety of the bendamustine-rituximab association in a group of patients with follicular lymphoma previously exposed to rituximab.
Methods: Retrospective analysis of patients with relapsed FL treated with BR in 7 spanish hospitals on behalf of the Spanish Lymphoma Group (GELTAMO). The study was approved by the reference Ethic Committee and by all of the participating centres. All patients acceded to the treatment through the compassionate use program.
Results: 41 patients were valid for analysis. Characteristics: 70% males with a mean age of 62 years (30-87). ECOG≤ 2 in 95% of cases, 73.2% in stages III-IV and FLIPI ≥3 in 48%. Bulky mass in 13% of patients, LDH and β2-microglobulin increased by 12% and 41.2% respectively and bone marrow involvement in 60%. 68% had received only one previous treatment, with an average of 1.7 (1-5) and the most frequent was CHOP-R in 66% followed by CVP-R in 11%. All patients had previously received rituximab and only 3 patients (7.3%) could be considered refractory. All patients received BR (B-90 mg / m2 D1-2, R-375mg / m2 D1). Median cycles 5.1 (1-6). Support with G-CSF was used in 27.5% of cycles. Maintenance with rituximab after obtaining a complete (CR) or partial remission (PR) was administered in 42% of patients. Response: The overall response rate was 95.1% (65.8% CR-iCR / 29.3% PR). With a median follow-up of 25 months (6-92) the median response duration was 41.9 months (32.8-51.1) and the median progression-free survival (PFS) was 57 months (27.4-86.5) with no impact neither by the number of previous treatments (1 *vs* ≥2) (P=0.69) nor by the age (<70 *vs* ≥70) (P=0.9). Patients who received maintenance with rituximab after BR had a significantly longer median PFS than without (NR *vs* 32) (p=0.004). Toxicity: No treatment-related death was recorded. 42% and 36.6% of the patients presented G3-4 neutropenia and thrombocytopenia respectively, although only 2 patients were admitted due to febrile neutropenia. 43% received cotrimoxazole prophylaxis and 3 opportunistic infections were recorded (1 *P. jirovecii* pneumonia in a patient without prophylaxis).
Summary/Conclusions: BR has a high efficacy and a good safety profile in this series of patients with relapsed FL previously exposed to rituximab. The number of previous treatments (1 *vs* ≥2) and the age had no impact in the results.

PB1888

USE OF RADIATION THERAPY FOR THE TREATMENT OF GASTRIC MALT LYMPHOMA
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Background: Gastric mucosa-associated lymphoid tissue (MALT) lymphoma is a rare disease however, the incidence is increasing and closely associated with helicobacter pylori (HP) infection. One choice of treatment of gastric MALT lymphoma refractory to HP sterilization is radiotherapy.
Aims: Our aim was to analyze the response to treatment with definitive radiotherapy in our department.
Methods: Between January 2014 and January 2017, 8 patients with gastric MALT lymphoma were treated with eradication therapy of HP, followed by definitive radiotherapy. The average total dose was of 38 Gy to the stomach in a once-daily scheme. Follow-up included computed tomography scan and

endoscopy with biopsies at regular intervals. The median follow-up was 14 months.
Results: In all patients we got complete responses (CR) with no tumor detectable by endoscopy or biopsy after initial treatment, but after 2 years one of them relapsed and required immunochemotherapy. The most common acute toxicities were fatigue and nausea, in our patients. In any case late toxicities were observed. The overall survival was 100% after 2 years.
Summary/Conclusions: In selected patients who are not responsive to HP sterilization, definitive radiotherapy can be an efficient therapy with tolerable complications, preservation of stomach and sustained response over time.

Infectious diseases, supportive care

PB1889

USE OF LIPEGFILGRASTIM IN CLINICAL PRACTICE FOR THE PROPHYLAXIS OF CHEMOTHERAPY-INDUCED NEUTROPENIA IN LYMPHOMA PATIENTS: INTERIM RESULTS OF A PAN-EUROPEAN NON-INTERVENTIONAL STUDY

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Background: Lipegfilgrastim (Lonquex®) is a long-acting fixed-dose glycopegylated granulocyte colony-stimulating factor administered once per chemotherapy cycle. It has been available in Europe since 2013. It was proven to be non-inferior with regard to duration of severe neutropenia compared with pegfilgrastim in breast cancer patients. However, data in patients with hematological malignancies are limited.
Aims: We aimed to evaluate the effectiveness of lipegfilgrastim in the cycle following the first lipegfilgrastim-supported treatment cycle in lymphoma patients.
Methods: This is a prospective observational cohort study. Patients with different tumor types treated with cytotoxic chemotherapy (CT) who received lipegfilgrastim in primary prophylaxis (PP) or secondary prophylaxis (SP) are being included in this study. CT dose modifications and neutropenia-related events are recorded and analyzed. Evaluation of effectiveness in the cycle following the first lipegfilgrastim-supported CT cycle in a lymphoma subpopulation is presented here.
Results: At the time of the interim analysis (December 2016), 249 patients diagnosed with lymphoma have been included. Mean age±standard deviation of lymphoma patients was 61.6±15.6 years and 56.6% were male. For the majority of patients (81.1%), intended use of lipegfilgrastim was in PP. Exposure to lipegfilgrastim has been documented for 228 patients with an average of 4.76 cycles per patient. Data on CT dose modifications and neutropenic events following the first lipegfilgrastim-supported cycle were available for 144 and 167 patients, respectively. CT dose was never omitted. CT dose delays were observed in 8.0% (PP) and 18.8% (SP) of patients and CT dose reductions in 4.5% (PP) and 12.5% (SP) of patients. In the first lipegfilgrastim-supported cycle, febrile neutropenia was recorded in 4.5% (PP) and 3.0% (SP) of patients; severe neutropenia was recorded in 7.5% (PP) and 9.1% (SP) of patients. Throughout the treatment, 22 (9.6%) patients exposed to lipegfilgrastim reported at least 1 adverse drug reaction (ADR). The most common ADRs were myalgia and musculoskeletal pain. Serious ADRs were reported by 11 (4.8%) patients.
Summary/Conclusions: Lipegfilgrastim is effective and well tolerated in the real-world setting in lymphoma patients, administered either in PP or SP. The results suggest that lipegfilgrastim administered in PP might give better outcomes in terms of dose delays and dose reductions than when administered in SP.

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TUBERCULOSIS IN ACUTE LEUKEMIA- AN ANALYSIS OF CLINICAL CHARACTERISTICS AND IMPACT ON MANAGEMENT IN 25 PATIENTS

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Background: Patients with acute leukemia represent an immuno-compromised population, with innate, humoral and cellular immune paresis. These patients are thus at high risk of development of new infections and reactivation of chronic infections. Despite the high prevalence of tuberculosis in the general population in endemic countries, it is rarely suspected and diagnosed in patients with acute leukemia.
Aims: To study the clinical manifestations of tuberculosis in patients with acute leukemia, as well as the impact of infection in the management of leukemia
Methods: A hospital database search was done to identify cases of acute leukemia and tuberculosis between a study duration of January 2013 to January 2017. All the medical records of the identified cases were retrieved from the central records department. A systemic analysis of characteristics pertaining to acute leukemia, treatment regimen, chemotherapy response, site of tubercular infection, mode of diagnosis and treatment response to anti-tuberculous therapy was conducted.
Results: A total of 25 patients with acute leukemia were identified who were also diagnosed with tuberculosis. 10 patients had Acute Myeloid Leukemia, 7 had Acute Promyelocytic Leukemia, 5 had Acute Lymphoblastic Leukemia, 2 had Mixed Phenotypic Leukemia while 1 had Myeloid Sarcoma. The mean interval between diagnosis of tuberculosis and acute leukemia was 37.2 weeks, with 2 patients being diagnosed after completion of therapy of acute leukemia

and one patient was diagnosed post mortem. The most common organ involved was the lung, which was seen in 80% of patients and 20% of patients had disseminated tuberculosis. The development of tubercular infection led to alteration of therapy for the acute leukemia in 24% of cases, while it was postponed in 44% of cases. In particular, hypomethylating agents were used successfully in two patients with AML as bridge therapy to high dose chemotherapy. 76% of patients were cured of tuberculosis with therapy, while 1 patient expired due to tuberculosis and 3 patients could not receive adequate therapy for tuberculosis. 3 patients went on to undergo HSCT post treatment for tuberculosis, and none had a flare of the disease post transplant.
Summary/Conclusions: The presence of tuberculosis infection in patients of acute leukemia has an impact on the overall management of the patient, and strategies such as utilization of hypomethylating agents as bridge therapy may help in successful management of the leukemia. A high index of suspicion is required to suspect and diagnose the presence of tuberculosis as the manifestations are more commonly attributed to fungal infections or to the leukemia per say. These patients usually have a favorable response to anti-tuberculous therapy and the presence of tuberculosis infection does not forego treatment options such as HSCT or high dose chemotherapy for these patients.

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INCIDENCE OF BACTEREMIA BY MULTI-RESISTANT BACTERIA IN HEMATOLOGY PATIENTS. A DESCRIPTIVE EPIDEMIOLOGIC STUDY FROM A THIRD LEVEL HOSPITAL

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Background: In recent years the incidence of multi-resistant bacteria (MRB) infections have notably increase. These infections are especially serious in hematological patients because of the immunosupression derived from their illness and their treatments. This increase is related to a high mortality rate and high health costs due to the severity of the infections and the difficulty in setting adequate therapy due to the lack of new antibiotics against these pathogens.
Aims: Define the MRB infections incidence and ways of presentation. As a secondary goal we try to determine if the isolation of these MRB has affected our empiric antibiotic therapy decision.
Methods: We retrospectively collected all positive blood stream cultures from hematologic patients from January 2012 to December 2016. We studied the characteristics, clinical features and pathogen isolates of our patients when the blood cultures were obtained.
Results: 1005 positive blood stream cultures were collected in 382 patients. The main characteristics of the patients are shown on Table 1.

Table 1.

SEX	MEN 55% WOMEN 45%
MEAN AGE	57.5 (10 month – 96 years old)
DIAGNOSTICS	AML / MDS 20% ALL 5% CLPS 7% CMPS 4% MM 19% NHL 35% HL 5% OTHERS (ITP, NPH, APLASIA AIHA...) 5%
HSCT (36% OF ALL PATIENTS)	ALOHSCT 60% AUTOHSCT 60%
NEUTROPHILS	< 500: 35% 500-1000: 8% >1000: 57%
INFECTION SOURCE	CVC: 48% RESPIRATORY: 18% ABDOMINAL: 8% SKIN/ SOFT TISSUE: 7% URINARY: 5% MULTIPLE LOCATION: 5%

The infection source was: central venous catheter (CVC) in 48% of patients (including tunneled, non-tunneled and PICC lines), respiratory 10%, abdominal 8%, urinary 5%, skin/soft tissue 7% and multiple location 5%. Regarding CVC isolation's, 11% were interpreted as contamination and 6% as colonization. Gram positive (G+) bacteria were more frequently isolated than Gram negative (G-) (72% *vs* 24%). Most common G+ bacteria was coagulase negative Staphylococcus, regarding G- E. Coli, Klebsiella sp and Pseudomonas aeruginosa. MRB were detected in 6.1% of blood cultures being the most frequent G- (85%). The main resistance mechanism was extended-spectrum beta-lactamases (ESBL) and carbapenemases (CP) production (Table 2). BMR infections increased significantly in last year, mainly associated to CP, 0.5% in 2012 up to 7.1% in 2016 (Figure 1). 29.5% of MRB infections developed in patients identified as chronic carriers of multiresistant organisms and 100% of them had extensive exposure to wide spectrum antimicrobials previously. 14% of infections began with a serious illness (persistent hyperthermia, hemodynamic disbalance and worsening), 5% needed intensive care assistance and 15% died because of the infection. In MRB infections, 44% were severe, 6% needed ICU and 25% died. The most common empirical antibiotic therapy was carbapenems - 12% in monotherapy, 17% with glucopeptids - followed by third generation cephalosporins in 7%. Related to febrile episodes in MRB known carriers: empiric treatment includes effective antibiotic against this MRB (including colistin and carbapenems in extended infusion) was started in 15% of patients, all with serious illness at diagnosis.

Table 2.

GRAM NEGATIVE MULTI-RESISTANTS BACTERIA	n
ESBL Escherichia coli	17
CP Klebsiella pneumoniae	11
ESBL Enterobacter cloacae	10
CP Pseudomona aeruginosa	8
ESBL Serratia marcescens	4
ESBL Proteus mirabilis	1
ESBL Citrobacter	1

Figure 1.

Summary/Conclusions: - Current antimicrobial resistance, especially concerning G- in our study, is particularly worrisome due to development of resistance to all available antimicrobial agents. The incidence of multi-resistant G+ is not very high. - Clinical presentation in MBR infections is more serious in our experience, and the mortality doubles in relation to the difficulty to establish appropriate treatment. - Severity sings at infection diagnosis in MRB carriers had led us to a change of empirical antibiotic therapy. - As reported in previous literature, prevention of transmission, a quick establishment of diagnosis and an effective treatment, along with a correct and limited use of antibiotic therapy could decrease the development of MRB.

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INFECTIONS PRESENTING IN THE BONE MARROW IN HIV POSITIVE PATIENTS AND THEIR MORPHOLOGICAL ASSOCIATIONS – SIX YEAR DATA FROM AN INDIAN TERTIARY CARE HOSPITAL

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Background: Centre of Disease Control enlists more than 20 infections considered as Acquired Immunodeficiency Syndrome(AIDS) defining. Progression of the disease and falling CD4 counts are the most important risk factors in acquiring these infections. Most of the cases present with non specific symtoms including fever, respiratory and gastrointestinal manifestations . A bone marrow examination is helpful in estabilishing the diagnosis in many of these cases.
Aims: The aim of this study was to evaluate the incidence of marrow infections in HIV positive patients and to study their morphological spectrum.
Methods: This is a six year retrospective study carried out in a tertiary care hospital in North India. All bone marrow aspirates and biopsies from HIV positive patients were retrieved and evaluated for the presence of infectious etiology. Cytochemical stains like Acid Fast Bacillus, Periodic Acid Schiff , Gomori Methenamine Silver and Mucicarmine were performed wherever needed. The associated morphological features which may assist diagnosis were noted.
Results: Bone marrow samples (either aspirates or biopsies or both) were available in 185 HIV patients. Out of these, fifty three cases (33.5%) were associated with infections. The most common infection in these patients was Mycobacterium Tuberculosis (22.7%). In addition, five cases of Histoplasmosis (2.7%), two cases of Cryptococcosis, two cases of Leishmania donovanii, and one case of Plasmodium falciparum, Parvovirus and Microfilaria each were noticed. The morphological spectrum associated with infections in these cases included lymphoplasmacytic infiltrate(68%) , granulomas(66%), macrophage infiltration, hemophagocytosis, gelationous marrow transformation and marrow hypoplasia. Two cases of M tuberculosis were associated with maturation arrest in the bone marrow. One case of Tuberculosis was associated with Non Hodgkin's Lymphoma. Myelodysplasia was seen in association with Leishmania infection.

Summary/Conclusions: A wide spectrum of infections may be observed in HIV positive patients in the bone marrow. Bone marrow aspirate and biopsy are essential, rapid and cost effective techniques to arrive at the right diagnosis in such cases . Features like hypoplasia, myelodysplasia and maturation arrest may be attributable to infections.

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UTILITY OF BONE MARROW BIOPSY IN FEVER OF UNKNOWN ORIGEN: A CRITICAL ANALYSIS OF A RETROSPECTIVE SERIE

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Background: The utility of bone marrow biopsy trephine (BMT) as a diagnostic tool in patients with fever of unknown origin (FUO) is a subject of controversy and debate. BMT has been shown to be safe and useful in patients with HIV/AIDS but its value in immunocompetent patients has not been sufficiently assessed. It's reported the use of diagnostic BMT as a rapid decision-making tool in patients with HIV/AIDS and FUO in the proper clinical setting. A BMT demonstrated infection-related evidence prior to positive bone marrow culture in 75% of cases. Special stains and blood cultures had similar diagnostic yield, but BMT offers faster results. Thus, this procedure assists in clinical decision-making and the refinement of treatment in a more timely manner.
Aims: determine the utility of Bone marrow biopsy in FUO patients.
Methods: We reviewed retrospectively the bone marrow biopsy results of the inpatients who underwent BMT from January 2010, to December 2016. Demographic, laboratory, diagnostic and outcome data were collected and retrospectively analyzed. We identified 31 patients who fulfilled the accepted classic Petersdorf criteria for FUO. The cohort included immunocompromise and immunocompetent patients.
Results: The BMT contributes to the diagnosis in only four cases (12.9%). In two patients (6%) the histology revealed the presence of granuloma and/or lymphohistiocytic aggregates; one secundary hemophagocytosis (3.2%) and one mastocytes infiltrate (3.2%). Six patients had a previous diagnosis of HIV/AIDS (19%). Sub analysis in HIV/AIDS patients revealed positive BMT culture in 2 of the patients (6,4%). Cultures demonstrated Mycobacterium tuberculosis and Mycobacterium avium intracellulare. There was one case in which a pathogen was grown in culture but that had a negative of 'direct examination'. The associations most likely related factor to contribute to the diagnosis in HIV/AIDS was male predominance (58% odds ratio [OR] 2.95; 95% CI, 1.19-4.25), clinical lymphadenopathy (OR 4.97; 95% CI, 1.90-2.44) or anemia (OR, 2.21; 95% CI, 1.26-3.84). Reactive myeloid hyperplasia was represented 15 cases (48%). Non- haematological diagnosis (lymphoma, Leukemia) was made on the exclusive bases of biopsy results.
Summary/Conclusions: Bone marrow examination is an integral part of investigation of FUO, however, morphological finding alone would not be sufficient to ascertain the diagnosis. In present study only two cases of established infections (Mycobacterium) were detected on bases of the marrow culture. Both were present in HIV/AIDS. These results are explained because a highly active antiretroviral therapy has reduced incidence of opportunistic infections. The percent of opportunistic infections diagnosed by BMT was very low and did not justify an invasive procedure. The presence of granulomas in trephine biopsy increases the likelihood of an aetiologic diagnosis in these patients. Bone marrow biopsy is still a useful ancillary procedure for establishing the diagnosis of FUO, only if used in the adequate context.

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THE OUTCOME OF PEDIATRIC CANCER PATIENTS ADMITTED TO THE INTENSIVE CARE UNIT OF A TERTIARY HOSPITAL IN GWANGJU-CHONNAM, KOREA

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Background: Recent advances in supportive care have considerably improved the prognosis of pediatric cancer patients. However, the use of aggressive cancer treatment is also associated with complications and life-threatening events that result in admissions to the intensive care unit (ICU).
Aims: This study aimed to analyze the outcome of pediatric cancer patients admitted to the ICU.
Methods: A retrospective analysis of 84 ICU admissions of cancer patients <21 years old between May, 2004 and Aug, 2016 at Chonnam National University Hwasun Hospital (CNUHH) was undertaken. The risk factors for short-term outcome (survival at the time of discharge from the ICU) were analyzed. After excluding scheduled perioperative admissions, the records of 81 admissions (75 patients) were reviewed.
Results: Hematologic cancer patients represented 71.6% of admissions. The mean duration of ICU stay was 10.7 days. Respiratory failure (39.5%) and septic shock (17.8%) were the most frequent indications for ICU admissions. Overall mortality rate was 46.9%. The mortality for hematologic cancer was 51.7% as compared to 34.8% for solid cancer (P >0.05). Mortality for individual indication was as follows: bleeding, 66.7%; respiratory failure, 59.4%; systemic infection 57.5%, anterior mediastinal syndrome, 50%, neurologic disorders, 37.5%, renal disorder, 37.5%, and so on. ICU mortality after hematopoietic stem cell transplantation was 66.7%, mostly within 100 days post-transplant. The median Pediatric Risk of Mortality Score (PRISM) III score of survivors was lower than that of non-survivors (11.3±5.1 *vs* 19.9±10.9, P<0.001). The mortality rates were 70.3% and 27.3% in patients with high (>15 points) and low (≤15 points) PRISM III score, respectively (P<0.001). Mortality rate was significantly related to the presence and number of organ system dysfunction (P<0.01 and P<0.001, respectively), positive inotropic support (P<0.01), and mechanical ventilation (P<0.001). By using multivariate logistic regressions, the independent risk factors were mechanical ventilation (OR, 6.0; 95% CI, 1.7-21.3; P<0.01), and ≥3 organ system dysfunction (OR, 18.5; 95% CI, 4.4-77.0; P<0.001). Hematologic cancer patients had higher mean PRISM3 score (16.5±9.4 *vs* 12.2±8.6; P=0.51) and higher risk of sepsis (39.3% *vs* 13.0%; P<0.05) compared to solid cancer patients.
Summary/Conclusions: These results revealed the current status of ICU care for pediatric cancer patients in a tertiary hospital in Korea. Further improvement of supportive care and earlier effective intervention should be translated in gradual reduction in mortality rate in these population.

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EFFICACY AND SAFETY OF TIGECYCLINE IN FEBRILE NEUTROPENIC PATIENTS WITH HEMATOLOGIC MALIGNANCIES AND CARBAPENEM RESISTANCE: A MULTICENTRE RETROSPECTIVE STUDY FROM CHINESE PEOPLE

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Background: Tigecycline has broad spectrum activity against multidrug-resistant (MDR) bacteria, but few investigations of tigecycline in febrile neutropenic (FN) patients with malignancy are available.
Aims: This study attempts to investigate the efficacy and safety of tigecycline in FN and carbapenem resisant patients with hematologic malignancies.
Methods: The study of 109 patients with hematologic diseases and FN were retrospectively analyzed. They are unresponsive to carbapenems for 3~5 days before receiving tigecycline (loading dose 100 mg; then 50 mg every 12 hours). Clinical response to treatment was defined as clinical cure, improvement or failure. Meanwhile, the adverse events were documented.
Results: The median duration of neutropenia was 15 days (ranged from 1 to 83d). Out of 109 patients, 96 (88.1%) had respiratory infection, while 33 (30.3%) had bloodstream infection. The total response rate of tigecycline was 65.1%. The bacterial eradication rates and bacterial hypothetical eradication were 25.9% and 24.1%, respectively. The clinical effective rate was 85.7% when tigecycline was administered for more than 9 days, while just 48.3% when administered for less than 9 days (p<0.001). Patients with bloodstream infection got a worse efficacy than those without (41.2% *vs* 69.6%, *p=0.024*). For patients whose absolute neutrophil counts were less than 0.1×10^9/L, the clinical effective rate decreased obviously (59.8%, *vs*.86.4%, p=0.019). The side-effects were well tolerated. No lethal adverse events were observed.
Summary/Conclusions: Our results demonstrated tigecycline was effective and safe for patients unresponsive to carbapenems with FN, combination and prolonged duration of tigecycline is recommended, and these results need to be further studied.

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BONE MARROW CYTOLOGICAL CHARACTERIZATION OF PATIENTS WITH HIPERREACTIVE MALARIAL SPLENOMEGALY

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Background: Hyperreactive malarial splenomegaly (HMS) is a common cause of massive splenomegaly in malarial-endemic areas. At present, diagnosis of patients with suspected HMS in tropical medicine departments of european hospitals is relatively frequent due to immigration and the return of missionaries and NGO workers after long periods in tropical countries.
Diagnostic protocols for HMS usually include a cytological study of bone marrow, because clinical similarities between HMS and lymphoproliferative disorders have been reported. However, there are no large series in the literature that estimate a bone marrow cytological standard associated to HMS. Another important issue is that patients with HMS are often multiinfected by other parasites, viruses and bacteria that may also induce splenomegaly.
Aims: The aim is to define the bone marrow cytological pattern of patients with confirmed HMS, as well as of HMS patients with associated viral (HIV, HBV, HCV) or parasitic diseases.

Methods: A retrospective cytological study of bone marrow aspirates from 95 patients with HMS (n=27), HMS+HIV (n=8), HMS+HCV/HBV (n=11) and HMS+intestinal parasitosis (n=49) has been performed.
Results: Bone marrow cellularity was normal in all the groups studied except in HMS+HIV patients, in which the cellularity was very diminished (statistically significant difference, p<0.01). Most frequent alterations observed in all samples (HMS and HMS+other entities) that could define the HMS-bone marrow cytological pattern, were: - Erythroid hyperplasia with dyserythropoiesis, which is reflected in a decreased myeloerythroid ratio. - Increased eosinophils percentage. - Increased lymphocytes percentage. - Increased plasma cells percentage and detection of Mott cells in a significant proportion of samples from all series (48.1% of HMS samples). Quantitative results for these variables are summarized in Table 1. Lymphocytosis was significantly increased in HMS+HBV/HCV bone marrow (p=0.04). Significant detection of atypical lymphocytes (>4%) varied widely between the groups, ranging from 14.8% of HMS bone marrows to 75.0% of HMS+HIV bone marrows (statistically significant difference, p<0.01). There was no lymphoma evidence in any case. No quantitative or qualitative alterations were detected in megakaryocytes, except for a slight decrease in HMS+HIV bone marrows (statistically non-significant difference) (Figure 1).

Table 1. Quantitative results (mean±standard deviation).

	Reference values	HMS	HMS+HIV	HMS+HBV/HCV	HMS+IP
Myelo:erytroid ratio	3.5:1	2.0:1±0.8	2.2:1±1.3	2.4:1±0.6	2.5:1±0.7
Eosinophils (%)	<5	12.4±10.0	8.6±4.9	9.4±8.9	12.3±8.3
Lymphocytes (%)	10-6	23.0±9.5	26.7±9.2	27.6±5.7	23.8±7.0
Plasma cells (%)	≤4	6.8±2.7	8.1±3.1	6.4±1.6	7.3±3.0

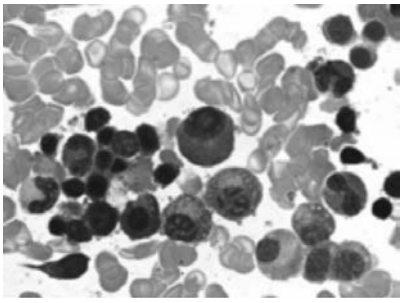

Figure 1.

Summary/Conclusions: As far as we know, this is the largest series of HMS bone marrow analyzed. Identification of common cytological findings in all the groups studied allows defining a characteristic cytological pattern for HMS. The reason for these findings could be related to an aberrant chronic immune response caused by a continuous exposure to malaria parasites. Only bone marrows of HIV coinfected patients present additional specific alterations (decreased cellularity and high proportion of atypical lymphocytes). Some authors hypothesize that HMS could eventually evolve to chronic lymphocytic leukemia, hairy cell leukemia or splenic lymphoma with villous lymphocytes, so a special follow-up would be advisable for those patients with a high proportion of atypical lymphocytes.

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ACUTE APPENDICITIS IN LEUKEMIA PATIENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION DURING THE NEUTROPENIC PHASE

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Background: Infectious complications arising from the gastrointestinal tract is common in neutropenic patients with hematologic malignances, especially during HSCT.
Aims: Sequential analysis of 776 HSCTs in single center, totally 10 cases of acute appendicitis were found out, the treatment and outcome were further analyzed.
Methods: The HSCT patients who occurred acute appendicitis during -10d~+60d in the Hematological Department of Nanfang Hospital from Jan. 2005 to July 2016 were analyzed. Patients were enrolled in our study based on the Modified Alvarado Scoring combined with ultrasonography (the MASS total score of 1-4: acute appendicitis very unlikely; Score 5-7: acute appendicitis probable; Score 8-10: acute appendicitis definite. We selected those cases with scores 5-10). And the follow-up lasted for 24 m.
Results: 776 HSCT patients were analyzed, in which 10 patients (7 male, 3 female) had acute appendicitis during -1d~+7d, which included two cases of sepsis. The median age was 18.5 (10-39) years of age. 7 patients were ALL and the other 3 were CML. All patients underwent allo-HSCT. 6 patients received conditioning regimen of IDA/TBI/CY/VP-16, and 4 others were treated with TBI/CY/VP-16/Ara-c, FA/TBI/CY/VP-16, Bu/Cy and Bu/Cy/Ara-c respectively. All the cases scored range from 5 to 10 of the MASS, and 6 patients showed positive findings on ultrasonography. All the patients had a mean value of neutrophil of 0.06×10^9/L (Table 1). 10/10 cases were cured with conservative therapy, mainly contained different kinds of full dose broad-spectrum antibiotics, such as piperacillin/ tazobactam, imipenem cilastatin, teicoplanin, meropenem, tigecycline, *et al.*. Within 24 m, 8 patients did not relapse, one patient died from gastrointestinal bleeding 2 m after without recurrence of appendicitis, while one patient relapsed 1 year later and was cured by appendectomy.

Table 1. Combined with the Modified Alvarado Scoring and ultrasonography to diagnose appendicitis during the neutropenic phase of HSCT.

	Symptoms*			Signs*			Total	Examination	Blood Routine Test	
Case	Migratory right ilia fossa pain	Anorexia	Nausea /vomit	Tenderness in right iliac fossa	Rebound tenderness	Elevated temperature	Score *	Ultrasound#	WBC (×10⁹/L)	NEU (×10⁹/L)
Case 1	1	1	1	2	1	1	7	-	0.09	0.03
Case 2	1	1	1	2	1	1	7	+	0.06	0.06
Case 3	1	1	0	2	1	1	7	-	0.06	0.06
Case 4	0	1	0	2	1	1	5	+	0.01	0.01
Case 5	1	1	0	2	1	1	6	+	0.12	0.01
Case 6	1	1	1	2	1	1	6	-	0.53	0.08
Case 7	1	1	1	2	1	1	7	+	0.17	0.02
Case 8	0	1	1	2	1	1	6	-	0.49	0.40
Case 9	1	0	0	2	1	1	5	+	0.05	0.01
Case 10	1	1	1	2	1	1	7	+	0.04	0.02

*Reference standard by the Modified Alvarado Scoring System, Total scores were 10, Score 1-4: acute appendicitis very unlikely; Score 5-7: acute appendicitis probable; Score 8-10: acute appendicitis definite. # "-": negative; "+": positive.

Summary/Conclusions: Acute appendicitis occurring during the neutropenic phase in HSCT patients could be diagnosed by the MASS and ultrasonography, and such cases could be cured by conservative therapy. This study could provide a further choice for the diagnosis and treatment of acute appendicitis in leukemia patients of HSCT.

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EPIDEMIOLOGY OF BLOODSTREAM INFECTIONS IN NEUTROPENIC AND NON-NEUTROPENIC PATIENTS WITH MALIGNANCY

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Background: Blood stream infections (BSI) in patients with malignancies remain associated with significant morbidity and mortality. The choice of an empirical antibiotic regimen is usually based on the local epidemiology of the microorganisms and their antibiotimicrobial susceptibility profile. Antimicrobial guidelines for the management of sepsis in cancer patients in East Sussex Healthcare Trust (ESHT) recommend piperacillin/tazobactam as monotherapy and gentamicin is added in case of septic shock. Vancomycin is also added as a first line therapy if there is a suspicion of central line sepsis. Alternative therapies are ceftazidime or meropenem plus aminoglycoside.
Aims: We intend to review the aetiology of BSI and check the effectiveness of the antibiotics used in ESHT in cancer patients.
Methods: This retrospective study was conducted at ESHT from January 2006 to December 2015. Demographic and laboratory data were collected from the Pathology information system.
Results: A total of 640 episodes of BSI occurred in 297 patients (159 male). Of the 297 patients, 239 had haematology malignancies while 54 had solid organ tumour. Four patients had both. The neutrophil count was <1 cells/10^9 in 383 episodes and majority of BSI occurred in this group. A total of 802 organisms (477 and 325 organisms from neutropenic and non-neutropenic respectively) were isolated. Of 802, 406 Gram positive and 386 Gram negative organisms were isolated. Seven Mycobacterium species and three *Candida* species were isolated. Most common organisms in neutropenic patients were Coagulase negative *Staphylococcus* (CoNS) (22%), *Klebsiella* species (14%), *Escherichia coli* (13%), *Streptococcus* species (10%), *Pseudomonas* species (10%), *Enterococcus* species (8%) and *Staphylococcus aureus* (4%). In non-neutropenic patients, CoNS (29%), *Escherichia coli* (11%), *Pseudomonas* species (8%), *Streptococcus* species (7%), and *Klebsiella* species (5%) were isolated. Twelve Glycopeptide resistant *Enterococci* were isolated. Four Methicillin resistant *Staphylococcus aureus* were isolated. In addition, 15 Extended Spectrum Beta-lactamase producing Gram negative bacilli were isolated. Among Gram negative organisms, more than 91% isolates were sensitive to piperacillin/tazobactam, ceftazidime and ciprofloxacin and higher sensitivity rates (>96%) were recorded in gentamicin and meropenem, Table1 summarises the effectiveness of antibiotics used.
Summary/Conclusions: This study highlights an on-going trend towards Gram positive organisms causing BSI in cancer patients. The antimicrobial regimens used in ESHT are highly effective against commonly isolated organisms. An early diagnosis and timely administration of appropriate antibiotics are imperative in managing BSI. The identification and the antimicrobial susceptibility of the microorganisms causing BSI in cancer patients remain important to develop antimicrobial treatment strategies, and to prevent the spread of antimicrobial resistance.

Table1. The sensitivity of antibiotic regimens used.

Antibiotics	Sensitivity rates	
	Neutropenic patients N=383	Non-neutropenic patients N=257
Piperacillin/tazobactam plus gentamicin	98%	99%
Meropenem plus gentamicin	99%	100%
Ceftazidime plus gentamicin	99%	99%
Ciprofloxacin plus gentamicin	98%	99%

PB1899

CHANGING TREND IN LOCAL BACTERIAL EPIDEMIOLOGY: EXPERIENCE IN ACUTE LEUKEMIA PATIENTS HOSPITALIZED IN SINGLE HEMATOLOGY UNIT

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Background: The intense chemotherapic regimens and hypometilant agents to treat acute leukemia induce prolonged neutropenia with high risk of infections.
Aims: To analize local microbial epidemiology we studied patients admitted to our ward.
Methods: All 100 cases of Acute Leukemia (AL) admitted in our ward from august 2013 to February 2017 received prophylactic antibacterial therapy with fluoroquinolones and were analized for weekly routine tissue colture screening and serial blood colture for fever. Six patients were Lymphoid AL and 94 were Myeloid AL. 41 patients were not elegible for intensive chemotherapy(for age and comorbidities) and were treated with hypometilant agents, while 59 were younger than 65 years and were treated with induction /consolidation chemotherapy 3 plus 7 regimen. Median age was 58 years with range from 27 to 88 years old.
Results: We found 28 patients (28%) bacterial septic shock during fever, of which 20 cases gram negative (71%) in particular 65% E.Coli, 15% Enterobacter, 10% Klembsiella, 5% Stenotrophomonas, 5% Pseudomonas; while 8 patients(29%) had a gram positive septic shock (S.Haemoliticus 38%, S.capitis 25%, S. hominis 25%, S epidermidis 12%). During intensive chemotherapy and prolonged severe neutropenia we took over the major incidence of septic shock (23 patients 82%) than hypometilant treatment in particular decitabine (5 patients 18%). During 2014 we had 3 mortal septic shock for multiresistant gram- klembsiella and Pseudomonas. Since than we adopted in our ward, isolation of patients with gram negative (klembsiella or pseudomonas)tissue colture positive, hygenic and sanitary practices with closing room for 48 hours and hand disinfection before entering and after leaving any patients room.We noticed a change of bacterial infections incidence in these 3 years in our ward.:reduction klembsiella /pseudomonas multiresistant infections and emergency of E.coli and Staphilococcus septic shock not multiresistant.
Summary/Conclusions: More epidemiological analysis in several haematological ward are necessary to understand if it is a changing local microbial epidemiology or is the different management of neutropenic patients with acute leukemia and/or a different antimicrobial strategy to determine this changing trend.

PB1900

UK SINGLE-CENTRE SERVICE EVALUATION TO DESCRIBE THE IMPACT ON HEALTHCARE RESOURCE USE OF LOCAL ANTIFUNGAL PROPHYLAXIS AND TREATMENT PROTOCOLS IN THE MANAGEMENT OF HIGH-RISK PATIENTS WITH NEUTROPENIA

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Background: Patients with neutropenia, including those with haematologic malignancies, are at high risk of invasive fungal infections (IFI). Pre-2014, there were no formal written guidelines but the guidance at Poole Hospital NHS Foundation Trust specified the use of posaconazole oral suspension for primary prophylaxis in all high-risk patients except those with acute lymphoblastic leukaemia (ALL). In 2014 formal guideline changes included the introduction of the tablet formulation of posaconazole, use of micafungin as first line empirical therapy and a focus towards improving diagnostics to guide management. MSD Ltd. has developed the Fungal Service Evaluation Tool (FSET), a secure database and analysis tool, to support UK clinicians managing patients at risk of breakthrough IFI (BIFI) to evaluate their antifungal management.
Aims: This service evaluation aimed to utilise the FSET to evaluate the impact of the antifungal management guidelines on healthcare resource utilisation associated with patients at risk of a BIFI.
Methods: An interim analysis of high-risk adult patients with prolonged neutropenia aged ≥18 years at initiation of antifungal prophylaxis/treatment was carried out. Retrospective data on patient characteristics, antifungal prophylaxis and treatment, IFI-related diagnostic tests, hospital attendance/admission during antifungal prophylaxis were collected for 12-month periods before and after 2014 (Cohort 1: 2013; Cohort 2: 2015). Anonymised data was entered into the FSET and this data was analysed using descriptive statistics.
Results: The evaluation included 24 patients in Cohort 1 (median age 66.8 [interquartile range (IQR): 47.5–72.2] years; 16 [67%] male; 5 [21%] ALL) and 22 patients in Cohort 2 (median age 66.8 [IQR: 51.7–73.4] years; 13 [59%] male; 1 [5%] ALL). At least one line of antifungal prophylaxis was recorded in 22 (92%) patients in Cohort 1 and 17 (71%) in Cohort 2. Posaconazole was the most commonly prescribed antifungal in Cohort 1 (18/24 [75%]) and Cohort 2 (17/22 [71%]). Other agents used included liposomal amphotericin B, fluconazole, and itraconazole. There were no patients in Cohort 1 and 2 (9%) patients in Cohort 2 (overall 4%) who experienced a BIFI: 1 was defined as confirmed and 1 as suspected. The mean 12 month costs per patient for all resource utilisation (including antifungal drug costs, hospitalisation costs [including admissions and attendances], investigations and tests) was £28,903 in Cohort 1 and £21,934 in Cohort 2 (Figure 1). Hospitalisation costs were a key determinant of overall costs, which is common in the management of people with complex underlying disease. There were 4 (17%) patients in Cohort 1 and 1 (5%) in Cohort 2 who had a period of ITU associated stay, which typically has greater costs than general wards. The most common investigations/tests were blood cultures (Cohort 1: mean 13.8; Cohort 2: mean 10.7) and chest x-ray (Cohort 1: mean 4.0; Cohort 2: mean 2.5), which are in-line with routine clinical practice. Once implemented, the guideline was adhered to in the management of 19 patients (86%) in Cohort 2.

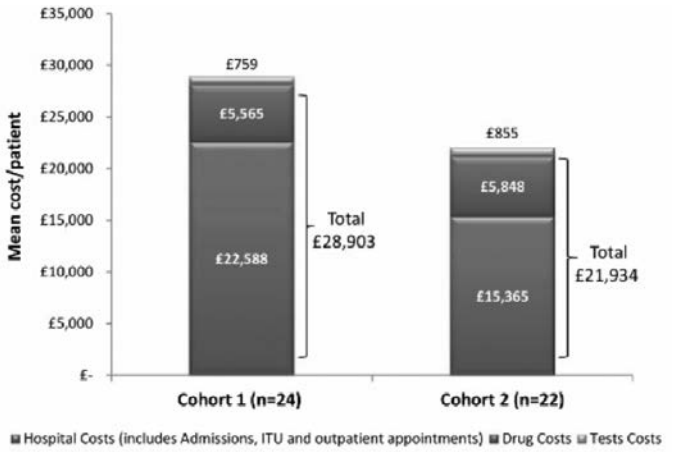

Figure 1. Breakdown of mean 12 month resource utilisation costs for cohorts 1 and 2.

Summary/Conclusions: These data show that rates of breakthrough IFI are low in complex patients receiving antifungal prophylaxis/treatment. Furthermore, the results in Cohort 2 indicate that the switch to recommending posaconazole tablets did not result in an increase in the mean cost per patient of antifungal prophylaxis and shows a lower overall mean cost per patient. A larger cohort study over a longer period is warranted to confirm these findings.

Iron metabolism, deficiency and overload

PB1901

REAL-LIFE FEASIBILITY OF AN IRON CHELATION PROGRAM WITH DEFERASIROX IN MYELODYSPLASIA AND OTHER ACQUIRED CHRONIC ANEMIAS: A SINGLE CENTRE EXPERIENCE

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Background: Prolonged red blood cell (RBC) transfusion support in patients affected by myelodysplastic syndrome (MDS) and other chronic anemias may cause vital organs damage due to accumulation of non-transferrin-bound iron with consequent increased oxidative stress. Retrospective studies have shown that iron chelation may prevent aforementioned mechanisms and improve survival in low-risk MDS patients. Iron chelation is usually recommended in patients who received at least 20 RBC units and/or have a serum ferritin level of 1000 ng/ml or higher. Deferasirox, an oral iron chelator, has widely replaced the use of deferioxamine, due to its greater manageability, especially in the elderly. However, an high dropout rate of approximately 50% of patients within one year was observed in the majority of clinical studies, the leading cause of discontinuation being gastrointestinal (G.I.) and renal toxicity and skin rash.
Aims: We aimed at evaluating the real-life feasibility of a program of prolonged iron chelation in a population of acquired chronic anemia patients. Thus, we performed a retrospective analysis to evaluate which is the percentage of patients who in our centre actually receive and tolerate deferasirox treatment, among the cohort of eligible patients.
Methods: Deferasirox treatment is considered at our centre in patients affected by MDS or other forms of chronic anemia (excluded chronic bleeding) who fullfill criteria for iron chelation (high transfusion burden, *i.e.* ≥20 RBC units and/or a serum ferritin ≥1000 ng/ml). Starting dose is usually 10 mg/kg, titrated up to 20-30 mg/kg as tolerated. The cohort of patients transfused at our centre during 2015 and 2016 was considered for analysis. Causes of unsuitability and of treatment discontinuation were extracted from our database.
Results: Our cohort consisted of 58 patients, mainly affected by MDS (45 pts); other diagnosis were myelofibrosis (6 pts), NHL (2) and multifactorial anemia, not related to blood cancer (7). Only 38 out of 58 potentially eligible patients were assigned to iron chelation (see the Figure 1). The leading cause of ineligibility in our cohort were a reduced life expectancy (5 pts), due to the hematologic disease itself or to comorbodities, and pre-exisiting renal failure (4). Importantly, in 6 cases patients were not offered iron chelation without a specific clinical reason: half of them (3/6) were non-MDS patients. Furthermore, 13/38 patients had to interrupt the treatment, due to toxicity (mainly renal failure, followed by gastrointestinal toxicity, see flow-chart). Overall, 25/58 (43%) potentially eligible patients, *i.e.* heavily transfused patients, initiate and continue an iron chelation program at our centre. The main cause for treatment discontinuation in our cohort was renal failure, while we had less difficulties in managing G.I. adverse events. Renal toxicity of deferasirox is known to be reversibile; however, in patients with pre-existing compromise and those who concurrently take nephrotoxic drugs, treatment may be difficult to carry on.

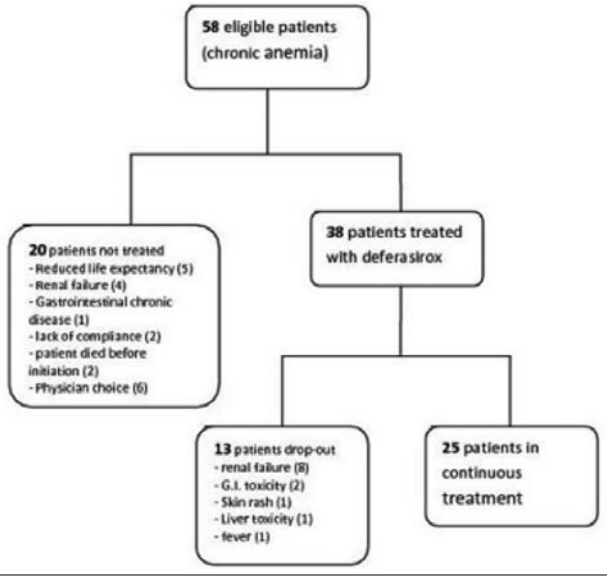

Figure 1.

Summary/Conclusions: Our data are in line with literature. However, there is still room for improvement, especially in the category of non-MDS patients, who are often under-treated. Furthermore, the introduction of a new formulation of deferasirox, which is forthcoming, may hopefully reduce G.I. toxicity and improve tolerance and patients adherence to therapy.

PB1902

NONINVASIVE TRANSCUTANEOUS SPOT-CHECKING OF TOTAL HEMOGLOBIN FOR THE SCREENING OF ANEMIA IN CAMBODIAN CHILDREN FROM REMOTE RURAL AREAS

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Background: Previous studies have reported a high prevalence of anemia among school-aged children from Cambodia, ranging from 21 to 64%. Although iron deficiency accounts for the majority of cases, additional nutritional and non-nutritional etiologies have been identified. Children living in rural or remote areas, with limited access to health facilities, are at high-risk of developing anemia, and therefore, painless, fast, and reduced cost screening tests are needed.
Aims: The aim of our study is to evaluate the role of a portable device for transcutaneous noninvasive spot-checking of total hemoglobin (SpHb) in children living in remote locations.
Methods: Transcutaneous noninvasive spot-checking of total hemoglobin (SpHb) was performed in children attending summer-school camps at 12 different locations in Cambodia. SpHb was measured in fingertips by using size adapted optic sensors. For the purpose of the study, three age groups were defined as follows: Group 1=less than 5 years, group 2=5 to 11 years, and group 3=11 to 14 years.
Results: A total of 476 otherwise healthy children were analyzed. Mean SpHb value was 11.9 ±0.93 gr/dl (range 9-16 gr/dl). Overall, the prevalence of anemia in the entire population was 34.5%. Anemia was present in 5/31 (16.1%) of the children within group 1, 97/189 (33,9%) in group 2, and 54/81 (40%) in group 3. (p=0.039, two sided Pearson's Chi square). There were no differences in the prevalence of anemia by gender in groups 1 and 2. In group 3, anemia was significantly more prevalent in females 32/65 (49,2%) than in males 22/48 (31,4%), p=0.035.
Summary/Conclusions: Taken together, our results demonstrate the feasibility of noninvasive transcutaneous spot-checking of total hemoglobin (SpHb) for the screening of anemia in children from remote rural areas with limited access to health services. Our results also confirm the high prevalence of anemia in this population.

PB1903

IRON DEFICIENCY ANEMIA IN INFANTS AND YOUNG CHILDREN

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Background: Iron deficiency anemia in infants and young children is easy to be underdiagnosed. Anemia and iron deficiency are usually corrected after aged 2-3 years old, but it causes complications. There is an association between IDA and impaired neurocognitive function and exercise intorance, even after treatment of IDA. Therefore, preventing the progression of iron deficiency is especially important during infancy and early childhood, when increased vulnerability is associated with rapid growth and development, especially of the brain.
Aims: To detect iron deficiency anemia early and to reduce the adverse impact by IDA, we assessed the characteristics of infants and young children with IDA, those at risk for IDA and those exhibiting associated characteristics of severe anemia.
Methods: Among 1,782 children with IDA aged 6 months to 18 years-old, we retrospectively analyzed medical records and laboratory data of 1,361 subjects aged 6–23 months with IDA who had been diagnosed between 1996 and 2013. We excluded patients with CRP ≥5 mg/dL.
Results: IDA was predominant in boys (2.14:1) during infancy and young childhood. Peak incidence was at 9 to 12 months of age. Only 7% of subjects were brought to the hospital with symptoms and/or signs of IDA, while 23.6% in subjects with severe IDA. LBW infants with IDA shows low adherence with iron supplementation. In a multivariate analysis, risk factors of severe anemia in infants included prolonged breastfeeding without iron fortification [odds ratio (OR) 5.70] and low birth weight (OR 6.49).
Summary/Conclusions: Many clinicians did not consider IDA as a real problem, so many children with IDA were not followed up. LBW infants need more attention to increase adherence of iron supplementation. For early detection of IDA, nutritional assessment should be evaluated in every infants and iron batteries in high risk infants (LBW infants, prolonged breastfeeding, picky eater and/or symptoms of IDA) at health screening visit.

PB1904

THE ROLE OF ZINC PROTOPORPHYRIN IN THE DIAGNOSIS OF SIDEROPENIC ANEMIA

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Background: Sideropenic anemia (IDA) is the main cause of anemia worldwide. Even though, its diagnosis is quite straightforward with the use of red blood cell indices, peripheral blood smear (PBS) and ferritin measurements, there are still some pitfalls, namely in the presence of inflammation. The chelation of iron by protoporphyrin constitutes the final reaction of heme biosynthesis. In the absence of iron, zinc becomes an alternative substrate for ferrochelatase leading to the formation of zinc protoporphyrin (ZPP). This compound can be quantified by fluorometry in blood samples, proving itself as a useful and easy parameter for the diagnosis of IDA. However, this technique is not broadly used in the clinical practice.
Aims: Determine the cut-off value of ZPP for the diagnosis of IDA. Evaluate the value of ZPP for the differential diagnosis between IDA and anemia due to inflammatory diseases (AID).
Methods: We have analyzed in our lab, from 1st to 15thFebruary 2017, all the consecutive samples (pediatric and adult) with anemia (as defined by WHO) which had sedimentation rate (SR) and serum ferritin evaluations.
We have defined three different groups: IDA: Anemia and Ferritin <20µg/L; AID: Anemia, Ferritin >20µg/L and SR>20mm/h; Group control (GC): Normal levels of Hb adjusted by age and sex, as defined by WHO, Ferritin 20-120µg/L and SR<20mm/h. ZPP measurement was performed by hematofluorometry (AVIV, Biomedica, Inc). Data were analyzed by SPSS v20.0 using Wilcoxon W and Man-Whitney to examine differences between groups and receiver-operating characteristic (ROC) analysis to determine the cut-off values of ZPP. We considered statistically significant a p-value <0.05.
Results: We have identified 204 samples that fulfilled the inclusion criteria: 104 with IDA, 51 with AID and 49 from control patients. IDA group: 73% female (F); mean age 32.3y in F [1.1-78], 28y in males (M) [1-78]; mean Hb was 10.6g/dL [SD 1.4]; mean ferritin was 9.3 ug/L [SD 4.85] and ZPP was 214.1 µmol [SD 121.3]; mean SR was 20.0 mm/h [SD12.9]. AID group: 75% F; mean age 47y in F [2-91] and 22y in M [1-85]. Mean Hb 11.0 g/dL [SD 1.2]; mean ferritin 150.3 µg/L[SD246.2] and ZPP 136.7 µmol [SD 107.8]; mean SR 47mm/h [SD 21]. GC: 69.4% F; mean age 44,8y in F [1.1-79], and 37y in M [2-65 years]; mean Hb 13.8 g/dl [SD 0.9]; mean ferritin 71.9ug/L [SD 49.9] and ZPP 78.6 µmol [SD 26.6]; mean SR 14mm/h [SD 4]. The mean serum ZPP in IDA and AID was significantly higher than in GC (95% CI; p<0.0005). The ROC analysis showed 83.7% sensitivity and 85% of specificity to identify IDA for ZPP ≥100.3 µmol (W=0.933) and 69% sensitivity and 70% of specificity to identify AID for ZPP ≥140 µmol (W=0.749) when compared with GC.
Summary/Conclusions: We have concluded that ZPP is a valid, quick, easy and cheap parameter to diagnose IDA in clinical practice, and we have defined in our cohort of patients a ZPP cut off of ≥100.3µmol as diagnostic of IDA with 83.7% sensitivity and 85% of specificity, independent of age. In AID patients we found a cut-off value of ≥140µmol, but with a low sensitivity and specificity. In our study ZPP was not a reliable method to differentiate IDA from AID. This could be due to a sample selection bias (since clinical data were missing and the number of patient with AID was substantially lower than with IDA). It would be important to enlarge the AID sample in order to obtain a more reliable result. Since ZPP measurement can be performed in capillary blood and it is a very quick and cheap method to diagnose IDA, this could be a powerful tool in underdeveloped countries.

PB1905

HYPERFERRITINEMIA AND SERUM INFLAMMATORY CYTOKINES IN 71 ADULTS WITH NEWLY DIAGNOSED HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS ASSOCIATED WITH HEMATOLOGICAL MALIGNANCY

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Background: Hemophagocytic lymphohistiocytosis (HLH) is an underdiagnosed but life-threatening syndrome of hyperinflammation which in adults is often caused by hematological malignancies. Release of inflammatory cytokines in HLH induces immune cells and cytokine production that cumulates in cytokine storm and hyperinflammation. Hyperferritinemia ≥500 µg/L is a diagnostic criterion for HLH. Prevalence of hyperferritinemia in HLH in the adult population is much less established than in children.
Aims: The aim of the present study was to evaluate the frequency and extent of hyperferritinemia as well as serum concentrations of selected inflammatory cytokines at the time of diagnosis of hematological malignancy-associated HLH (hM-HLH) in adults.
Methods: The study included 71 adults with hM-HLH, aged 22–84 years, and diagnosed between 2009 and 2016. Hematological malignancy was defined as a neoplasm of lymphoid or myeloid origin. In all studied patients, the diagnosis of HLH was based on the HLH-2004 criteria. Since the majority of patients in this study had severe lymphopenia, we decided to not perform functional analyses of NK-cells for HLH diagnosis. Thus, we included in this analysis all patients with hematological malignancies and suspected HLH who fulfilled at least four of seven HLH-2004 criteria as well as at least two of three additional features: sIL-2Rα ≥2400 U/mL, hemophagocytosis in BM, and hyperferritinemia ≥10,000 µg/L. Serum concentrations of inflammatory cytokines IL-1β, IL-6, IL-8, IL-10 and TNF-α were analyzed using chemiluminescence (IMMULITE® One, DPC Siemens). Serum levels of sIL-2Rα were determined by ELISA, using the quantitative 'sandwich' enzyme immunoassay, on the IMMULITE® 1000 Immunoassay System (DPC Siemens).
Results: Lymphoid malignancy was diagnosed in 42 patients and myeloid malignancy in 29 patients. Fifty-four (76%) patients developed HLH as a first manifestation of an unknown malignancy, during progressive disease, or at malignancy relapse. The remaining 24% of patients developed HLH during chemotherapy. Serum ferritin concentration (ref.: 30–350 µg/L) at the time of hM-HLH diagnosis was elevated in all but one patient (70/71, 98%). Mean ferritinemia was 37,281±84,440 µg/L, median value 14,727 µg/L, and ferritinemia range 96–645,291 µg/L. As HLH-2004 criterion, hyperferritinemia ≥500 µg/L was present in 69 of 71 patients (97%) at the time of HLH diagnosis. Hyperferritinemia of ≥2000 µg/L was noted in 67 (94%) patients, hyperferritinemia of ≥5000 µg/L in 56 (79%) patients, and hyperferritinemia of ≥10,000 µg/L occured in 42 (59%) patients. Serum levels of sIL-2Rα (sCD25) were measured in 69/71 patients, of whom 91% (63/69) had values ≥2400 U/mL. Moreover, in 3 more patients sIL-2Rα was clearly elevated to 2179, 2233, and 2345 U/mL, respectively. Concentrations of TNF-α, IL-6, and IL-10 in serum were each elevated in over 85% of the examined hM-HLH patients. IL-8 concentration was increased in half of all tested patients at the time of HLH diagnosis. However, IL-1β concentration was above reference range only in 12% of patients (7 of 58). Results of the inflammatory cytokine analyses in patients with newly diagnosed hM-HLH are presented in Table 1.

Table 1. Inflammatory cytokines in patients with newly diagnosed hM-HLH.

Inflammatory cytokine	Reference range	Number of tested patients	Number (%) of patients with elevated cytokine concentration	Elevated cytokine concentration		
				range	mean	median
IL-1β	<5 ng/L	58	7 (12%)	5.5 – 40	15.1	11.5
IL-6	<5 ng/L	57	50 (88%)	5.2 – 10,101	505	70.8
IL-8	<60 ng/L	58	29 (50%)	64 – 6,256	680	184
IL-10	<5 ng/L	39	38 (97%)	5.9 – 10,001	710	80.0
TNF-α	<12 ng/L	50	43 (86%)	12.5 – 173	45.1	27.6

Summary/Conclusions: Hyperferritinemia at the time of HLH diagnosis was common in Swedish adult patients with hM-HLH. Hyperferritinemia ≥500 µg/L was present in vast majority (97%) of them. We would like to emphasize that serum ferritin level fluctuates and can differ significantly from one day to another. Ferritinemia should be repeatedly measured in cases of suspected HLH. Serum concentrations of TNF-α, IL-6, IL-8, and IL-10 were frequently elevated in the examined hM-HLH patients and these can become important markers supporting HLH diagnosis in equivocal cases. On the other hand, IL-1β seems to be less usefull in confirming a cytokine storm in this patient group.

PB1906

REDUCING UNNECESSARY BLOOD FILMS USING AN IRON DEFICIENCY ALGORITHM

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Background: In 2015, Wellington SCL (WSCL) was selected to provide integrated laboratory services in the Wellington region, New Zealand (NZ). This involved merging services from previous community laboratory Aotea Pathology Ltd. (APL) with the three regional hospital based District Health Boards (DHB) laboratories - Capital & Coast (CCDHB), Hutt Valley and Wairarapa. On the 1st of November 2015, WSCL would launch its new integrated service with a merged workforce, new technology, processes and procedures. Considered to be the biggest laboratory integration project undertaken in NZ, every effort needed to be made to reduce the workload without compromising patient care.
Aims: In the Haematology laboratory, one of the most common triggers for

blood films are iron deficient pictures with the characteristic finding of reduced Haemoglobin (Hb), MCV and MCH. Above certain thresholds, the blood film adds little or no value to the CBC in these patients, apart from correlating with the iron studies results or suggesting iron studies when unavailable. One initiative used to manage the workload was based on this logic and aimed to reduce the blood film review rate using IT3000 technology (Roche).
Methods: An algorithm was designed in IT3000 to encourage testing and treatment for iron deficiency using a series of automated educational comments, while minimising unnecessary laboratory work. The impact that this algorithm had at WSCL was investigated by retrospective analysis of all the patient results from 1st November 2015 to the 1st of May 2016.
Results: In the first six months of operation, WSCL performed 232,192 CBCs and 30,204 blood films with an average review rate of 13.01%. Had this algorithm not been employed, 2,434 extra blood films would have been reviewed, bringing the review rate up to 14.05%.
Summary/Conclusions: Incorporation of an algorithm specific for iron deficiency in IT3000 has significantly reduced the review rate without any negative impact on patient care.

PB1907

THE RELATIONSHIP ENDOTHELIAL MICROPARTICLES AND ASYMMETRIC DIMETIL ARGININE IN CHILDREN WITH IRON DEFICIENCY AND IRON DEFICIENCY ANEMIA

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Background: Iron deficiency anemia and iron deficiency without anemia increase the risk of atherosclerosis by increasing oxidative stress and inflammation. Endothelial dysfunction is an important factor of the pathogenesis of atherosclerosis.
Aims: Endothelial micro particles (EMPs) are considered as markers of endothelial dysfunction. Asymmetric dimetil arginine (ADMA) is known as another marker of endothelial dysfunction. In this study; we aimed to evaluate circulating EMPs and ADMA in children with iron deficiency and iron deficiency anemia and to disclose iron deficiency with the strongest relation with EMPs, ADMA and carotid atherosclerosis.
Methods: This study included 30 children with iron deficiency anemia, 30 children with iron deficiency without anemia and 30 healthy children whose anthropometrics measurements were recorded. Hemoglobin, serum iron level, iron binding capacity, ferritin, and lipid profile were studied. Circulating EMPs (CD144, CD146, and CD105) were measured by flow cytometry. ADMA was measured by ELİSA. The carotid artery intima media thickness (CIMT) and left ventricular mass index (LVMI) were measured using echocardiography.
Results: CD144 and CD105 EMP levels were lower in the iron deficiency without anemia group than in the control group and statically lower than in the iron deficiency anemia group ($p<0.05$). There were no significant differences in ADMA level between groups. Any significant variety in ADMA level was not observed between groups. CIMT was negative correlated with ferritin and high density lipoprotein and positive correlated with body weight.
Summary/Conclusions: In this study**,** endothelial dysfunction which occurs as a result of iron deficiency were observed. According to our result, CD144 and CD105 EMP levels in the iron deficiency without anemia group were lower than the iron deficiency anemia and control group; these levels in iron deficiency anemia group were higher than control group. In addition, when the level of ferritin has decreased, CIMT has increased. This study show that CD144 and CD105 may be related to endothelial dysfunction which occurs by iron deficiency.

PB1908

INVESTIGATION OF IRON METABOLISM FOR REGULATING MEGAKARYOPOIESIS AND PLATELET COUNT ACCORDING TO THE MECHANISMS OF ANEMIA

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Background: Iron deficiency anemia (IDA) is characterized by depletion of total body iron stores. By contrast, chronic inflammation makes iron unavailable for hematopoiesis through a cytokine-mediated cascade, resulting in anemia of chronic disease (AOC). However, the laboratory data regarding the regulatory role of iron metabolism on platelet count has not been fully discussed yet.
Aims: In this study, we investigated the relationship between iron status and platelet production according to different anemic mechanisms representing different iron metabolisms.
Methods: The study included total of 759 blood specimens from 537 different patients. The complete blood count with various CBC index were measured using Advia 2120 (Siemens, USA). Biochemical indexes including iron level were estimated using Toshiba chemical analyzer (Toshiba, Japan).
Results: We found a significant relationship between platelet count and serum iron level in AOC group ($p<0.27$), whereas there was no correlation in IDA group. In AOC group, platelet count was significantly correlated to serum iron level only in AOC group with decreased serum iron level ($p<0.0001$), unlike AOC group with normal serum iron level.
Summary/Conclusions: Reactive thrombocytosis in inflammatory states occurs by cytokine cascades involving interleukin-6 and thrombopoietin in AOC. Moreover, iron deficiency in AOC involves upregulated hepcidin production induced by increased inflammatory cytokines. It can cause increased iron sequestration in macrophage and decreased iron absorption for bone marrow. The condition of decreased megakaryocytic iron supply makes megakaryocytes with higher ploidy which can release more platelets than lower ploidy. These two features may enhance thrombocytosis in patients of AOC with decreased iron level. In the future, the further study should be performed to elucidate underlying mechanism involving the tight regulation between iron metabolism and megakaryopoiesis in anemic patients.

PB1909

SOLUBLE TRANSFERRIN RECEPTOR LEVELS OF APPARENTLY HEALTH ADULTS IN PORT HARCOURT NIGERIA

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Background: Soluble transferrin receptor (sTfR) is an early marker of tissue iron deficiency before onset of anaemia. sTfR is one of the noninvasive tools for early diagnosis of iron deficiency anaemia, which is one of the most prevalent causes of anaemia in our environment. However, there is not established reference range of this diagnostic marker sTfR in our environment. There is an important need to devise a screening tool for early detection of iron deficiency before onset of anaemia.
Aims: To determine the sTfR levels of apparently healthy adults in Port Harcourt, Nigeria, determine the reference range of sTfR in the study population.
Methods: This is a descriptive cross sectional study conducted at the University of Port Harcourt Teaching Hospital. one hundred and fifty participants (107 males and 43 females) who satisfied the inclusion criteria were enrolled in this study. The ages of the study population were grouped into four: 21-30, 31-40, 41-50, 51-60. Full blood count and sTfR concentration were assyed on anticoagulated blood samples using a 3- part autoanalyser (sysmex -KX2IN) and Human ELISA kit by biovendor respectively. The results were analysed using SPSS version 21. P value of <0.05 was considered significant.
Results: The mean sTfR concentration of the study population was 0.89±0.46ug/mL with a range of 0.3- 3.05ug/ml. The mean value of sTfR for males and females were 0.92±0.49ug/ml and 0.82±0.37ug/ml. The mean sTfR for age group 21-30 was 0.88+/-0.48ug/ml and 0.80+/-0.47ug/ml for males and females respectively, while the mean sTfR for age group 51- 60 was 0.98+/-0.53 ug/ml and 0.97+/-0.13ug/ml for males and females respectively. The mean sTfR levels did not differ significantly for age and sex.
Summary/Conclusions: This is the first cross sectional study in our environment to determine reference value of sTfR levels in healthy adults in Port harcourt. This reference value was established as 0.3-3-05ug/ml. This study found no statistically significant relationship between different ages and sexes.

Myelodysplastic syndromes - Biology

PB1910

ROLE OF PRO-PHAGOCYTIC CALRETICULIN AND ANTI-PHAGOCYTIC CD47 IN MDS AND MPN MODELS TREATED WITH AZACYTIDINE OR RUXOLITINIB

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Background: Myelodysplastic syndrome (MDS) and Myeloproliferative neoplasms (MPN) are clonal myeloid disorders with the tendency to progress into acute myeloid leukaemia. Previous studies in solid tumours have shown an increase in expression of both pro-phagocytic calreticulin (CALR) and anti-phagocytic CD47, as they act in response to one another, reflecting a possible apoptosis *vs* survival mechanism in response to chemotherapy.
Aims: The aim of our study is to assess the changes in CALR and CD47 levels during treatment of MDS and MPN with azacytidine (AZA) or ruxolitinib (RUXO), in a series of model cell systems.
Methods: CALR and CD47 gene and protein expression was measured in MDS cell line models (MOLM-13 and SKM-1), MPN cell line models (HEL-92 and GDM-1) and in an intermediate MDS/MPN cell line (K562) before and after treatment with AZA and RUXO. Drug titrations were completed, resulting in dosing regimens of 0.05μM/ml for both AZA and RUXO, with re-drugging occurring at 24 hours. Cells were then harvested, cDNA was synthesized for use in qPCR and protein levels determined by Western blot analysis.
Results: When treated with AZA, MDS cell models showed a 7-10 fold increase in CALR expression and 4-6 fold increase in CD47 expression. In contrast, the MDS/MPN intermediate cell model (K562) showed a 4.5 fold increase in CALR but only a 0.5 fold increase in CD47 expression. In the MPN model HEL-92, a 9 fold CALR increase and 5 fold CD47 increase was seen, whereas in the other MPN model (GDM-1 cells) expression was more evenly matched between CALR and CD47 (5.3 and 4.8 fold increases, respectively). After treatment with RUXO, MPN models showed a 9.5-16 fold increase in CALR expression and a 6-9 fold increase in CD47, which would be expected as RUXO is used to treat MPN in humans. When the MDS/MPN cell model or pure MDS models were treated with RUXO, the ratio of CALR/CD47 decreased substantially (with CALR expression only increased 2.4-3.7 fold compared to CD47 increasing 4.6-6.9 fold) showing resistance to treatment and a significant anti-phagocytic response. Interestingly one of the MDS cell line models (MOLM-13) showed an unexpectedly good response to RUXO therapy with high CALR/CD47 ratio (8 fold *vs* 4.8 fold, respectively).
Summary/Conclusions: In line with results in solid tumours, we have shown that treatment for MDS and MPN leads to an up-regulation of CALR and, to a lesser extent, CD47 in cell lines models. The ratio of CALR/CD47 seems to correlate with specific treatment response, significantly increasing when given diseases models are treated with the appropriate drug. We postulate a role of CALR expression in leukaemia cell phagocytosis, with CD47 co-expression in synergy as a protective instinct within the cell to try and prevent apoptosis. Some models showed an excessive rise in CD47 expression and low expression of CALR. This indicates that the CD47 mediated anti-phagocytosis takes control and supresses the CALR expression, leading to cancer cell survival and ineffectiveness of treatment. Those results need to be validated in human samples at different stages of disease to allow a better understanding of treatment response and/or resistance to chemotherapy within these diseases.

PB1911

GENETIC VARIANTS OF MSH3 AND BLM GENES MAY INFLUENCE MYELODYSPLASTIC SYNDROME SUSCEPTIBILITY AND PROGNOSIS

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Background: Myelodysplastic syndrome (MDS) is a heterogeneous group of hematopoietic stem cell disorders, characterized by peripheral cytopenias, ineffective hematopoiesis and frequent transformation into acute myeloid leukemia (AML). Several mechanisms are involved in disease development and progression as a consequence of stepwise accumulation of DNA mutations, which infers a defect in DNA repair mechanisms. Mutations in DNA repair genes of the nucleotide excision repair (NER) group, and affecting the mismatch repair (MMR), and DNA crosslink repair genes, among others, are the cause of inherited cancer syndromes. On the other hand, genetic variants in genes involved in these mechanisms have been identified for their potential role in cancer susceptibility. However, in MDS, the relevance of these variants remains to be fully established and correlated with prognosis.
Aims: In the present study we investigate the influence of polymorphisms in DNA repair genes (*XRCC5, RMI1, RAD52, XRCC3, BLM, TOP3A, OGG1, LIG1, ERCC2*, and *MSH3*) as risk factor for MDS development as well as prognostic factors of acute leukemia transformation.
Methods: We performed a hospital-based case control-study to investigate the association of DNA repair genes with MDS susceptibility and prognosis in a group of Portuguese patients. To that end, we genotyped by TaqMan real-time PCR 10 SNPs (one per gene: *XRCC5, RMI1, RAD52, XRCC3, BLM, TOP3A, OGG1, LIG1, ERCC2*, and *MSH3*) in 60 MDS patients and 120 age-sex matched controls. Frequencies of alleles, genotypes, and genotypic profiles were estimated and compared between patients and controls. The role of these genes in MDS susceptibility was studied by logistic regression analysis. The influence in MDS prognosis was evaluated by estimating, through Kaplan Meier analysis, the rate of MDS transformation into AML and the overall survival.
Results: There was no significant difference in frequencies of *XRCC5, RMI1, RAD52, XRCC3, BLM, TOP3A, OGG1, LIG1* and *ERCC2* variants between patients and controls. In contrary, we found that heterozygous individuals for MSH3 c.2655+5137C>G had an increased susceptibility to MDS development (OR=6.882, 95% CI 1.789-26.479, p <0.003), being the increased risk attributed to G allele (OR=6.405, 95% CI 1.552 30.469, p <0.003). In addition, MDS patients homozygous for BLM c.-4-889A>C showed higher rate of MDS transformation into AML (HR=7.646, 95% CI 1.362 24467, p <0.023).
Summary/Conclusions: The present study suggests that MSH3 c.2655+5137C>G variant influences MDS susceptibility, and BLM c.-4-889A>C variant may be implicated in the propensity to AML transformation observed in MDS patients. Thus, these gene variants could be used as a risk and prognostic biomarkers for MDS, if these associations were replicated in a larger case-control study and/or with other populations.

PB1912

LOW RPS14 EXPRESSION IN MDS PATIENTS WITHOUT 5Q- ABERRATION SEEMS NOT TO BE RELATED WITH GENOMIC ALTERATIONS IN 5Q REGION

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Background: Heterozygous deletion of RPS14 occurs in isolated interstitial deletion of chromosome 5q in patients with myelodysplastic syndrome (MDS). 5q- MDS has been linked to impaired erythropoiesis and it is characterized by a constant macrocytic anemia and normal or high platelet counts associated with hypolobulated megakaryocytes. Previous studies have detected reduced RPS14 expression in more than 50% of non-5q-patients. Recently, the pivotal role of RPS14 in human erythropoiesis during 5q- MDS pathology has been demonstrated: RPS14 haploinsuficiency produces the activation of p53 and its target p21 in erythroid cells, resulting in cell cycle arrest and apoptosis. Based on these results, non-5q- patients expressing low levels of RPS14 will be potentially beneficed by lenalidomide therapy. In this work, we explore the origin of the altered RPS14 expression in non-5q- patients and its potential link with 5q- pathology.
Aims: The objective of this work was to explore the origin of RPS14 low expression in non-5q- MDS patients and its link with 5q- pathology. In order to do this, we analysed potential mutations in RPS14 gene. We also studied expression changes in other key genes involved in the development of the 5q- disease, including the tumour suppressor gene SPARC and the putative tumour suppressor gene CSNK1A1, contained in the commonly deleted region. Moreover, other 32 genes related with MDS disorders were evaluated in relation with RPS14 levels. Finally, in order to establish if this group of patients could be beneficed by lenalidomide therapy, p21 expression levels were also analysed.
Methods: DNA and RNA were extracted from the bone marrow of 89 non-5q- MDS patients. Ten controls and nine 5q- MDS patients were used as negative and positive controls, respectively. RPS14, SPARC, CSNK1A1 and p21 mRNA levels were analysed by real time PCR using Taqman probes in a 7500 RT PCR System. β-glucuronidase gene was used as endogenous reference to normalize data. Samples were classified by RPS14 expression levels and differences in SPARC, CSNK1A1 and p21 expression mean values between the two groups were analysed using the Mann-Whitney U test. RPS14 and 32 genes related with MDS were analysed using Ion Proton sequencing.
Results: Non-5q- patients expressing low levels of RPS14 presented higher survival probability in the IPSS lower risk group. This data, in addition with a tendency for increased p21 expression, suggests that this group could be beneficed by lenalidomide therapy. Nevertheless, we did not observe a significant decrease in SPARC and CSNK1A1 expression in patients with low levels of RPS14, discarding alterations in the adjacent genes commonly deleted in 5q- MDS patients. In addition, the majority of patients analysed did not present any mutation in RPS14 gene. Only two MDS patients showed mutations upstream, downstream or within intronic regions of the gene. Then, the origin of RPS14 decreased expression seems not to be related with genomic alterations in 5q region. On the other hand, mutations in FLT3, U2AF1, DNM3A and CBL were frequently observed in this group of patients.
Summary/Conclusions: Although the important role of RPS14 in MDS pathol-

ogy has been recently demonstrated, the origin of RPS14 downregulation in about 50% non-5q- patients remains unknown. Our results suggest that the origin of RPS14 decreased expression is not related with genomic alterations in 5q region. Further studies are necessaries in order to establish a link with 5q- pathology and demonstrate the potential use of lenalidomide in this group of patients.

PB1913

INTEREST OF THE XN-10® ANALYZER TO SCREEN FOR MYELODYSPLASTIC SYNDROMES ON COMPLETE BLOOD COUNTS

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Background: A prospective study was performed over one year in order to investigate whether suspected myelodysplastic syndromes (MDS) could be detected on a complete blood counts (CBC), the fastest laboratory investigation, performed on the recently developed XN-10® (Sysmex, Kobe, Japan).
Aims: The primary end point was to discriminate MDS patients from normal samples and the secondary end-point was to to distinguish MDS with excess blasts (MDS-EB), MDS with multilineage dysplasia (MDS-MLD), MDS with single lineage dysplasia (MDS-SLD) and MDS with ring sideroblasts and single lineage dysplasia (MDS-RS-SLD) within the MDS group and by comparison with controls as described by the WHO 2016 classification.
Methods: One hundred and thirteen patients were enrolled in the study, for whom a diagnosis of MDS was concluded based on CBC, bone marrow smears examination and karyotype. All patients were free of treatment, including transfusions, at inclusion. They were 63 men and 50 women with a median age of 82 years (range 36-96 yo). CBC were performed on a Sysmex analyzer XN-10®, including classical parameters (hemoglobin level, Mean Corpuscular Volume (MCV), reticulocytes, platelets, neutrophils and extra-parameters *i.e.* platelets by fluorescene (PLT-F), immature platelets fraction (IPF%), immature reticulocytes fraction (IRF%) and the neutrophils median position on the three axes as well as their dispersion (Neut-WX). For comparison with normal values, results from 707 healthy subjects over 50 years old, for whom CBC were performed on the same analyzer and generated no flag, were used. All had parameters within the normal range according to age. According to the WHO, 37 patients in the cohort had MDS-EB, 35 MDS-MDL (among whom 7 had ring sideroblasts [RS]), 26 MDS-SLD- RS, 12 MDS-SLD without RS and 3 MDS with isolated del(5q). Sixty-two patients had a normal karyotype, 24 displayed anomalies classically reported in MDS, and 8 had complex karyotypes. Among the latter, 7 were associated with MDS-EB.
Results: Both classical and extra parameters indeed showed significant differences between the subgroups tested. Among the whole group of MDS patients, a number of parameters of all lineages were statistically different from the healthy cohort. The median level of hemoglobin was 9,92±1,96 g/dL (p<0,0001), the median MCV (99,24±10,56 fL p<0,0001), reticulocyte counts 44,3x10^9/L (range 8-165,9; p=0,041) and IRF% 16,7% (range 2,4-50,9; p<0,0001). The median platelet count was 194 ±128x10^9/L (p<0,0001) and the median IPF% 8,8% (1,2-42; p<0,0001). Among leukocyte parameters, the MDS median neutrophil count was significantly lower at 3,08±2,58x10^9/L (p<0,001) while Neut-WX was significantly higher (387±71;p<0,0001). The latter, allowed to predict a diagnosis of MDS with 73% sensitivity and 97% specificity. For patients over 50 years old, 4 parameters (Neut, Neut-WX, hemoglobin level and MCV) in a score allow to diagnose MDS with 92% sensitivity and 81% specificity . When considering MDS subgroups, although each of them was significantly different from controls for hemoglobin levels, MCV and IRF% and (p<0,0001), they could not be discriminated by these parameters. In the subgroup of MDS with single lineage dysplasia and ring sideroblasts, platelet counts were similar to those of controls, yet significantly higher than for MD-EB or MDS-MLD (p=0,004 and p=0,029 respectively). Moreover, neutrophils counts were significantly lower in MDS-DML or MDS-EB than in MDS-SLD-RS.
Summary/Conclusions: This study demonstrates that a simple CBC allows to screen for MDS using a multiparameter score including Neut-WX. Blood smear examination should be performed in this situation even if the XN-10® analyzer does not raise any alarm, especially in unknown patients older than 50.

PB1914

PROGRESSION SCORE FOR ACUTE LEUKEMIA – A NEW PROGNOSTIC SCORE IN MDS?

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Background: Since 1997, the *International Prognostic Scoring System* (IPSS) has been the standard for stratifying patients with Myelodysplastic Syndrome (MDS). Although other models were proposed to improve this stratification, some issues remain, notably the identification of low-risk patients with poor prognosis who may benefit from earlier and/or aggressive therapy.
Aims: The aim of our work was the conception of a new prognostic score in MDS, based in the cellular and molecular disease characterization.
Methods: Our sample consisted of 102 patients diagnosed with MDS de novo. The median age was 74 years (22-89), with a 0.8 Male to Female ratio. The subtypes, according to the World Health Organization 2008, were Refractory Cytopenia with Multilineage Dysplasia (RCMD) (n=52), Refractory Cytopenia with Unilineage Dysplasia (RCUD) (n=12), Refractory Anemia with Excess Blasts type 1(RAEB-1) (n=8), RAEB-2 (n=8), Refractory Anemia with Ringed Sideroblasts (n=6), 5q- syndrome (n=4) and Chronic Myelomonocytic Leukemia (n=12). The IPSS based stratification was: low (n=37), intermediate-1 (n=39), intermediate-2 (n=10) and high (n=1). Several variables were analyzed: hematological (leukocytes, neutrophils, hemoglobin, platelets, blasts and ring sideroblasts), biochemical (erythropoietin, β2-microglobulin, folic acid, vitamin B12, ferritin, LDH), imunophenotypic (hematopoietic stem cell characterization by flow cytometry, FC, using the markers, CD34 / CD117 / CD123 / GlicoP / IL-6 / TNFa) and molecular characteristics (methylation profile of genes *p15*, *p16*, *DAPK*, *R1*, *R2*, *R3* and *R4* performed by PCR-MS, and evaluation of expression levels of regulatory molecules of apoptosis BCL-2, BAX, TRAIL, R1, R2, R3, R4, FAS, Survivin, Caspase 3, Cit C, GlycoP and p53, by FC).
Results: In the 60-month follow-up, 11 patients progressed to Acute Myeloblastic Leukemia (AML), 7 with RAEB-2, 2 with RCMD, 1 patient with RAEB-1 and another with CMML. These patients had a higher% of ring sideroblasts and blasts; higher scores on IPSS, IPSS-R and WPSS; lower platelet counts, higher erythropoietin levels and greater expression of CD34 / CD117 / IL-6. Assigning a value (+1) to each altered variable a new prognostic score was obtained, which we named Progression Score for Acute Leukemia. We observed that patients belonging to subtypes with the highest scores were those that progressed to AML, namely RAEB-1, RAEB-2 and RCMD.
Summary/Conclusions: In conclusion, we believe that this score may contribute to evaluate the risk of progression to AML, by reflecting the heterogeneity of MDS and its multifactorial etiology. The coexistence of many altered variables not only contributes to the etiopathogenesis of MDS but also allows the assessment of potential leukemic transformation.

PB1915

CORRELATION OF PATIENT PROGNOSIS WITH PU.1 AND JDP2 PROVIDES POTENTIAL NOVEL PROGNOSTIC/DIAGNOSTIC MARKERS IN MDS

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Background: PU.1 is a key transcription factor in haematopoiesis that plays important roles in various haematological malignancies. Previously, significant down-regulation of PU.1 has been reported in high risk myelodysplastic syndrome (MDS) and acute myelogenous leukemia (AML) patients.
Aims: To clarify PU.1 molecular function we investigated the gene expression of PU.1 and JDP2 (c-Jun dimerization protein-2), a member of the activating protein-1 family located downstream of PU.1, in bone marrow from 12 MDS patients stratified according to IPSS-R score (6-low, 3-intermediate, 3-high risk), 1 AML patient and 10 normal controls.
Methods: Samples were enriched for the mononuclear fraction by Ficoll separation. Total RNA was extracted and analysed by Real Time PCR for PU.1 and JDP2 expression relative to the housekeeping gene GAPDHusing the 2-ΔΔCT method. Western blot has been perfomed using anti-PU.1 and anti-JDP2 (Abcam)according to manifacture instructions.
Results: We revealed both PU.1 and JDP2 are down regulated in MDS. In addition, our data suggests that PU.1 and JDP2 expression inversely correlates with disease, with expression of these genes consistently reducing according to IPSS-R groups. Furthermore, a positive correlation of PU.1 and JDP2expression <R=O.9333, s=0.0004 >, provides additional evidence that suppression of JDP2 by PU.1 could contribute to the pathogenesis of AML. Notably, PU.1 and JDP2 do not correlate to the same extent in normal HSCs, indicating that cofactors are required for PU.1 to exert its JDP2-regulating function and that such cofactors are not present under normal conditions. To confirm that JDP2 suppression is a direct result of reduced PU.1 we performed PU.1-knockdown in K562 cells stably expressing PU.1 short interfering RNAs *versus* control cells. These analyses reveal only a partial reduction in JDP2 expression when analysed by RT-PCR and Western blot, suggesting a more complex regulatory mechanism. Additionally, both PU.1 and JDP2 expression was recovered by treatment with azacitidine, which is routinely used to treat MDS, suggesting an involvement in treatment response.
Summary/Conclusions: PU.1 and JDP2 expression correlates with patients prognosis highlighting a potential role as new diagnostic and prognostic markers in MDS.

PB1916

DECREASED EXPRESSION OF DECORIN, A WNT-PATHWAY RELATED PROTEIN, IN MESENCHYMAL STEM CELLS OF PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Background: Myelodysplastic syndromes (MDS) are clonal disorders of the haematopoietic stem cells (HSCs) characterized by inefficient bone marrow (BM) haemopoiesis and increased risk for leukaemic evolution. Ineffective BM haemopoiesis in MDS has also been linked with an abnormal microenvironment that may sustain or even induce the aberrations within the HSC compartment. We have previously shown that the stroma progenitor cells, namely the mesenchymal stem cells (MSC), in MDS patients display impaired clonogenic and proliferative potential, reduced haemopoiesis supportive capacity and down-regulation of the canonical Wnt-signaling pathway.
Aims: Decorin, a small leucine-rich proteoglycan, and galectin-3, a member of b-galactosidase specific lectin family, are components of the extracellular matrix of the BM microenvironment. Both proteins have been implicated in the canonical Wnt-pathway participating therefore in cell growth and proliferation. The aim of the study is to assess the expression of decorin and galectin-3 in MSCs of MDS patients, evaluating their implication in the abnormal Wnt-signaling previously reported in MDS.
Methods: BM MSCs were isolated from 12 patients with lower risk MDS aged 51 to 75 years (median 67.5 years) and 12 haematologically healthy subjects aged 50 to 73 years (median 63.3 years), after informed consent. The study has been approved by the Ethics Committee of the University Hospital of Heraklion. BM MSCs were characterized according to international system for human cytogenetic nomenclature (ISCN) criteria, expanded and re-seeded for two passages (P). Total RNA was extracted from culture-expanded P2 MSCs and amplified by real-time PCR for the evaluation of decorin and galectin-3. Relative gene expression was calculated by the ΔCt method.
Results: A statistically significant decreased expression of decorin was identified in MSC of MDS patients (mean 1.338, SD 0.84) compared to the healthy individuals (mean 1.830, SD 0.71). ($P<0.05$). Galectin-3 expression was also decreased in MDS patients (mean 0.6758, SD 0.50) compared to controls (0.9395, SD 0.50), although not at a statistically significant levels.
Summary/Conclusions: MSCs from MDS patients display statistically significant decreased expression of decorin and a tendency towards decreased expression of galectin-3 in BM MSCs compared to healthy individuals. These preliminary data indicate that extracellular matrix proteins may have a role in the disturbed Wnt-pathway signaling and abnormal MSC function in MDS patients. The underlying mechanisms are currently under investigation.

PB1917

CLINICAL FEATURES, CYTOGENETIC STUDY AND OUTCOME OF ADULT MYELODYSPLASTIC SYNDROMES: REVIEW OF 101 CASES, A SINGLE CENTER EXPERIENCE IN ALGERIA

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Background: Myelodysplastic syndromes (MDS) are heterogeneous disorders defined as clonal diseases involving hematopoietic stem cells and even characterized by cytopenias, with a high risk of leukemic transformation. Morphological analysis of peripheral blood (PB) and marrow aspirates or bone marrow biopsies is the first step that ensures a diagnosis of MDS. Cytogenetic studies are important means of defining different prognostic groups and even of showing how patients are eligible for this or that treatment. We conducted the first study including conventional karyotyping and fluorescent *in situ* hybridization (FISH) for MDS in our country.
Aims: Our study was aimed to evaluate outcome of MDS regarding IPSS and IPSS-R classification in an emerging country.
Methods: Between January 2012 to December 2016, 101 patients with MDS were consecutively diagnosed. Frequent genetic abnormalities in MDS were screened by R-banding karyotype and metaphasic and interphasic FISH using a panel including six probes (5q-,7q-,20q-, del(17p13), MLL, Inv(3) t(3;3). Patients were stratified into risk groups according to IPSS and IPSS-R scores; survival probabilities were estimated using the Kaplan-Meier method.
Results: Among these 101 pts, 58 were male with a sex ratio=1, 35; range in age is from 18 years to 94 years with a median of 61, 6 years. Median hemoglobin value was 80 g/L (29-150), more than 60% of patients had severe anemia mainly in the low risk group; the median absolute neutrophil count was 3 G/L (0,060-13, 5), and the median platelet count was 144 G/L (5-659). Median bone marrow blast value was 4% (0-18). Cases were classed by cytomorphology FAB as RA (N=45), REAB (n=34), RARS (n=16), other (n=6). Classification by WHO 2008 included CRDU (n= 31 of which RA : 18, RT : 10, RN : 3), CRDM (n= 16), RAEB-1 (n=22), RAEB-2 (n= 13), RARS (n= 15), Isolated 5q- (n=4). Among 101 patients, cytogenetic abnormalities by R banding karyotype (n= 84) and FISH (n=101) were found in 41 cases (41%) distributed as single anomaly (n= 19) , double anomaly (n=5) and complex (n=17). The main cytogenetic abnormalities seen were isolated 5q deletion (n=4), isolated 7q deletion (n=2), isolated 20q deletion (n= 6), isolated trisomy 8 (n=2), 17p13 deletion (n=6), - Y (n=1), complex aberrations ≥3 (n=6) , complex aberrations ≥5 (n=6), complex aberrations ≥7 (n=5), others (n=3). IPSS was assessed in 84 patients: 27% (low risk), 44% (intermediate 1) , 24% (intermediate 2), 5% (high risk). IPSS-R was assessed in 84 patients (18% very low risk, 30% low risk, 22,5% intermediate, 15,5% High risk, 14% very High risk). Leukemic transformation into AML occurred in 33% of patients in a median time of 12 months. According to IPSS, the median OS time survival is not reached for low risk group, 41 months (m) for Intermediate 1 risk, 11 m for Intermediate 2 risk, and 4 m for High risk. According to IPSS-R, the median OS time survival is not reached for Very low risk, 43 m for low risk, 24 m for Intermediate risk, 18 m for High risk and 4 m for very high risk.
Summary/Conclusions: Our results are in agreement with those previously published regarding demographic features, distribution of recurring cytogenetic abnormalities and prediction of survival. Myelodysplasias are among the most difficult haematological diseases to treat. Treatment of low risk and high risk myelodysplasia are completely different, the last group carrying a great risk of leukemic transformation. For all these reasons, application of the new tools to classify MDS is of an major importance. This is especially true in emerging countries where few therapeutic means are available, hence the need to predict the prognosis of these diseases in order to better target treatments. To the best of our knowledge, it is the first study conducted in our country.

Myelodysplastic syndromes - Clinical

PB1918

CLINICAL EVOLUTION OF ACUTE MYELOID LEUKEMIA WITH MYELODYSPLASIA-RELATED CHANGES

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Background: Acute myeloid leukemia (AML) with myelodysplasia-related changes (MRC) is usually classified associated to worse clinical course and poor prognosis compared other AML subtypes. Differences between treatment modalities according to age, and the response to treatment, would help to provide specific anti-AML treatment in this difficult scenario.
Aims: The objetive of this study is analyze the clinical features and course of patients with AML with MRC, in order to evaluate the impact of different therapeutic regimens in this subgroup.
Methods: We report an unicentric retrospective study of 76 patients with AML with MRC, over the past ten years in a single institution in Spain. We analyzed the overall survival (OS) among the subgroup of patients with over or under 65 years, and the different types of treatment that has been offered.
Results: Median age was 69 years with a male predominance, and 66% was preceded by a known myelodysplastic syndrome with a median interval of 18 months to progress to AML. The more frequent genetic abnormalities in descending order were trisomies, del(5q), and del(7q)/-7. The patients aged >65 and <65 were 70% and 30%, respectively. The patients aged >65 received DNA hypomethylating agents (40%), anthracycline-cytarabine combinations (9%), low-dose cytarabine or hydroxyurea (17%), and supportive measures (34%). The patients aged <65 received induction chemotherapy with anthracycline-cytarabine combinations so as to continue with post-consolidation management with allogenic transplantation, but the 44% died over the induction chemotherapy (OS: 2.2 months). The OS in patients aged <65 was 20,2 months in chemotherapy plus allogenic transplantation. The OS in patients aged >65 was 10,3 months in the group of anthracycline-araC combinations, 3,81 months in the DNA hypomethylating agents group, 2,8 months in the low-dose of AraC/hydroxiurea, and 0,5 months in supportive measures group (Figure 1).

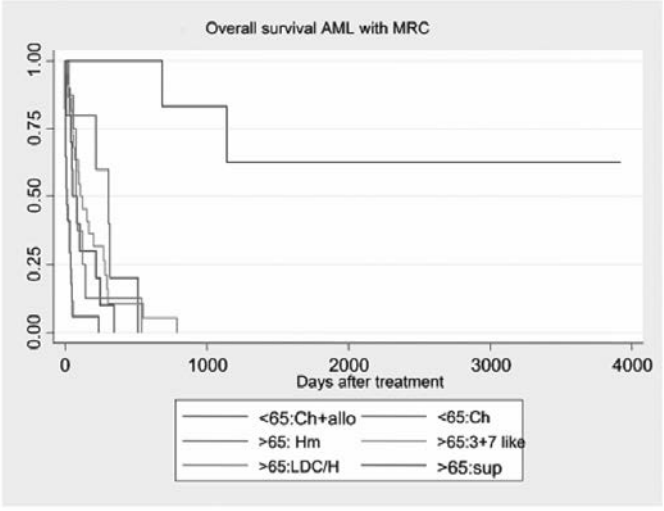

Figure 1.

Summary/Conclusions: The AML with MRC patients is a group with difficult treatment decisions and poor prognosis, in whom only the chemotherapy plus allogenic transplantation treatment manage long-term survival. In patients aged >65, there is not a significant difference among groups, although the chemotherapy with anthracycline-cytarabine seems to reach a better OS *versus* other available treatments.

PB1919

SAFETY, EFFICACY, AND PHARMACOKINETICS OF INTRAVENOUS RIGOSERTIB IN JAPANESE PATIENTS WITH RECURRENT/RELAPSED OR REFRACTORY MYELODYSPLASTIC SYNDROMES: A MULTICENTER, OPEN-LABEL, PHASE I STUDY

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Background: Rigosertib, a novel phosphoinositide 3/polo-like kinase pathway inhibitor, selectively induces the apoptosis of cancer cells and is safe and well tolerated in pts with recurrent/relapsed or refractory MDS.
Aims: We conducted a multicenter, open-label, Phase I study of intravenous rigosertib to evaluate its safety, efficacy, and pharmacokinetics and to determine the recommended dose (RD) for Japanese pts.
Methods: The key eligibility criteria were as follows: recurrent/relapsed or refractory MDS; age: 20 or older; FAB classification (RA, RARS, RAEB, RAEB-t, and CMML), excepting patients at IPSS low- or Int-1 risk with respect to RA; ECOG PS of 0 to 2; no major organ dysfunction; and written informed consent. Rigosertib (1,200 and 1,800 mg daily) was administered intravenously for 72 h, followed by 11-day monitoring in one 14-day cycle. The primary endpoint was dose-limiting toxicity (DLT). The secondary endpoints were 1) safety as assessed with adverse events (AEs) and laboratory results; 2) efficacy as assessed with the International Working Group 2006 criteria; and 3) pharmacokinetics.
Results: Between June 2012 and February 2015, 7 male and 2 female pts (median age: 70; range: 63-84) were enrolled, and 3 and 6 pts were eventually assigned to the 1,200 and 1,800 mg arms, respectively. According to the FAB classification, 6, 2, and 1 pts were categorized to RAEB, RAEB-t, and RA, respectively. There were 3 pts each in the IPSS Int-1, Int-2, and high-risk groups, with 1 and 2 pts in each risk group in the 1,200 and 1,800 mg arms, respectively. The median numbers of delivered cycles in the 1,200 and 1,800 mg arms were 4 (2 to 4) and 2 (1 to 8), respectively. DLT occurred not in the 1,200 mg arm but in the 1,800 mg arm: 5 episodes of ≥grade 3 non-hematologic toxicities in 2 pts. One pt developed 2 episodes of sepsis and meningitis, and the other 3 episodes of hypochloremia, pustular rash, and hyponatremia. Thus, 2 among 6 pts in the 1,800 mg arm developed DLT, which led us to conclude that 1,800 mg/day is the RD for Japanese pts. No deaths occurred during the study period. However, 5 pts died during follow-up, 4 of whom died from primary disease progression. Furthermore, 1 pt died of grade 5 bacterial pneumonitis that was rated to "Unrelated". In the 1,200 mg arm, 2 cases each of grade 3/4 thrombocytopenia, grade 4 neutropenia, and grade 3/4 leukopenia, as well as 1 case of grade 3 lymphopenia developed. In the 1,800 mg arm, 3 cases of grade 3/4 leukopenia, 2 cases each of grade 3 CD4 lymphopenia, grade 4 thrombocytopenia, and grade 3/4 neutropenia, as well as 1 case each of grade 4 lymphopenia, increased C-reactive protein, erythropenia, and hypochloremia developed. Three cases of SAEs, including grade 4 meningitis, grade 4 sepsis, and grade 3 catheter-related infection, developed in the 1,800 mg arm. Stable disease was obtained in 2 pts in the 1,800 mg arm. Hematological remission, hematological improvement, and cytogenetic response were not obtained in the two arms. The C_{max} values in the 1,200 and 1,800 mg arms were 5.99±1.50 and 6.74±2.39 μg/mL, respectively. The $AUC_{0-\infty}$ values were 314.6±142.7 and 324.8±83.9 μg × hr/mL, respectively.
Summary/Conclusions: This Phase I study showed that intravenous rigosertib (1,800 mg daily) for consecutive 72 h was well tolerated, indicating that this is the RD for Japanese pts with MDS similar to a Phase III study in the U.S. Based on these clinical outcomes, Japanese pts with MDS are participating in a global randomized Phase III study to compare rigosertib with physicians' choice of treatment.

PB1920

IRON CHELATION THERAPY IMPROVES HAEMATOLOGICAL RESPONSE IN HIGH-RISK MYELODYSPLASTIC PATIENTS TREATED WITH AZACITIDINE

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Background: The goals of treating older patients with Myelodysplastic Syndrome (MDS) are different than for younger patients. Few elderly patients are able to pursue an allogeneic stem cell transplant. Azacitidine (AZA) improves long-term outcomes of higher-risk MDS patients and is now the reference front-line therapy of higher-risk MDS not eligible for allogeneic stem cell transplant. Anaemia is the most common symptom of MDS and most patients become transfusion-dependent with the risk of iron overload. Deferasirox is an orally available iron chelator administered once-daily in transfusion-dependent patients with various chronic anaemias. Its efficacy has been established in controlled clinical trials.
Aims: We report our experience on using the azacitidine in patients with high-risk MDS, evaluating the efficacy and safety. Concomitant treatment with deferasirox was performed in a routine clinical setting following Consensus Guidelines on Iron Chelation Therapy.
Methods: In our Institution from October 2009 to January 2017 we have

treated 32 elderly patients (19 male and 13 female, median age 76 years, r. 71-88) affected by HIGH-RISK MDS (IPSS INT-2/HIGH). Patients received subcutaneous azacitidine at 75mg/m(2) daily for 7 days every 4 weeks. All patients completed at least 6 cycles of therapy. 12/30 (40%) patients underwent more than 8 cycles of therapy. 18/30 patients underwent as well iron chelation therapy with deferasirox receiving a starting dosage of 10 mg/kg/day, subsequently titrated according to serum ferritin (SF) measured monthly.
Results: Complete response (CR), partial response (PR), and hematologic improvement (HI) were observed in 2 (7%), 5 (17%), and 12 (40%) patients, respectively. The median number of cycles to clinical response was 4 (range 4-8). The 2-year rate of acute myeloid leukemia-free survival was 48%. Five serious adverse events occurred in five patients with one fatal outcome. 16 out of 18 patients who showed any hematologic response (CR+PR+HI) meeting International Working Group 2006 criteria had also performed deferasirox therapy. No increased toxicity was noted when deferasirox was used concomitantly with azacitidine.
Summary/Conclusions: Our results confirm the effectiveness of the therapy with azacitidine in HIGH-RISK MDS elderly patients with acceptable toxicity profile. Peripheral cytopenias were the most commonly occurring adverse event, with gastrointestinal adverse events and injection-site reactions among the most commonly occurring non-haematological adverse events. In conclusion, azacitidine is an important agent for use in the treatment of elderly patients with MDS. Furthermore concurrent use of deferasirox in patients with iron overload seems to significantly improve the hematologic response by reducing transfusion requirement.

PB1921

EXPLORING THE RISK OF RED CELL ALLOIMMUNIZATION IN MYELODYSPLASTIC SYNDROMES. TO WHAT EXTEND COULD CYTOGENETIC ANALYSIS AT DIAGNOSIS PREDICT THIS RISK?
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Background: Red cell alloimmunization poses a huge burden for the blood transfusion services as it may be associated with crossmatching difficulties, haemolytic transfusion reactions and potentially severe clinical consequences for the transfused patient. Collectively, alloimmunization appears to be higher in patients with myelodysplasia (MDS) and chronic myelomonocytic leukaemia (CMML) with a rate somewhat around 15%. Identification of patients at risk of developing alloantibodies would be of clinical significance as antigen negative red cells could be crossmatched in advance for use in clinical practice. Largely, studies have failed to predict this cohort of patients and little is known regarding identifiable risk factors.
Aims: To this end, we focused on exploring the cytogenetic profile from patients with MDS and CMML along with demographic characteristics as risk factors for alloimmunization.
Methods: A retrospective analysis was performed in 360 transfused patients with MDS (74.4%) and CMML (25.6%) registered in our local database between 1980 and 2016. Prognostic variables (age, sex, disease subtype) were assessed using a multivariate prediction model in SPSS statistical software. Cytogenetics at diagnosis were available in 228 of the above patients and univariate analysis was performed separately.
Results: The mean age at diagnosis was 73 years (range 20-95) with 58.3% male patients. Overall, 45 patients (12.5%) formed 76 antibodies [68 alloantibodies, 8 autoantibodies] with 42% of them developing more than 1 antibody. 5 additional patients developed autoantibodies without alloantibodies. Alloantibody specificities were as follows: E (22 cases), C (8), K (7), Cw/ Jka/ Kpa (5 cases each), Lua (4), e/ Fya (3 cases each), M (2), c/ D/ Chido/ Bga (1 case each). Collectively, alloantibodies against the Rh and Kell systems encountered in 69% of this cohort. 6 out of 8 patients with anti-C had also developed a second antibody. In a regression model, none of the following variables reached statistical significant level as predictors for immunization; age (p=0.59), sex (p=0.07), MDS WHO subtype (p=1.0). 228 patients had known cytogenetics at diagnosis. Normal profile (46, XY or 46, XX) was encountered in 58.8%. Similarly, univariate analysis of this cohort (normal *versus* abnormal cytogenetics) showed odds ratio 1.1 with no statistical significant point (p=0.64). Further subgroup analysis was performed to explore whether the risk was increased in patients with poor or very poor cytogenetics as per IPSS-R. Descriptive statistics showed; very good/ good risk cytogenetics 69.7%, intermediate 12.7% and poor/ very poor 17.5%. Logistic regression analysis revealed no association between cytogenetic groups and risk of alloimmunization (p=0.89, p=0.96 and p=0.84 respectively).
Summary/Conclusions: The rate of alloimmunization in our cohort of patients was 12.5%, slightly lower compared to published studies. The most common alloantibody found was anti-E. Prognostic variables included in analysis (age, sex, MDS type but also cytogenetic profile) are not significant predictors of alloimmunization and further studies are needed to investigate other possible risk factors. Prophylactic Rh and Kell antigen matched cells, when possible, would be a reasonable strategy until further knowledge is acquired.

PB1922

PROGNOSTIC MARKERS THAT PREDICT THE OUTCOME OF REDUCED INTENSITY CONDITIONING TRANSPLANT IN ADULT PATIENTS WITH MYELODYSPLASTIC SYNDROMES: A SINGLE CENTER EXPERIENCE
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Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of hematologic diseases, characterized by a clonal abnormality of hematopoietic stem cells. The incidence of MDS is age-dependent. The treatment approach is to categorize patients into lower or higher risk MDS and to select a suitable treatment accordingly. HCT offers potentially curative therapy for patients with MDS. A reduced intensity conditioning (RIC) regimen was used to reduce the toxicities associated with transplant procedure. The main concept of RIC relay upon adoptive immunotherapy especially in the low risk patients allowing the graft *versus* leukemia to occur.
Aims: This study aimed to investigate the outcome of allogeneic peripheral blood stem cell transplantation after reduced intensity conditioning regimen for adult patients with MDS, the effect of different prognostic factors on outcome and the effect of chronic GVHD according to IPSS risk.
Methods: A retrospectively study analyzed the fifty-one patients with MDS who underwent transplantation at the BMT unit at Nasser Institute during a period of 10 years, by the RIC regimen from HLA identical donor using peripheral blood stem cell. Outcomes analyzed the incidence of acute and chronic GVHD, disease free survival (DFS) & overall survival (OS).
Results: They were 31 males (60.8%) and 20 females (39.2%). Their ages ranged from 17 to 60 years, with mean age±SD of 34.5±10.1 years, including refractory cytopenia with multiple dysplasia (RCMD) in 14 patients (27.5%), MDS –U in 13 patients (25.5%), refractory anemia (RA) in 12 patients (23.5%), refractory anemia with excess blasts II (RAEB II) in 6 patients (11.8%) and MDS with hypocellular bone marrow in 4 patients (7.8%) and refractory anemia ring sideroblasts (RARS) in 2 patients (2%). According to IPSS classification, 11 patients (21.6%) were low risk, 28 patients (54.9%) were intermediate-I risk group, and 9 patients (17.5%) were intermediate-II & 3 patients (5%) were high risk group. The incidence of acute and chronic GVHD were 51.1% and 28.6% respectively. The 5- year estimate for overall survival of the whole group was 21.8%. In univariate analysis, covariates associated with a better OS were recipient age <40 years (p=0.02) and the presence of cGVHD (p=.002). On multivariate analysis the presence of cGVHD is significant predictor of survival (p=0.04). Also cGVHD significantly improve the OS for low and high risk MDS group (p= 0.02 and 0.03 respectively). While presence of acute GVHD, IPSS & interval between diagnosis and transplant weren't significantly affect OS (p>0.05). The 5- year estimate for DFS of the whole group was 28.6%. On multivariate analysis the presence of cGVHD significantly reduce relapse (p=0.029).
Summary/Conclusions: The presence of cGVHD significantly improved OS and reduced the risk of relapse in patients with MDS. We also found that the presence of cGVHD significantly improved OS especially in high-risk patients group, which suggests that the GVL effect may be beneficial in high-risk patients who do not receive intensive preparative regimens.

PB1923

MANAGEMENT OF MYELODYSPLASTIC SYNDROMES WITH ERYTHROPOIESIS STIMULATING AGENTS IN REAL-LIFE EXPERIENCE: AN UPDATE FROM RECAMDS
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Background: Erythropoiesis stimulating agents (ESAs) are the frontline treatment in low-risk anemic MDS patients and an employment of this therapy in the earlier stage of the disease can delay the need for RBC transfusion, hypothetically by slowing the disease course. It's matter of debate whether the clinical response is a result of proliferation and maturation of the dysplastic clone or stimulation of residual normal erythropoiesis by ESAs.
Aims: Macrocytosis is one of the cytological hallmarks of dyserithropoiesis in MDS: an analysis of the erythropoietic response to ESAs therapy in a cohort of anemic non trasfusion-dependent MDS patients, enrolled in a retrospective register, RECAMDS, subgroup of Italian register, was performed.
Methods: 183 patients, treated with standard-dose ESAs, have been retrospectively analyzed (Table 1). Data analysis was performed, according to IWG 2006 criteria, at the baseline, after 3 and 6 months of continuous treatment, with a subanalysis of the patients according to WHO and R-IPSS risk stratification. ESAs were started at mean Hb concentration of 9.31 g/dl, mean serum EPO concentration: 51 mU/L, after a mean time from diagnosis of 6 months (r.1-118).
Results: Overall response rate(ORR) was 83.6% (153/183), no difference among WHO and IPSS subgroups was found: 132/183 (72.1%) achieved response after 3 months of treatment, while other 21/183 (11.2%) after 6 months. 19 patients with stable disease (non-responders, according to IWG

criteria), in which treatment was continued, achieved response after 9 months. In the macrocytic-responders group 83.2% exhibits again macrocytosis after 3 months, while 16.8% become normocytic. In the normocytic-responders group 89.8% exhibits again normocytosis, while 10.2% become macrocytic: in these patients, after 3 months, there was a contemporary worsening in neutropenia and thrombocytopenia, with transfusion-dependence, regarded as first signs of progression of disease. Non-responders were 30/183 (16.3%): in the macrocytic non-responders group 89% exhibit again macrocytosis after 3 months, while 11% become normocytic; in the normocytic group 76% exhibits again macrocytosis, while 24% become normocytic.

Table 1.

MDS PATIENTS	183
M	89 (49%)
F	94 (51%)
ERYTHROPOIESIS	
BASELINE HB (mean, g/dL)	9.31 g/dL (r. 7.1-11.3)
BASELINE SERUM EPO (mean, mU/mL)	51 mU/mL (r.3-84)
OVERALL RESPONSE RATIO	
RESPONDERS	153/183 (83.6%)
RESPONDERS AT 3 MONTHS	132/183 (72.1%)
RESPONDERS AT 6 MONTHS	21/183 (11.4%)
RESPONDERS AT 9 MONTHS (NON RESPONDERS IN IWG 2006)	19/183 (10.3%)
NON RESPONDERS	30/183 (16.3%)

Summary/Conclusions: These preliminary data can suggest that, in the majority of MDS patients responsive to ESAs, the increase of Hb concentration occurs mainly stimulating erythroid production in MDS clones; in the minority of patients probably it happens recruiting residual polyclonal erythropoiesis. It is interesting to note that stimulating effects of ESAs last even when the expression of dysplasia progresses.

PB1924

CHARACTERIZATION OF MYELODYSPLASTIC SYNDROMES WITH TRANSFORMATION TO ACUTE LYMPHOBLASTIC LEUKAEMIA

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Background: Myelodysplastic syndromes are heterogeneous diseases with variable probability of developing a transformation to acute leukaemia. The vast majority of these cases present a transformation to acute myeloid leukaemia. We here describe a series of 4 cases of MDS/CMML with evolution to acute lymphoblastic leukaemia. These events are very rare and are to date only published as single cases.
Aims: The aim of these study is to better define cases of MDS transforming to ALL.
Methods: We describe 4 cases of patients suffering from MDS who in the course of their disease presented with ALL. Three of these cases presented in 1 centre, 1 in the other, all cases were documented in a 17-year time span.
We than performed a literature research including at the moment 37 cases of MDS transforming to ALL described as case reports.
Results: Subtypes of MDS are varying from low risk MDS with deletion (5q) (del(5q)) to refractory anaemia with excess of blasts in transformation (RAEB-T), classified as AML in newer WHO classifications (2008 and 2016) and CMML, classified as MDS/MPS nowadays. Even if MDS subgroups are manifold, cytogenetic results are less so. Two of the 4 patients described demonstrated KMT2A rearrangements, 1 already at MDS presentation, the other at ALL presentation. One patient presented with del(5q). Of the 37 cases we identified in the literature, 7 presented with del(5q) and 2 showed with anomalies of the 11q23 locus.
Summary/Conclusions: KMT2A is known to be a gene involved in myeloid neoplasms as well as in acute lymphoblastic leukaemia. In a small series of cases like this one, it is not excluded that ALL following MDS is only by chance and "bad luck", but at least in the patient showing the same translocation at MDS presentation and at ALL presentation, both diseases seem to be related. MLL as a cytogenetic event enabling the disease a switch from one phenotype (myeloid) to the other (lymphoblastic) could be a possible explanation for this phenomenon. KMT2A rearrangement in MDS is an extremely rare event, but could explain part of these rare changelings of MDS transforming to ALL. Further studies are needed to confirm this hypothesis, and molecular examination is needed to characterise the event enabling a myeloid phenotype to switch to a lymphoblastic one. The reason why del(5q) seems to be present in a high proportion of MDS patients transforming to ALL is not clear, further studies need to be performed.

PB1925

IMMUNOSUPPRESSIVE THERAPY AS FIRST-LINE TREATMENT OF PATIENTS WITH PRIMARY MDS

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Background: Myelodysplastic syndromes (MDS) are included into a heterogeneous group of clonal blood diseases characterized by peripheral cytopenias, dysplastic features of hematopoietic precursors, progressive deterioration and a high risk of transformation into leukemia. MDS occur in several versions that differ in frequency of appearance, the duration of the course and the probability of transformation into acute leukemia. The choice of therapy for a particular patient is determined by the morphological variant of the disease, the prognostic group, age and comorbidity. In hypoplastic cases of MDS are often used immunosuppressive therapy.
Aims: Analysis of the effectiveness of immunosuppressive therapy in patients with primary MDS
Methods: The research included 19 patients with primary MDS from 22 to 58 years (median age 46 years, 11 male, 8 female). The diagnosis was made according to the criteria of the WHO classification of myeloid neoplasms in 2008. The materials were taken only after signing by patients informed consent form to participate in the research. The calculations are performed in the R version 3.1.3 statistical package.
Results: There were patients with defined MDS subtypes: RA in 52,6%, RCMD in 31,6 and RAEB in 15,8%. Hypoplastic form of MDS were diagnosed in 63,2% patients. The increased number of lymphocytes in the bone marrow of patients were 52,6%, accumulation of lymphocytes in the bone marrow biopsy – in 36,8%. Cytogenetic abnormalities were found in 21% of patients (in 5,3% complex and in 15,7% isolated). All patients used immunosuppressive therapy as a first-line treatment: Antithymocyte globulin and Cyclosporine A (CsA) in 15,8%, monotherapy with CsA in 84,2%. CsA therapy started at a dose of 5 mg/kg per day. Dose correction performed depending on the concentration of CsA in the serum and toxicity. Median treatment was 143 days (36…1253 days). The response rate to CsA treatment was considered a complete remission (normalization of blood and bone marrow), partial remission (improvement of blood counts for more than 50% and no dependence on transfusions of blood components) or improvement (reduction in transfusion requirements by 50% or more). Complete remission was achieved in 10,5% of patients (only variant RA). Partial remission was obtained in 31,6% (variants RA and RCMD) and improvement in 36,8% (variants RA, RCMD and RAEB). There was no response to treatment in 21,1% of patients (variants RCMD and RAEB). Positive effect on immunosuppressive therapy significantly more likely achieved in patients with hypoplastic forms MDS (57,9%) and the presence of clusters of lymphocytes in the bone marrow biopsies (36,8%). Dependence of treatment efficiency and cytogenetic abnormalities not detected.
Summary/Conclusions: The effectiveness of immunosuppressive therapy in MDS associated with a variant of the disease, bone marrow cellularity and the bone marrow lymphoid infiltration. The greatest effect of the immunosuppressive therapy can be expected in patients with hypoplastic MDS and accumulation of lymphocytes in the bone marrow biopsy.

PB1926

VITAMIN D IS ASSOCIATED WITH SEVERITY OF DISEASE AS EXPRESSED BY SUBDIAGNOSIS AND IPSS-R IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES AND RELATED DISEASES

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Background: Recent findings indicate that vitamin D (VD) might impact hypomethylating therapy of myelodysplastic syndromes (MDS). Epigenetic activity of VD is mainly mediated through interaction with its nuclear receptor (VDR). Activated VDR binds to specific genomic sequences (VD response elements) which influence gene transcription by histone modification, mainly acetylation but also demethylation. Among genes affected by VD/VDR is BGLAP encoding for the non-collagenous protein osteocalcin (OCN) produced by osteoblasts and implicated in osteogenesis. Furthermore, it has been shown that OCN is expressed by activated hematopoietic stem cells in hematological malignancies.
Aims: We initiated an exploratory study, collecting patients' data on serum VD, and osteocalcin (OCN)-levels in 59 unselected patients with MDS, MDS/myeloproliferative neoplasm (MPN) and secondary acute myeloid leukemia (sAML).
Methods: Serum VD levels were assessed by measuring 25-hydroxyvitamin D (25(OH)D), the biochemical indicator of VD status. Analysis was done by chemiluminescence immunoassay. Intact OCN is unstable due to protease cleavage between amino acids 43 and 44. The N-MID-fragment, resulting from cleavage, is considerably more stable. Measurement of both intact OCN and the stable N-MID-fragment was effectuated by electrochemiluminescence immunoassay.
Results: We found median serum 25(OH)D levels (normal range 30-100 ng/ml) of 16 ng/ml (RA, RARS, n=35), 23 ng/ml (RAEB-1/2, sAML, n=16), and 20 ng/ml (MDS/MPN, n=8) (p=0.273).When classified by IPSS-R, median serum 25(OH)D levels were 18 ng/ml in "(very) low" (n=20), 16.5 ng/ml in

"intermediate" (n=14), and 29.5 ng/ml in "(very) high" (n=6) with p=0.102.Regarding cytogenetic risk classification median serum 25(OH)D levels were 18 ng/ml in "(very) good" (n=48), 19 ng/ml in "intermediate" (n=8), and 18.5 ng/ml in "(very) poor" (n=4) cytogenetic risk patients (p=0.738).Median serum OCN levels (normal range 11-46 ng/ml) were 19 ng/ml (RA, RARS, n=33) and 16.2 ng/ml (higher-risk MDS/sAML, n=16), (p=0.136). IPSS-R risk classification resulted in median serum OCN levels of 17.4 ng/ml in IPSS-R "(very) low" (n=17), 16.2 ng/ml in "intermediate" (n=15), and 21.7 ng/ml in "(very) high" (n=6), (p=0.701). Cytogenetic risk classification had no impact on median serum OCN levels (p=0.271). We found median serum 25(OH)D levels (normal range 30-100 ng/ml) of 16 ng/ml (RA, RARS, n=35), 23 ng/ml (RAEB-1/2, sAML, n=16), and 20 ng/ml (MDS/MPN, n=8) (p=0.273). When classified by IPSS-R, median serum 25(OH)D levels were 18 ng/ml in "(very) low" (n=20), 16.5 ng/ml in "intermediate" (n=14), and 29.5 ng/ml in "(very) high" (n=6) with p=0.102. Regarding cytogenetic risk classification median serum 25(OH)D levels were 18 ng/ml in "(very) good" (n=48), 19 ng/ml in "intermediate" (n=8), and 18.5 ng/ml in "(very) poor" (n=4) cytogenetic risk patients (p=0.738). Median serum OCN levels (normal range 11-46 ng/ml) were 19 ng/ml (RA, RARS, n=33) and 16.2 ng/ml (higher-risk MDS/sAML, n=16), (p=0.136). IPSS-R risk classification resulted in median serum OCN levels of 17.4 ng/ml in IPSS-R "(very) low" (n=17), 16.2 ng/ml in "intermediate" (n=15), and 21.7 ng/ml in "(very) high" (n=6), (p=0.701). Cytogenetic risk classification had no impact on median serum OCN levels (p=0.271).

Summary/Conclusions: In summary, our cohort of patients with MDS, MDS/MPN and sAML show clearly decreased serum VD levels. The preliminary results suggest a tendency of serum VD levels to increase with higher risk MDS/sAML which is supported by positive Kendall's tau (0.210). Serum OCN levels lie below normal limits, but seem not to be affected by disease risk. These results suggest specific hypotheses regarding the pathomechanism that shall be investigated on an enlarged data set, which we are continuously collecting.

PB1927

JUVENILE MYELOMONOCYTIC LEUKEMIA IN TURKEY: A RETROSPECTIVE ANALYSIS OF 65 PATIENTS

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Background: Juvenile myelomonocytic leukemia (JMML) is a chronic malignant myeloproliferative disease of early childhood
Aims: To define the status of juvenile myelomonocytic leukemia (JMML) patients in Turkey, in terms of time of diagnosis, clinical characteristics, mutational studies, clinical course and treatment strategies.
Methods: Data including clinical and labarotory characteristics and treatment strategies of JMML patients were collected retrospectively from pediatric hematology-oncology centers in Turkey.
Results: Sixty-five children with JMML diagnosed between 2002 and 2016 in 18 institutions throughout Turkey were enrolled into the study. The median age at diagnosis was 17 months (range, 2-117 months). Splenomegaly was present in 92% of patients at the time of diagnosis. The median WBC, monocyte and platelet counts were, $32.9x10^9$/L, $5.4x10^9$/L and $58.3x10^9$/L, respectively. Monosomy 7 was present in 18% of patients. JMML mutational analysis was performed in 32 out of 65 patients (49%), *PTPN11* was the most common mutation. Hematopoetic stem cell transplantation (HSCT) could only be performed in 28 (44%) patients, majority of being after the year 2012. The most frequent reason for not performing HSCT was the inability to find a suitable donor. The median time from diagnosis to HSCT was 9 months (range,2-63 months). The 5-year cumulative survival rate was 33% and median estimated survival time was 30±17.4 months (95% CI:0-64.1) for all patients. Survival time was significantly better in the HSCT group (log-rank p=0.019). Older age at diagnosis (>2 years), platelets less than $40x10^9$/L and *PTPN11* mutation were the factors significantly associated with shorter survival time.
Summary/Conclusions: Although there has recently been improvement in terms of definitive diagnosis and HSCT in JMML patients, the overall results are not satisfactory and it is necessary to put more effort into this issue in our country.

PB1928

THE PRECURSOR B CELLS AS A PROGNOSIS FACTOR IN MYELODYSPLASTIC SYNDROMES

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Background: Recently, an immunosuppressive enviroment with low number of precursor B cells at the bone marrow has been related with poor survival in patients with very low/low and intermediate risk myelodysplastic syndrome (MS), but this negative impact is unclear yet.
Aims: The objetive of this study is to establish if there is an negative association between the percentage of precursor B cells (%PBC) at the time of diagnosis of MS and progression-free survival.
Methods: We analyzed 48 patients with IPSS-R very low/low risk (VL/L) and 34 patients with intermediate risk (INT) in the past 10 years in a single institution in Spain. We reviewed the%PBC CD34+ (CD34+CD10+ or CD34+CD117-) over total marrow cells at diagnosis measured by flow cytometry, and we calculated the time of progression-free survival (PFS) defined as the time between inclusion until progression to refractory anemia with excess blasts type 2 (RAEB-2) or acute myeloid leukemia (AML). The Competing risks regression test was used to assess the predictive value of PBC in relationship to PFS.
Results: Median age in both groups was 69 years, and median of progression to RAEB-2 or AML was 1,96 years in VL/L group and 0,64 years in INT group. The%PBC was not a predictor of PFS in VL/L group with a sub-hazard ratio (SHR) of 0,23 (95% CI: 0,003-13,96, P=0,485) neither in the INT group with a SHR 0,14 (95%CI: 0,001-4,52, P=0,211). We also performed a median split analysis fo the%PBC with a median value of 0,1% in both groups. In the VL/L group, patients with a%PBC above the median had a median PFS of 2,48 years *versus* 1,99 years for the patients with%PBC below the median. In the INT group, patients with a%PBC above the median had a median PFS of 1,14 years *versus* 0,83 years for the patients with%PBC below the median (Figure 1).

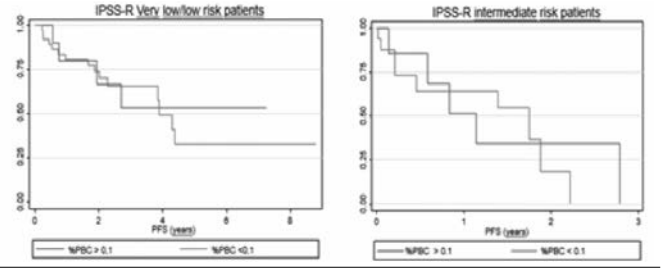

Figure 1.

Summary/Conclusions: Our results not provide evidence in order to establish a prognostic value between%PBC at diagnosis in IPSS-R very low, low or intermediate MS.

PB1929

TO INFINITY AND BEYOND: NGS IN MDS

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Background: Myelodysplastic syndrome (MDS) constitutes a heterogeneous group of hematopoietic stem cell disorders, characterized by peripheral blood cytopenias in the presence of a dysplastic and hypercellular bone marrow. This biological heterogeneity is reflected in the clinical course, ranging from an indolent disease to entities with high risk of progression to AML and dismal prognosis. Genetic and epigenetic abnormalities are at the core of myeloid neoplasias development and despite the degree of dysplasia and blast percentages still being the main features for the WHO classification, a large amount of data has recently become available on recurring mutations in MDS, mainly due to massive parallel sequencing techniques.
Aims: Our aim was to search for genetic mutations in a cohort of patients with MDS.
Methods: We studied a total of 33 patients diagnosed with *de* novo MDS (WHO 2008 classification), using a Next Generation Sequencing panel comprising 45 myeloid genes.
Results: Patients were 15 male and 18 female, with a median age at diagnosis of 76 years (52 – 93 years). The MDS subtypes distribution was 16 patients (48,5%) with RCMD, 4 patients with RARS, 4 with RAEB -1 and 4 with RAEB-2 (12,1% for each subtype), 3 patients (9,1%) with 5q-Syndrome and 2 patients (6,1%) with RCUD. These patients were stratified according to the IPSS as Low-risk (24,2%), Int-1 (33,3%) and Int-2 (18,2%), without any high-risk

patients. All patients required erythropoiesis stimulating agents and 9 patients received treatment with azacytidine (AZA), including all the Int-2 patients and 3 lower risk patients who progressed to a higher risk MDS. Estimated cumulative survival at 46 months was 67% with a median OS not reached and median follow-up time of 34 months. Patients receiving AZA revealed a trend towards survival benefit (mean survival 54,2 *vs* 50 months), independent of IPSS and R-IPSS, but not statistically significant. Mutational analysis revealed that 75,8% of patients had at least one gene mutation and it was most frequently related to DNA methylation genes (n=14), particularly in TET2 (n=7 patients) and DNMT3A (n=6 patients, 7 different mutations) genes. We found a statistically significant difference between mutations in these genes and lower absolute neutrophil counts (mean 2,42±0,47 G/L *vs* 1,33±0,18 G/L; p=0,42). The second most frequently mutated genes were related to signal transduction pathways (n=11; JAK1, JAK2, NRAS, CBL, GATA2, SH2B3, CSFR). Patients with these mutations had significantly lower serum EPO levels (p <0,001; median 32,35 *vs* 42,70 U/L). Furthermore, patients with such mutations demonstrated a clear disadvantage in survival analysis, with a median OS of 19 months *vs* not reached in patients without mutations (p<0,001), being these results independent of the IPSS and R-IPSS risk groups. We were also able to identify a trend towards worst survival in patients with previously described high risk mutations (TP53, EZH2, ASXL1, RUNX1 and ETV6 genes).

Summary/Conclusions: We conclude that the most frequently detected mutations were related to DNA methylation genes, as described in the literature, which was independent of the IPSS risk group, being observed in both low-risk and high-risk patients. These results raise the question whether hypomethylating agents may also be of benefit for lower-risk patients.

We also observed a high frequency of mutations in signal transduction pathways which was related to a clear survival disadvantage across all risk groups of the IPSS and R-IPSS. This unveils the question whether we may be facing a shift towards the molecular level in MDS risk stratification and if therapies targeted to such molecules may improve the outcome of these patients.

PB1930

CLINICAL FEATURES, CYTOGENETIC STUDY AND OUTCOME OF ADULT MYELODYSPLASTIC SYNDROMES: REVIEW OF 101 CASES, A SINGLE CENTER EXPERIENCE IN ALGERIA.

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Background: Myelodysplastic syndromes (MDS) are heterogeneous disorders defined as clonal diseases involving hematopoietic stem cells and even characterized by cytopenias, with a high risk of leukemic transformation. Morphological analysis of peripheral blood (PB) and marrow aspirates or bone marrow biopsies is the first step that ensures a diagnosis of MDS. Cytogenetic studies are important means of defining different prognostic groups and even of showing how patients are eligible for this or that treatment. We conducted the first study including conventional karyotyping and fluorescent *in situ* hybridization (FISH) for MDS in our country.

Aims: Our study was aimed to evaluate outcome of MDS regarding IPSS and IPSS-R classification in an emerging country.

Methods: Between January 2012 to December 2016, 101 patients with MDS were consecutively diagnosed. Frequent genetic abnormalities in MDS were screened by R-banding karyotype , metaphase and interphase FISH using a panel including six probes (5q-,7q-,20q-, del(17p13), MLL, Inv(3) t(3;3). Patients were stratified into risk groups according to IPSS and IPSS-R scores; survival probabilities were estimated using the Kaplan-Meier method.

Results: Among these 101 pts, 58 were male with a sex ratio=1, 35; range in age is from 18 years to 94 years with a median of 61, 6 years. Median hemoglobin value was 80 g/L (29-150), more than 60% of patients had severe anemia mainly in the low risk group; the median absolute neutrophil count was 3 G/L (0,060-13, 5), and the median platelet count was 144 G/L (5-659). Median bone marrow blast value was 4% (0-18). Cases were classed by cytomorphology FAB as RA (N=45), REAB (n=34), RARS (n=16), other (n=6). Classification by WHO 2008 included RCUD (n= 31 of which RA: 18, RT : 10, RN : 3), RCMD (n= 16), RAEB-1 (n=22), RAEB-2 (n= 13), RARS (n= 15), Isolated 5q- (n=4). Among 101 patients, cytogenetic abnormalities by R banding karyotype (n= 84) and FISH (n=101) were found in 41 cases (41%) distributed as single anomaly (n=19), double anomaly (n=5) and complex (n=17). The main cytogenetic abnormalities seen were isolated 5q deletion (n=4), isolated 7q deletion (n=2), isolated 20q deletion (n= 6), isolated trisomy 8 (n=2), 17p13 deletion (n=6), - Y (n=1), complex aberrations ≥3 (n=6), complex aberrations ≥5 (n=6), complex aberrations ≥7 (n=5), others (n=3). IPSS was assessed in 84 patients: 27% (low risk), 44% (intermediate 1) , 24% (intermediate 2), 5% (high risk). IPSS-R was assessed in 84 patients (18% very low risk, 30% low risk, 22,5% intermediate, 15,5% High risk, 14% very High risk). Leukemic transformation into AML occurred in 33% of patients in a median time of 12 months. According to IPSS, the median OS time survival is not reached for low risk group, 41 months (m) for Intermediate 1 risk, 11 m for Intermediate 2 risk, and 4 m for High risk. According to IPSS-R, the median OS time survival is not reached for Very low risk, 43 m for low risk, 24 m for Intermediate risk, 18 m for High risk and 4 m for very high risk.

Summary/Conclusions: Our results are in agreement with those previously published regarding demographic features, distribution of recurring cytogenetic abnormalities and prediction of survival. Myelodysplasias are among the most difficult hematological diseases to treat. Treatment of low risk and high risk myelodysplasia are completely different, the last group carrying a great risk of leukemic transformation. For all these reasons, application of the new tools to classify MDS is of a major importance. This is especially true in emerging countries where few therapeutic means are available, hence the need to predict the prognosis of these diseases in order to better target treatments. To the best of our knowledge, it is the first study conducted in our country.

PB1931

IS PRE-TRANSPLANT THERAPY A KEY FACTOR IN INFLUENCING POST TRANSPLANTATION RELAPSE INCIDENCE IN EXCESS BLAST MYELODYSPLASTIC SYNDROMES? A SINGLE CENTRE EXPERIENCE

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Background: The importance of pre-transplant disease burden in myelodysplastic sindromes (MDS) as a factor influencing post hematopoietic cell transplantation (HCT) outcome is an important argument of debate. It has been reported that relapse rate (RR) after transplant is reduced in patients entering HCT with a blast cell count <5%. However, the effect of a pre-transplant debulking therapy in reducing RR has not been clearly demonstrated.

Aims: Here we review our data to evaluate if the intensity of pre-transplant therapy may have influenced post transplant RR.

Methods: In our Institute, we treat all patients with a blast cell count of 10% or higher with a debulking therapy pre-transplant. This is usually an AML-like, cytarabine and anthracycline based, intensive chemotherapy (I.C.). In selected cases fludarabine and cytarabine containing regimens are also used. In the last ten years, in the context of a clinical trial, a series of patients have received a less intensive, hypometilating therapy (repeated courses of 5-azacytidine 75 mg/m^{2}subcutaneously for 7 days), as bridge to transplant. Conditioning regimen used in MDS patients is busulfan based in younger patients (Bu-Flu, BU-Cy); in the elderly or less fit patients a RIC regimen (thiotepa 5 mg/kg e.v., fludarabine mg/m^{2}x 3 and L-PAM 100 mg/m^2) is administered.

Results: In the last ten years we performed 14 HCT (between June 2008 and september 2016) in patients with MDS with excess blasts. Median patients age was 63,5 years (range: 49-69), male/female ratio was 9/5. According to IPSS, 12 out of 14 patients were high/ int-2 (2 int-1), 11/14 had >10% blast cells (EB-2). According to our centre protocol, we treated 11 patients with EB-2 and 1 patient with EB-1 (with hypercellular bone marrow) with a debulking therapy. This was I.C. in 6 patients and 5-AZA in 6 patiens. Two patients with EB-1 did not receive any therapy pre-transplant. However, both of them are not evaluable, due to early mortality. Transplant conditioning was RIC in 11/14 patients, myeloablative in 3 cases. The donor was a sibling in 9/14, MUD in 5/14. Four out of six patients treated with I.C. achieved a pre-transplant CR (67%), compared to one out of six in the 5-Aza cohort (17%). Four patients experienced a relapse post HCT, after a median of 8,5 months (4-11). With a median follow up of 21 months (6-68), post transplant RR was 4/12 (33,3%) and was not influenced by debulking therapy (I.C. *vs* 5-Aza, p=0,54), nor by pre-transplant disease state (CR *vs* noCR, p=0,22). In fact, 3 out of 6 patients treated with I.C., but only 1 out of 6 treated with 5-Aza relapsed after transplant. Three out of four patients who subsequently relapsed had recived RIC transplant; type of transplant was not assocaited with relapse (P=1,0) The only variable that showed a trend for reduced RR was MUD transplant (p=0,08).

Summary/Conclusions: Extreme caution must be used in considering our data, given the very small patients number. In our cohort, pre-transplant intensive debulking chemotherapy, although obtained an high rate of CR, showed no effect in preventing relapse. Larger studies are necessary to assess the real utility of I.C. in this subset of frail patients.

PB1932

IRON CHELATION THERAPY IN MYELODYSPLASTIC SYNDROMES AND IN OTHER TRANSFUSION-DEPENDENT CHRONIC ANEMIAS. RETROSPECTIVE STUDY OF 69 PATIENTS FROM A SINGLE INSTITUTION

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Background: Although several recent guidelines recommend iron chelation therapy (ICT) for iron overload in transfusion-dependent patients (pts) with lower-risk myelodysplastic syndromes (MDS), several barriers may limit the initiation or the continuance of ICT: older age, comorbidities, poor tolerance and compliance.

Aims: Therefore, with the aim of assessing the safety and efficacy of ICT in the daily clinical practice, we retrospectively analyzed our single-center experience on ICT in MDS and other chronic anemias.
Methods: From October 1997, in our Institution, 69 pts (48 males), median age: 74 (23-96) yrs, with transfusion-dependent anemia, received ICT, because of a diagnosis of iron overload, *i.e.* both a transfusion history of at least 20 units of RBC and a serum ferritin (SF) higher than 1000 ng/ml.
Results: 40 pts (58%) were affected by lower-risk MDS (IPSS risk: low or intermediate-1), while 13 pts (18.8%) showed a higher-risk MDS (IPSS risk: high or intermediate-2) but were considered for ICT because of responsiveness to hypomethylating therapy and/or elegibility for allogeneic SCT. 16 pts (23.2%) were affected by other diseases (chronic myelomonocytic leukemia: 2 pts; idiopathic myelofibrosis: 3 pts; aplastic anemia: 9 pts; pure red cell aplasia (PRCA): 2 pts). 45 pts (65.2%) received deferasirox (DFX) as first-line treatment, 12 pts (17.4%) received DFX after a previous treatment with deferoxamine (DFO), while 9 pts (13%) received DFO and 3 pts (4.3%) received DFO after DFX because of contraindications to DFX or toxicity. Median time from diagnosis to the start of ICT: 18 months. Median number of RBC transfusions before the start of ICT: 37.5. Median SF level pre-ICT: 1964 ng/ml; median SF after ICT (last value): 1858 ng/ml; median duration of ICT: 12 (range 1-230) months. 36 pts (52.2%) continued ICT for a period ≥12 months, and 25 pts (36.2%) for a period ≥24 months. 27 pts (39.1%) showed a drop of SF ≥500 ng/ml, 11 pts (15.9%) showed a drop of SF <500, 13 pts (18.8%) showed an increase of SF <500, in spite of ICT, and 18 pts (26.1%) showed an increase of SF ≥500. 12 pts (17.4%) achieved a SF value <1.000, and 48 pts (69.6%) a SF value <2.500. Adverse events possibly related to DFX were observed in 30 pts (43.5%): renal (increase of serum creatinine): 14 pts (20.3%) (grade >2: 1 pt: 1.4%); gastrointestinal : 14 pts (20.3%) (grade >2: 1 pt: 1.4%); cutaneous: 2 pts (2.9%) (grade >2: no pts). Permanent discontinuation of ICT: 40 pts (58%), because of toxicity (16 pts: 23.2%), worsening of clinical condition (6 pts: 8.7%), discontinuation of transfusions (9 pts: 13%), allogeneic transplantation (9 pts: 13%). 5 pts (7.2%) (4 MDS and 1 PRCA) (with DFX: 4 pts; with DFO: 1 pt) showed an erythroid response following ICT, after 2, 4, 7, 32 and 112 months, respectively, and one of them (with PRCA) achieved complete remission. 35 pts (50.7%) died, because of infection (9 pts), AML (4 pts), cachexia (4 pts), other neoplastic diseases (3 pts), hemorrhage (2 pts), heart failure (2 pts), stroke (2 pts) and other causes (9 pts). 10 pts (14.5%) are still receiving ICT. With a median follow-up of 34 (2-230) months, median overall survival (OS) was 64 months for all pts, 51 months for MDS pts, 87 months for lower-risk MDS (IPSS risk: low and intermediate-1) and 24 months for higher-risk MDS (IPSS risk: intermediate-2 and high).
Summary/Conclusions: In conclusion, in our experience ICT appears feasible and effective, in terms of reduction of SF and OS, even in a population of elderly pts, if carefully selected.

Myeloma and other monoclonal gammopathies - Biology

PB1933

VCAM-1 AS A NOVEL DRUG THERAPY TARGET OF BONE MARROW MESENCHYMAL STEM CELLS IN MULTIPLE MYELOMA

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Background: Multiple myeloma is characterized by the clonal proliferation of malignant plasma cells in the bone marrow microenvironment. The pathogenesis consists, in part, in critical interactions between myeloma cells and the mesenchymal stem cells (MSC). The interactions between myeloma cells and bone marrow cells are establish through surface receptors (*e.g.* integrins, cell adhesion molecules, etc.), which determine tumor growth, survival, migration and drug resistance. Mesenchymal stromal cells modulate the pattern of myeloma markers on the cellular surface *in vitro* towards a less differentiated phenotype. However, the exact mechanism by which mesenchymal stromal cells carry out their functions is not yet fully understood.
Aims: To evaluate the effect of MSCs from healthy donors and myeloma patients over malignant plasma cells and the molecular changes produced for the interaction each other.
Methods: Interactions between both cell types were studied through different co-cultures studies. We evaluate differences between culturing primary MSC cells and MM cell line RPMi 8226. Pathological MSCs were extracted from the bone marrow of newly diagnose MM patients. On the other hand, purified healthy MSCs will be isolated from donor patients. Pathological or healthy MSCs were cultured and co-cultured 24h after seeding with MM plasma cells RPMI 8226 for duplicates at 24, 48 and 72h. The phenotypical and molecular effect of the interaction of both cells were characterized by viability through trypan blue, cell apoptosis percentage (7AAD) and variations on expression of cell surface proteins (MSCs: CD90, CD105, CD106 and CD54. MM cell: CD138, CD38, CD49d and CD11a) using flow cytometry, and statistically analyzed with GraphPad.
Results: We observed a decrease of apoptosis of MM plasma cells when are in co-culture with pathological MSCs at short-term (24h, 7AAD positive cells MM alone: 4.8%, MM in co-culture: 0.4%) and mid-term (72h, 7AAD positive cells MM alone: 16.4%, MM in co-culture: 10.7%) compared with MM plasma cells alone. However MM plasma cells not decreases the level of apoptosis at mid-term with healthy MSCs in co-cultures (72h, 7AAD positive cells MM alone: 16.4%, MM in co-culture: 18.0%). The molecular analysis showed a correlation between MSC lack of protection over MM plasma cells and the decrease in the levels of expression of VCAM-1 (CD106).
Summary/Conclusions: As reported in literature CD106 expression increase when MSCs are co-cultured with plasma cells. Adhesion of tumor cells to BMSC activates many pathways resulting in upregulation of cell cycle and anti-apoptotic proteins. MM pathophysiology is supported by a strong interaction between CD106/CD49d. Changes in VCAM-1 and VLA-4 expression have been demonstrated in cell lines assays, and were corroborated with primary cells in the context of MSCs protection over MM plasma cell. Thus, MM pathological MSCs did not change VCAM-1 levels and MM plasma cell protection be held. However, healthy MSCs have the capacity to modulate the VCAM-1 in mid-term to avoid the protection effect. Therefore, these results suggest MSCs VCAM-1 as potential drug therapy target in MM disease.

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RALA AND RALB MEDIATE CELL SURVIVAL INDEPENDENTLY OF ONCOGENIC RAS AND PROVIDE POTENTIAL THERAPEUTIC TARGETS IN MULTIPLE MYELOMA

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Background: Genetic mutations and the bone marrow microenvironment contribute to disease progression, aggressive phenotype, and shorter survival in multiple myeloma (MM). Oncogenic RAS is one of the most common mutations in MM. Pathway activation through oncogenic RAS is associated with promotion of disease progression and shorter survival. Cell survival and proliferation in MM are mainly mediated via classical signaling pathways such as MEK/ERK and PI3K/Akt. Since there is a lack of specific RAS-inhibitors for clinical use, it is important to identify and analyze associated pathways, which may provide useful alternative targets for MM therapy. The small GTPase Ral has previously

been implicated in putative downstream signaling of RAS, and may therefore promote proliferation, survival and drug resistance of MM cells.
Aims: We used shRNA-mediated knockdown of RalA and RalB isoforms to appraise their role as potential therapeutic targets and to analyze their connection to important signaling pathways, which regulate MM cell survival and proliferation. Because oncogenic RAS is a potential activator of the Ral pathways, we investigated a possible link between oncogenic RAS and Ral.
Methods: Immunohistochemical stainings of bone marrow trephines of MM patients and Western analysis of primary MM cells and MM cell lines were performed to evaluate Ral protein expression. Transient or stable knockdown of RalA or RalB was achieved by electroporation of MM cell lines and the effect on cell survival and apoptosis was measured with flow cytometry using annexin V/propidium iodide staining. Ral pulldown assays were applied to test potential dependence of Ral activation on oncogenic RAS. Furthermore, RNA sequencing was performed to compare RAS and Ral gene expression signatures after respective knockdowns.
Results: Both Ral isoforms were expressed in primary MM cells and MM cell lines, with RalA showing the most prominent and consistent protein expression levels. ShRNA-mediated knockdown of RalA strongly induced apoptosis in two thirds of the tested cell lines, whereas RalB depletion did impair MM cell survival in less than half of the cell lines. Western analysis revealed no alteration of classical MEK/ERK and PI3K/Akt pathway activation after Ral knockdown. Ral activity appears to be independent of oncogenic KRAS or NRAS. In addition, RNA sequencing revealed differing gene expression signatures for RAS and Ral.
Summary/Conclusions: Ral and its effector network constitute potential therapeutic targets in MM, which are activated independently of oncogenic K- or NRAS. Therefore, investigation of the functional network of Ral may be important to identify useful clinical targets.

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CXCR4 MUTATIONS FOUND BY USING DEEP SEQUENCING WITHOUT SORTING B CELLS, AND PROGNOSTIC IMPLICATION IN WALDENSTROM MACROBULINEMIA

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Background: Waldenstrom macroglobulinemia (WM) is a lymphoplasmacytoid lymphoma with IgM monoclonal gammopathy. Most of WM harbor *MYD88 L265P* and one thirds of WM with *MYD88* present *CXCR4* mutations. Currently, frequency of *CXCR4* mutations and its clinical implication is not reported in Asian patients with WM.
Aims: We investigated the profiles of *CXCR4* and *MYD88* mutation in correlation with prognostic implication. To detect minor cell population with *CXCR4* mutation, we adopted a ultra-deep sequencing strategy for *CXCR4*, which can detect specific variants <1% of the cell population.
Methods: Allele-specific PCR for *MYD88* was performed on 37 patients with WM, along with 161 patients with B-cell neoplasms [diffuse large B-cell lymphoma (DLBCL), B-cell acute lymphoblastic leukemia (B-ALL), chronic lymphocytic leukemia (CLL)]. Deep-sequencing for *CXCR4* and interphase fluorescent *in situ* hybridization (FISH) for 6q deletion was performed on 31 patients with WM. Clinicopathologic features were compared among 3 groups according to *MYD88* and *CXCR4* mutation status (Group 1, $MYD88^{WT}$ and $CXCR4^{WT}$; group 2, $MYD88^{L265}$ and $CXCR4^{WT}$; group 3, $MYD88^{L265P}$ and $CXCR4^{Mutation}$; statistical comparison, Fisher exact test, one-way ANOVA).
Results: *MYD88 L265P* mutation was detected in 81.3% (26/32) patients with WM, 10.8% (9/83) in patients with DLBCL, 9.5% (6/63) in patients with CLL, 0% (0/15) in patients with B-ALL, and 0% in 200 healthy persons. Among the 31 WM patients, 6 patients have *CXCR4* mutation (19.4%) in the c-terminal domain (Figure 1); 1 frameshift mutation and 5 nonsense mutations. Two variants, K327X and K331X, are novel and the rest were known reported sites. All of them had *MYD88* L265P mutation. FISH revealed 6q21 deletion in 14 patients (43.8%), and *IGH* rearrangement in 9 patients (28.1%). There was no correlation aming cytogenetic aberrations and genetic mutation (*MYD88* and *CXCR4*). IgM levels of group 2 ($MYD88^{L265}$ and $CXCR4^{WT}$) were significantly higher than that of group 1 ($MYD88^{WT}$ and $CXCR4^{WT}$) (*P*=0.024). Meanwhile, IgG level was significantly lower in group I, compared to group 3. Other clinical characteristics such as age, Hb, platelet, adenopathy, hyperviscosity showed no significant difference among 3 groups. Group I showed adverse survival and 1 year survival rate of group 1 (66.7%) was lower than group 2 (94.7%), though it was not statistically significant (*P*=0.410). There were no death events in group 3 ($MYD88^{L265P}$ and $CXCR4^{Mutation}$) patients during the research period.
Summary/Conclusions: The frequency of *CXCR4* mutation in Korean WM was similar to those of Caucasian. We suggest that ultra-deep sequencing using next generation sequencing can replace B cell sorting for the detection of *CXCR4* mutation. Patients with $MYD88^{WT}$ and $CXCR4^{WT}$ showed higher IgM level and lower survival, suggesting an adverse prognostic implication. This is the first report on *CXCR4* mutation in Korean WM patients.

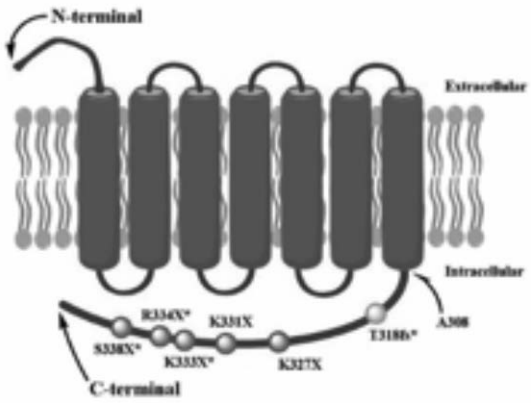

Figure 1.

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THE CLINICAL IMPACT OF CHROMOSOMAL TRANSLOCATION T(14;16)(Q32;Q23) IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Translocation t(14;16)(q32;q23) in plasma cells is considered as a strong negative prognostic factor in patients with multiple myeloma (MM). The oncogenic potential of this chromosomal aberration is based on the overexpression of the c-*MAF* protooncogene (located at 16q23) under strong enhancer of *IgH* gene (14q32). Although the *IgH/MAF* positive cases comprise just 2-4% of MM patients, the evaluation of this aberration is an integral part of the cytogenetic risk stratification according the RISS. The International Myeloma Working Group (IMWG) proposed a model of high risk MM as having at least one of the following aberrations: deletion of 17p13 (*TP53* gene), translocation t(4;14)(p13;q32) and translocation t(14;16)(q32;q23) determined by FISH. However, the unequivocal poor prognostic value of t(14;16)(q32;q23) was not confirmed in several MM series thus further studies are needed.
Aims: The aim of our study was to assess the impact of t(14;16)(q32;q23) on event free (EFS) and overall survival (OS) in cohort of *IgH/MAF* positive MM patients in comparison with control group of 30 MM *IgH/MAF* negative cases.
Methods: During the years 2004 to 2016, we examined 870 bone marrow samples of MM patients on immunofluorescently labeled plasma cells (clg FISH). The basic FISH panel included 4 specific DNA probes (Abbott-Vysis, Kreatech and MetaSystems) detecting: the *IgH* gene rearrangement (1), deletion 13q14 (*RB1* gene)/monosomy 13 (2), gain of 1q21/deletion of 1p32 (3) and deletion of *TP53* gene (4). Cases with rearranged *IgH* gene were gradually examined for 3 specific translocations- 1) t(11;14)(q13;q32), 2) t(4;14)(p16;q32) and 3) t(14;16)(q32;q23). Kaplan-Maier analysis was performed to evaluate OS and EFS.
Results: Translocation t(14;16) was identified in 19 out of 870 patients (2.2%). Eighteen patients were examined at the time of diagnosis and one at the time of the progression of asymptomatic myeloma to symptomatic disease. Relapse and/or disease progression occurred in 15 patients. The median event-free survival (EFS) was 13 months in t(14;16) carriers (range 3 – 62 months) and 22.5 months in controls (range 3-71 months, p=0.285). Fourteen t(14;16) positive patients died. The median overall survival (OS) was 25 months (range 10-204 months) in comparison with 52 months in control group (range 3-132 months). However, the difference in OS was not statistically significant (p=0.155). In 15 t(14;16) positive patients (83.3%), two or more additional chromosomal changes were detected by FISH (monosomy/deletion of chromosome 13 being the most frequent). In four cases, (14;16) was detected together with another high risk chromosomal change - deletion of *TP53* gene - and all these patients died within median of OS 12.5 months (range 10-16).
Summary/Conclusions: Beside its supposed negative clinical impact, the examination of t(14;16) is not always included in routine diagnostics of chromosomal changes and its prognostic significance should be proved in large series of MM patients. Our data substantiate the trend of worse clinical outcome (shorter OS) in t(14;16) positive group compared to *IgH/MAF* negative MM controls. The detailed analysis of other clinical parameters, type of therapy, combination with other chromosomal aberrations will be performed to prove its role of as independent prognostic factor.
Supported by grants RVO-VFN64165, ProgresQ28 and GACR P302/12/G157.

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THE ROLE OF NEUROTROPHINS AND ANGIOGENIC CYTOKINES IN THE PATHOPHYSIOLOGY OF PERIPHERAL NEUROPATHY IN PATIENTS WITH MULTIPLE MYELOMA

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Background: The introduction of new treatment modalities have changed significantly the prognosis of multiple myeloma (MM) patients. The novel drugs and schemes of treatment of MM have contributed to substantial extend of the overall survival time of patients. However, the administration of some of the treatments, *e.g.* thalidomide or bortezomib, is also associated with occurrence of a serious and common side-effect problem, which is the drug-induced peripheral neuropathy. The mechanism of the development of the peripheral neuropathy is poorly understood. Nevertheless, one of its potential cause, could be inadequate concentrations of crucial trophic factors, including neurotrophic and/or angiogenic factors, which are responsible for proliferation, differentiation, survival and death of neuronal and nonneuronal cells.
Aims: The aim of this study was to elucidate the potential relationship between concentration of neurotrophic and angiogenic factors and development of peripheral neuropathy in the natural clinical course of the disease and, especially, induced by treatment regimen: VMP (bortezomib, melphalan, prednisone) or VTD (bortezomib, thalidomide,dexamethason) in patients with MM.
Methods: Peripheral blood samples were collected from patients classified into two groups: i) patients with multiple myeloma, without neuropathy and before therapy; and ii) patients with peripheral neuropathy 3^{o}or 4^{o} induced in the course of VMP or VTD therapy. The control group consisted healthy aged matched subjects. Assessment of concentrations of neurotrophins (BDNF, NSE) and angiogenic factor (PDGF) were performed using Luminex technology, which utilize microbeads coated with fluorescently labeled antibodies.
Results: Concentration of BDNF, PDGF and NSE were significant decreased in patients after treatment regimen involving VMP or VTD who have developed peripheral neuropathy grade 3 or 4, compared with patients with newly diagnosed MM without neuropathy, before therapy and control healthy group. Additionally, plasma levels of both neurotrophins and PDGF in patients before therapy were higher, then in control group. Obtained results may be caused by the changes in an activity of the transcription factor NF-κB during the treatment of MM, since reduction of NF-kB concentration is associated with decrease in the transcription of genes encoding BDNF, NSE and PDGF.
Summary/Conclusions: Alterations in the concentration of BDNF, PDGF and NSE suggest the cause and effect relationship between these factors and the development of neuropathy in patients with MM. Comprehensive elucidation of this phenomenon may contribute to the extension of the knowledge concerning the pathogenesis of neuropathy, and might well lead to reduction of the incidence of polyneuropathy in MM patients in the future.

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INFLUENCE OF XRCC5, XRCC4, NFKB2, AND BIRC5 GENES POLYMORPHISMS IN THE RISK AND PROGNOSIS OF MONOCLONAL GAMMOPATHIES

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Background: Monoclonal gammopathies (MG) are a group of disorders characterized by the proliferation of monoclonal plasma cells, which produce and secrete monoclonal immunoglobulin (M protein). Symptomatic multiple myeloma (MM) is characterized by the clonal proliferation of plasma cells. MM is consistently preceded by a pre-neoplastic entity, called monoclonal gammopathy of undetermined significance (MGUS), with an intermediate phase of indolent multiple myeloma (MMI). This disease is a heterogeneous hematological neoplasm characterized by the proliferation of clonal, long-lived plasma cells within the bone marrow (BM) secreting monoclonal proteins and by the presence of so-called CRAB criteria and/or biomarkers of malignancy (as clonal BM plasma cells ³ 60%, involved:uninvolved serum free light chain ratio ³100, >1 focal lesion in MRI studies). Genetic instability and several molecular abnormalities are hallmarks of MM cells. Alterations in DNA repair pathways, namely abnormal activity of non homologous end–joining (NHEJ) repair pathway, are involved in the disease onset and progression. Moreover, it has been observed that virtually all primary MM samples have constitutive nuclear factor-κB (NF-κB) pathway activity, having this pathway a well–established role in MM pathogenesis.
Aims: In this context, we hypothesized that polymorphisms of genes involved in NHEJ repair pathway (XRCC5, XRCC4) and in NF-κB pathway (NFKB2, and BIRC5) may have impact in MG susceptibility and prognosis.
Methods: In the present, a hospital-based case-control study, we analyzed eight polymorphism in four genes (XRCC5, XRCC4, NFKB2, and BIRC5), by genotyping 189 individuals (63 MG patients and 126 controls) using TaqMan qPCR. Results are expressed in terms of frequencies of allele, genotype, haplotype, and genotypic profiles, and their correlation with MG susceptibility. The strength of association between polymorphisms and disease risk was assessed by odds ratio (OR) with 95% confidence interval (CI95%) calculated by logistic regression. We also investigated the association of these SNPs with overall survival through Kaplan Meier curves. All statistical analyses had a significance levels of 95%.
Results: In the patient group, 51% (32/63) of the individuals were females and 49% (31/63) were males; the mean age was 70.11±10.25 years old. Among the control group, 52% (65/126) of the individuals were females and 48% (61/126) were males, and the mean age was 69.90±10.06 years old. Most of patients were diagnosed with multiple myeloma (84%, 53/63) and the remaining ones (16%, 10/63) were diagnosed with smoldering multiple myeloma. According to the ISS classification, 43% (27/63) of patients are in stage III. The data analysis revealed two associations of the studied gene polymorphisms with MG. First, the analysis by gender stratification suggested a decreased predisposition to MG in male carriers of NFKB2 rs12769316 GA and AA genotypes (OR 0.346, 95%CI 0.124–0.965, p=0.043). Second, we observed that patients with BIRC5 rs9904341 CC genotype had a highly significant lower overall survival (recessive model: HR 4.89, 95%CI 5.06 199.70, p<0.01). BIRC5 GGC haplotype (rs4789551, rs9904341, and rs8073069) was found in one patient and absent in controls.
Summary/Conclusions: The present study suggests that NFKB2 gene variant (rs12769316, allele A) may be associated with MG susceptibility in males, and BIRC5 (rs9904341) CC genotype may negatively influence MG prognosis. Nonetheless, further studies are needed to validate these findings, enlighten the role of genetic polymorphisms in MG susceptibility and prognosis.

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SILENCE OF LONG NONCODING RNA MALAT1 BY RNA INTERFERENCE INHIBITS PROLIFERATION AND INDUCES APOPTOSIS IN MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) is a neoplastic plasma-cell disorder characterized by abnormal proliferation of monoclonal plasma cells in bone marrow leading to various end-organ damages. Altered long non-coding RNAs (lncRNAs) levels can result in aberrant expression of gene products that may contribute to cancer biology. Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), an evolutionarily highly conserved mRNA-like lncRNA was originally identified with high expression in metastatic non-small-cell lung cancer and reported to be up-regulated in many other cancers. However, the function of *MALAT1* in MM remains unknown.
Aims: Our study aimed to evaluate the role of *MALAT1* on proliferation as well as apoptosis in MM cells *in vitro* and tumorigenic ability *in vivo*, following transfection with MALAT1-specific short hairpin RNA (shRNA) expression plasmids.
Methods: Levels of *MALAT1* in human myeloma cell lines were detected by real-time polymerase chain reaction (RT-PCR) analysis. The effects of *MALAT1* shRNA in MM were investigated *in vitro* and *in vivo*.
Results: We found that *MALAT1* was high expressing in RPMI8226 and U266 cells. Silencing of *MALAT1* by shRNA significantly inhibited the proliferation through cell cycle arrest at G1 phase and induced apoptosis, which was closely associated with activation of caspase-3/-9, downregulation of Bcl-2 and upregulation of Bax. Study *in vivo* revealed that silencing of *MALAT1* delayed the tumor growth and led to apoptosis in mice bearing myeloma xenograft.
Summary/Conclusions: *MALAT1* may serve as a promising novel therapeutic target in human MM. Notably, the inhibition of *MALAT1* by shRNA may prove to be an effective genetic therapeutic strategy for MM treatment.

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LONG NON-CODING RNA MEG3 FUNCTIONS AS A COMPETING ENDOGENOUS RNA TO REGULATE PTEN EXPRESSION BY SPONGING MIR-181A IN MULTIPLE MYELOMA

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Background: Long non-coding RNA maternally expressed gene 3 (MEG3) plays a critical role in cancer progression and metastasis. However, the overall biological role and regulatory mechanism of MEG3 in multiple myeloma (MM) development and progression remains largely unknown.
Aims: To explore the tumor suppression role of lncRNA MEG3 in MM and further reveal the mechanism of MEG3 functions as ceRNA to contribute to MM pathogenesis.
Methods: MEG3 expression was measured in MM patients by real-time PCR.

The effect of MEG3 on cell apoptosis, cell proliferation and angiogenesis were gained from CCK-8, flow cytometric analysis and transwell invasion assays in MM cell lines ARP-1 and LP-1. Insights of the mechanism of competitive endogenous RNA (ceRNA) were gained from bioinformatic analysis, luciferase reporter assays and RNA binding protein immunoprecipitation (RIP) assay.
Results: MEG3 expression was significantly decreased in MM patients with advanced stage disease (ISS II and III) and poor prognosis.Overexpression of MEG3 promoted cell apoptosis and inhibited cell proliferation, migration and angiogenesis in MM ARP1 and LP-1 cell lines. Furthermore, MEG3 increase the expression of phosphatase and tensin homolog (PTEN) and consequentially inhibit MM cell proliferation and angiogenesis through sponging miR-181a *in vitro*. miR-181a mimics can limit the promotion of PTEN by MEG3.
Summary/Conclusions: MEG3 functions as a tumor suppressor in MM. High expression of MEG3 is a marker for good survival. We reveal a novel mechanism that MEG3 as a ceRNA of the PTEN gene by competing for miRNA-181a binding sites and thereby regulate the expression of the PTEN mRNA.

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IMPROVE RISK-STRATIFICATION OF MULTIPLE MYELOMA PATIENT WITH MICROFLUIDIC DEVICES

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Background: Cytogenetic alterations are required for risk stratification of multiple myeloma (MM); however, current pathology assays performed on bone marrow samples directly can produce false negatives due to the unpredictable distribution and rarity of MM cells. A more accurate method is needed for MM diagnosis and risk-stratification. We develop a new microfluidic device to facilitate CD45-depletion for enhancing the detection of cytogenetic alterations in plasma cells.
Aims: Improve accuracy of risk stratification for multiple myeloma patients
Methods: Bone marrow samples from 48 MM patients were divided into two parts each. One part was directly detected by classic flow cytometry and FISH while the other part was first enriched by microfluidic size selection and then underwent CD45-cell depletion (MF-CD45-TACs). The enriched samples were then analyzed by flow cytometry and FISH and compared to the classical analysis.
Results: MF-CD45-TACs significantly increased the percentage of CD38+/CD138+ cells to 37.7%±20.4% (P<0.001) compared to 10.3%±8.5% in bone marrow. After the MF-CD45-TACs enrichment, the detection rate of IgH rearrangement, del(13q14), del(17p) and 1q21 gains rose to 56.3%(P<0.001), 37.5%(P<0.001), 22.9%(P<0.001) and 41.7% (P=0.001), respectively, all significant increases compared to untreated samples.
Summary/Conclusions: We have developed a rapid, simple assay for improved diagnostics and risk-stratification for MM. With more precise diagnosis, the clinical outcomes of MM will be significantly improved.

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SERUM FREE LIGHT CHAIN RATIO IS AN INDEPENDENT RISK FACTOR FOR PROGRESSION IN MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE

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Background: Monoclonal gammopathy of undetermined significance (MGUS) is a premalignant plasma cell proliferative disorder found in approximately 3% of the general population 50 years of age and older. MGUS is associated with progression to multiple myeloma or related malignancy at a rate of 1% per year. Thus the risk of malignancy for a 50-year-old patient with a 25-year life span is 25%.
Aims: We hypothesized that the presence of monoclonal free kappa or lambda immunoglobulin light chains in monoclonal gammopathy of undetermined significance (MGUS), as detected by the serum free light chain (FLC) assay increases the risk of progression to malignancy.
Methods: 90 Patients seen at the Hematology consultation from 2010 to 2015 with MGUS have a serum Mprotein less than 30 g/L, bone marrow plasma cells less than 10%, and no anemia, hypercalcemia, lytic bone lesions, or renal failure that would be indicative of a malignant plasma cell disorder.
The prognostic effect of abnormal kappa-to-lambda FLC ratio on progression of MGUS was studied. We also examined whether the risk of progression varied depending on the extent to which the FLC ratio was abnormal (the normal reference range of κ/λ ratio 0.26 to 1.65).
Results: The median age at diagnosis of MGUS was 59 years (35-92years). 62 Womans and 28 Mans Sex ratio=2.2. Serum electrophoresis and immunoelectrophoresis or immunofixation was done in 85 patients. Of these, The median serum M protein size at diagnosis was 12 g/L (1.7-28.5g/L). IgG monoclonal : 68 patients (75%), and non IgG monoclonal : 22 patients (25%). A monoclonal light chain was detected in 62 patients, as detected by the serum free light chain (FLC) assay increases the risk of progression to malignancy. An abnormal FLC ratio (kappa-lambda ratio <0.26 or >1.65) was detected in 27 (30%) patients. At a median follow-up of 5 years, malignant progression had occurred in 6 patients (6,6%) with an abnormal serum FLC ratio.
Summary/Conclusions: A novel, highly sensitive serum free light chain (FLC) assay is now available for clinical practice.The risk of progression in patients with an abnormal FLC ratio was significantly higher compared with patients with a normal ratio, and was independent of the size and type of the serum monoclonal (M) protein. Patients with an abnormal serum FLC ratio, non–immunoglobulin G (non-IgG) MGUS, and a high serum M protein level (>15 g/L) had a major risk of progression.

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INTENSITY OF EXPRESSION OF MULTIDRUG RESISTANCE GENES AFFECT ON THE OVERALL SURVIVAL OF PATIENTS WITH MULTIPLE MYELOMA WERE TREATED WITH BORTEZOMIB AND ASSOCIATED WITH THE INITIAL MULTIDRUG RESISTANCE

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Background: Bortezomib is an important drug in multiple myeloma (MM) treatment, but the resistance to this treatment exist. Many conflicting data suggests that cellular overexpression of multidrug resistance (MDR) genes may reduce the effectiveness of bortezomib - containing treatment. The main indicator of the effectiveness of the treatment of MM is the overall survival of patients.
Aims: We evaluated the changes of intensity of expression of MDR genes in patients with newly diagnosed and refractory/relapsed multiple myeloma and the effect of expression of MDR genes such as MDR 1, MRP 1, BCRP, LRP on the overall survival of patients after treatment with bortezomib.
Methods: The bone marrow aspirates of 30 patients (12 men and 18 women) aged 48 to 77 years (median 60 years) with stage III MM by classification Durie-Salmon were studied. 15 patients were included in a group of newly diagnosed (ND) MM. 15 patients were in group of a clinically refractory/ relapsed (RR) MM. The bone marrow in this group of patients were studied after treatment with alkylating agents at the time of registration of resistance to the given therapy. In the future, all patients were treated by bortezomib - containing chemotherapy regimens. mRNA expression studied genes were determined by semi-quantitative polymerase chain reaction reverse transcription. The degree of expression was assessed by semi-quantitative visual assessment from 0 (no electrophoretic strips) to 4 points (bright glow of the transcript). The overall survival (OS) was analyzed by the Kaplan-Meier method, with the use of Cox-Mantel test. Differences were considered statistically significant at p <0.05.
Results: In both groups of patients had comparable expression of all studied MDR`s genes. The development of clinical resistance to treatment with alkylating agents were accompanied by an increase in mRNA expression of all studied genes. However, the statistically significant increase the expression of the intensity obtained for LRP gene only (the average intensity of the expression of mRNA LRP gene in ND MM 0.9±0.24, with RR MM 1.93±0.34, p <0.05). The MDR 1 mRNA expression was 1.50±0.34 in the group of ND MM and 1.67±0.31 in the group of RR MM, p>0.05. The expression of mRNA of MRP 1 and BCRP are 1.07±0.21 and 1.63±0.15 respectively before treatment and increased to 1.73±0.31 and 2.13±0.35 respectively in the group of RR MM, p=0,06. OS was negatively associated with high LRP gene expression only in group of ND MM (median of OS in patients with high LRP gene expression was 17 months and in those with low expression 62 months, p <0.05).
Summary/Conclusions: High expression of LRP gene is associated with worse overall survival in patients with newly diagnosed MM treated with bortezomib- containing chemotherapy programs. “Genetic resource MDR” in MM is due mainly to the initial multidrug resistance. The treatment of MM by alkylating drugs increase the existing at the time of diagnosis of MDR activity of genes.

PB1944

ASSOCIATIONS OF IL-1, IL-4 AND TGF-B1 POLYMORPHISMS WITH CYTOGENETIC PROFILES IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) is a plasma cell malignancy characterized by complex cytogenetic and molecular genetic aberrations. Those cytogenetic abnormalities occur at different stages of the disease. The chromosome ploidy status and Ig rearrangements are two genetic criteria that are used to help stratify patients into prognostic groups.
Aims: The aim of the study was to analyse correlation between cytogenetic profiles and some cytokine genotypes in 24 patients with MM (Caucasoid inhabitants of the North-West region of Russia).
Methods: Genomic DNA was extracted from the peripheral blood; gene geno-

typing (IL-4, TGF-β1, IL-1α, IL-1β) was performed by PCR-SSP; study of cytogenetic abnormalities was performed by standard GTG-method and interphase FISH analyses with DNA probes: LSI 13(RB1)13q14, IGH/CCND1, IGH/FGFR3, LSI TP53 (17q13.1); p-values less than 0.05 were considered statistically significant.
Results: Previous results allow us to describe some cytokine genotype markers associated with the development of MM (IL-1α -889 TT, IL-1β +3962 TT, IL-6 -174 GG and IL-6 nt565 GG; gr. 1) as additional negative prognostic markers but IL-4 -33 CC and TGF-β1 codon 25 GG genotypes as additional positive prognostic markers (gr. 2). However, in some MM patients we found presence of negative and positive markers together (mixed markers; gr. 3). We analyzed cytogenetic profiles in MM patients with different prognostic markers in their genotypes (Table 1).

Table 1.

Genotypes with prognostic markers	Abnormal cytogenetic profile	Normal cytogenetic profile
1st gr. - MM patients with negative prognostic markers in genotype: IL-1a -889TT, IL-1b +3962TT, IL-6 -174GG, IL-6 nt565GG	0.778*	0.222
2nd gr. - MM patients with positive prognostic markers in genotype: IL-4 -33CC, TGF-b1 codon25GG	0.111*	0.889*
3rd gr. - MM patients with mixed prognostic markers in genotype	0.667	0.333
*- p<0.05		

The frequency of abnormal cytogenetic transformations in the 2nd gr. was noticeably lower compare to patients from the 1st and 3rd gr. (0.11 *vs* 0.78 *vs* 0.67 respectively; p<0.05). Similarly, significant differencies in the frequency between patients with positive prognostic markers and normal cytogeneic profile (0.89) compare to MM patients with negative (0.22) or mixed (0.33) genotypes but normal cytogenetic profiles were also observed (p<0.05). In the 1st gr. frequency of cytogenetic abnormalities was noticeably higher compare to patients with normal profile (0.78 *vs* 0.22; p<0.05). Vice versa, in patients with positive prognostic markers in the genotype frequency of normal cytogenetic profiles was remarkably higher (0.89) compare to patients with aberrations (0.11; p<0.05).
Summary/Conclusions: Thus, our results allow to describe IL-1α -889 TT, IL-1β +3962 TT, IL-6 -174 GG and IL-6 nt565 GG as markers associated with the presence of cytogenetic abnormalities in MM patient cells. However, IL-4 -33 CC and TGF-β1 codon 25 GG indicate the normal cytogenetic profile in patients with MM from the North-West region of Russia. Although, if MM patients have both negative and positive prognostic markers associated with the development of multiple myeloma (mixed genotype) it seems that the chance of finding cytogenetic abnormalities is much higher compare to patients with positive prognostic markers only.

PB1945

CORRELATION DEPENDENCE OF CHRONIC LYMPHOPROLIFERATIVE DISORDERS, MULTIPLE MYELOMA FROM CHANGES OF IMMUNE RESPONSE GENES PROFILE

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Background: Hematological malignancies are multifactorial diseases in the development of which play a role as environmental factors and genetic determination of leukemic process. Such genetic factors include the presence in human genome of allelic variants of the regulatory regions of the innate immune response genes. At present time, they are considered as real risk factors for these diseases in a person with a certain set of genetic variants. Their distribution among the population corresponds to the population laws and has its ethnographic features. Analysis of the individual associations of genes polymorphic variants involved in the implementation of the immune response does not sufficiently complete answer about their role in the formation of predisposition to the development of chronic lymphoproliferative disorders (CLD) and multiple myeloma (MM). It is noted that in the pathogenesis of hematological diseases contribute significantly to certain combinations of immune response genes.
Aims: Analysis of interactions between genes based on the distribution of immune response genes combinations in chronic lymphoproliferative disorders and multiple myeloma.
Methods: The study included 176 patients aged 22-86 years (median - 61 year), identifying themselves as Caucasians residing in one region in the north-east of the Russian Federation. This group consisted of 80 patients with chronic lymphocytic leukemia/small lymphocytic lymphoma (45%), 72 with multiple myeloma (41%), 10 with diffuse large B-cell lymphoma (6%) six with marginal zone lymphoma (3%) four with mantle cell lymphoma (2%), three with lymphoplasmacytic lymphoma (2%) and one patient with follicular lymphoma (1%). Genotyping of polymorphism of the innate immune response genes *TLR2* (rs5743708), *TLR3* (rs3775291), *TLR6* (rs5743810), *TLR9* (rs5743836), *IL1β* (rs2856841), *IL2* (rs2069762), *IL4* (rs2243250), *IL6* (rs1800795), *IL10* (rs1800871), *IL17A* (rs2275913), *CD14* (rs34424920), *TNFα* (rs1800629), *FCGR2A* (rs1801274) was performed by polymerase chain reaction with allele-specific primers (Litech, Russia). Analysis of interactions between genes was performed using nonparametric GMDR program (Generalized Multifactor-Dimensionality Reduction) [Lou X.Y. *et al.*, 2007.; http://www.healthsystem.virginia.edu/internet/addiction-genomics/Software/].
Results: In the analyzed group of patients with CLD and MM identified almost 78 753 combinations of multi-locus genotypes of the 13 immune response genes is 1 594 323 theoretically possible, indicating the non-random nature of the combination of allelic variants of analyzed genes. A statistically significant two-, three-, four-, five-, six, seven- and eight-loci model of inter-gene interactions at the investigated hematological malignancies: - *IL4* (C-589T) and *CD14* (C-159T) (χ^2=8.39, p=0.0038); - *IL4* (C-589T) and *CD14* (C-159T) and *IL6* (C-174G) (χ^2=12.14, p=0.0005); - *IL4* (C-589T) and *IL17A* (G-197A) and *CD14* (C-159T) and *IL6* (C-174G) (χ^2=17.30, p<0.0001); - *IL4* (C-589T) and *IL17A* (G-197A) and *IL10* (C-819T) and *CD14* (C-159T) and *IL6* (C-174G) (χ^2=16.98, p<0.0001); - *IL4* (C-589T) and *IL17A* (G-197A) and *IL10* (C-819T) and *TNF* (C-308A) and *CD14* (C-159T) and *IL2* (T-330G) (χ^2=16.98, p<0.0001); - *IL4* (C-589T) and *IL17A* (G-197A) and *IL10* (C-819T) and *TNF* (C-308A) and *TLR9* (T-1237C) and *CD14* (C-159T) and *IL2* (T-330G) (χ^2=16.98, p<0.0001); - *IL4* (C-589T) and *IL17A* (G-197A) and *IL10* (C-819T) and *TNF* (C-308A) and *TLR9* (T-1237C) and *CD14* (C-159T) and *IL2* (T-330G) and *IL1b* (T-31C) (χ^2=16.98, p<0.0001); - *IL4* (C-589T) and *IL17A* (G-197A) and *IL10* (C-819T) and *TNF* (C-308A) and *TLR9* (T-1237C) and *CD14* (C-159T) and *IL2* (T-330G) and *IL1b* (T-31C) and *TLR2* (Arg753Gln) (χ^2=16.98, p<0.0001).
Summary/Conclusions: The findings suggest an important role of immune response genes in the development of a number of chronic lymphoproliferative disorders and multiple myeloma, and can later be used as diagnostic and prognostic markers of different types of hematological malignancies. In addition, provided bioinformatics model will reveal not only the genetic criteria for high and low risk of hematological malignancies studied, but also to determine their prognostic significance in the clinical course of these diseases.

PB1946

FEATURES OF STROMAL ELEMENTS OF HEMATOPOIETIC BONE MARROW NICHE IN MULTIPLE MYELOMA

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Background: Structure of bone marrow stroma – mesenchymal stromal cells (MSC), endosteal stromal cells, and microvessels forming the hematopoietic niche and regulate the development of hematopoietic stem cells (HSC). Analysis of morphological changes of these elements of the hematopoietic niche is important to clarify the pathogenesis of multiple myeloma (MM).
Aims: To investigate the morphological and functional characteristics of stromal elements of the hematopoietic niche in bone marrow of patients with MM, as well as the characteristics of culture of mesenchymal stromal cells (MSC) and hematopoietic stem cells (HSC).
Methods: 42 trepanobiopsy of bone marrow from patients diagnosed with MM were used for the study. The age of the patients ranged from 53 to 72. The study applied histological, histochemical, immunohistochemical (IHC) and morphometric methods (VideoTest®). Also 20 patients from this group conducted cultural studies for the determination of colony-forming ability of HSC and morphofunctional status of MSC.
Results: Myeloma cellular composition of infiltrates were polymorphic. The surveyed patients were allocated to 3 types of infiltration is nodular, interstitial, diffuse. The histogenesis of infiltration was confirmed by IHC research with antibodies 79α, CD 138, CD 38.Regardless of the type of infiltration in all patients were recorded focal destructive changes of bone tissue. The density of microvessels in IHC studies with antibodies to CD 31, CD34 cl.II (Dako) was increased. A greater number of microvessels were detected in the endosteal area at all types of the bone marrow involvement (compared to normal), the total number of microvessels could statistically do not exceed such normal. A reduction in the expression of type I collagen bone matrix and the increase of collagen type IV expression, which is associated with increased microvascular density. Intratrabecular collagen type I was mellowed, ossification was reduced, most notably it revealed in areas of trabecules resorption. At the same time increased the amount of collagen type IV in endosteal spaces of the bone marrow. A typical feature was the appearance of focal network of reticulin fiber in subendosteal and perivascular spaces.Cultural studies have shown a significant decrease of colony-forming ability of HSC mobilized peripheral blood of MM patients after cryopreservation. *In vitro* studies preliminary data on lack of differences in the phenotype of MSC bone marrow of patients with MM and phenotype from healthy individuals, but also, a decrease in the proliferative ability of the MSC of patients with MM.
Summary/Conclusions: Analysis of parenchymal-stromal relationships in trepanobiopsy bone marrow of patients with MM evidence of their violation in the context of malignancy of lymphopoiesis, while cultural studies have shown a decrease of colony-forming ability of HSC and proliferative capacity of MSC. Regardless of the prevalence of neoplastic lesions, and myeloma infiltration noted the response of the stromal microenvironment, forming the hematopoietic niche.

PB1947

Abstract withdrawn.

Myeloma and other monoclonal gammopathies - Clinical

PB1948

Abstract withdrawn.

PB1949

IMPACT OF RENAL IMPAIRMENT IN NEWLY DIAGNOSED MULTIPLE MYELOMA IN A REAL WORLD SETTING

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Background: Renal impairment (RI) is a frequent complication of patients with newly diagnosed multiple myeloma (NDMM), reported in 15-40% with 10% requiring hemodialysis (HD). It is associated with higher early mortality (EM) and lower overall survival (OS). Early diagnosis and treatment with new agents improve these results.
Aims: Analyze renal response, OS and EM in NDMM with RI and compare them to patients with MM without RI.
Methods: All consecutive and unselected NDMM patients treated at Hospital de Clínicas, Montevideo, Uruguay, from January 2011 to June 2015 were included. Our database was completed prospectively and included clinical and laboratory characteristics of the disease, treatment, treatment-related adverse events, response, HD requirement, renal response and mortality.
Diagnosis of MM, response to treatment and degree of renal function recovery were based on the International Myeloma Working Group criteria. RI was defined as an estimated glomerular filtration rate (eGFR) <40 ml/min/1.73m^2, calculated by MDRD (*Modification of Diet in Renal Disease*) equation. Patients whose RI was explained by other causes were excluded. Early treatment was defined by initiation within 7 days after diagnosis. EM was defined as death within 3 months of diagnosis.
Results: MM was diagnosed in 52 patients, median age was 67 years (range 39-90), 61,5% were male, 38,5% had RI. The characteristics of the patients and front-line treatment are shown in Figure 1.

Characteristics (mean)		Pts with RI (n=20)	Pts without RI (n=32)
Age (years) median		68,5 (49-90)	63 (39-85)
Male		12 (60%)	20 (62,5%)
eGFTR (ml/min/1,73m^2)		19,3	72,7
Hemoglobin (g/dl)		7,8	9,7
Calcium (mg/dl)		10,2	9,5
Serum M Spike (mg/dl)		3,4	2,8
Urinaria Proteins (g/24hs)		1,4	0,04
M-component type:	IgG, IgA, IgM	17 (85%)	27 (84,4%)
	Light chain	3 (15%)	2 (6,2%)
	No secretor	0	3 (9,4%)
Light chain type:	Kappa	10 (50%)	19 (59,4%)
	Lambda	10 (50%)	10 (31,2%)
Stage III Durie-Salmon		19 (95%)	26 (81,3%)
ISS 3		18 (90%)	9 (28,1%)
Treatment:	Steroids	20 (100%)	32 (100%)
	Thalidomide	5 (25%)	22 (68,8%)
	Bortezomib	11 (55%)	5 (15,6%)

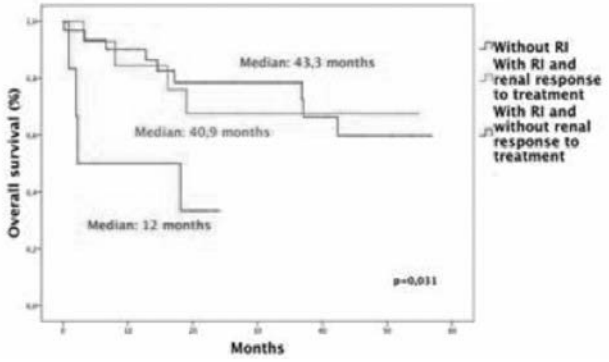

Figure 1. Characteristics of patients and overall survival according to renal function.

Overall response to first line treatment was 70% for those with RI (CR 20%) and 68,8% in patients without RI (CR 15,4%). Treatment related adverse effects were higher in patients with RI (45% *vs* 28,2%), being polyneuropathy the most common side effect. Patients with RI required more dose adjustments (40% *vs* 6,3%). Renal response: 50% reversed RI, 10% achieved renal PR and 40% renal CR, all before the 4th month from diagnosis; 77,8% started early treatment and 70% received bortezomib (bz). Patients whose RI did not reverse had had late initiation of treatment in 78% and 40% received bz. Six patients (30%) remained in chronic HD, all had late initiation of treatment. Two of the 6 patients who required HD at diagnosis obtained later independence; both received bz and one was consolidated with autologous stem cell transplantation. Impact of RI on OS and EM: median OS in patients with RI was not significatively different to that of MM without RI (35,3 *vs* 43,3 months, p=0,346). Patients without RI had higher OS compared to those who had reversible renal failure and those who never recovered (43,3; 40,9 and 12 months, respectively, p=0,031). OS was higher in patients with RI who received bz *vs* other therapeutic schemes (42,5 *vs* 25,8 months, p=0,137). With a mean follow-up of 26 months, mortality was 40% and 28,1% in patients with and without RI, respectively. EM were also higher in patients with RI at diagnosis (50% *vs* 22,5%). The main cause of EM was infection in both groups.
Summary/Conclusions: RI was frequent in NDMM and was associated with advanced disease and higher tumor mass (>90% stage III Durie-Salmon and ISS3), revealing a late diagnosis. Prompt institution of treatment and use of bz relates to higher recovery of renal function and dyalisis independence. Although toxicity and dose adjustements were higher in patients with RI this was not associated with lower response to treatment. Reversal of renal failure associates with better OS, similar to those without RI at diagnosis. EM are more prevalent in patients with RI at diagnosis. Even when the number of patients is small, this real life data supports the need of planning local strategies that lead to early diagnosis and initiation of treatment, which are crucial to reduce morbidity and mortality associated to RI in NDMM.

PB1950

THE EXPRESSION OF THE TRYPTASE POSITIVE MAST CELLS AND THE LEVELS OF IL-17, CORRELATE WITH ANGIOGENIC FACTORS IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Angiogenesis in the bone marrow plays a very important role in the progression of multiple myeloma(MM). The procedure of angiogenesis is supported by several factors such as VEGF, FGF-2 and metalloproteases that are secreted straight from the tumor cells. The presence of IL-6 in the microenvironment, induces the production and the secretion of several angiogenic factors that activate inflammatory cells of the matrix, like macrophages and mast cells to secrete more angiogenic factors. IL-17 is among the most important cytokines that have an important role in the development of myeloma tumor. IL-17 is a proinflammatory cytokine that is secreted primarily by CD4 (activated memory cells) and stimulate macrophages, fibroblasts and other cells that release several cytokines. It has been reported that IL-17, induces angiogenesis in humans by stimulating the migration of vessel endothelial cells and adjusting the production of various proangiogenic factors. In a previous study, it was found that increased levels in stage II and stage III, resolved after therapy. Additionally, blocking the receptor of IL-17, with an antibody, cancels the effects of IL-17.
Aims: Aim of this study is to assess the relationship of the MCD and IL-17, in angiogenesis of MM, as well as their correlation with known angiogenic factors in disease progression.
Methods: We studied 52 newly diagnosed patients with MM, 32 women and 20 men, aged 67,5±9,6 years. According to the ISS stage, 19 were stage I, 17 stage II and 16 stage III . Regarding the type of paraprotein that had been found, 31 IgG, 17 IgA and 4 patients with light chains. 20 age and sex-matched healthy volunteers, were used as controls. Serum samples and bone marrow biopsy samples were obtained from all patients prior to the initiation of treatment. Patients with impaired hepatic and renal function, chronic or acute infections, chronic inflammatory or autoimmune disorders or other malignancies were excluded from the study.We also excluded patients who were taking anti-inflammatory drugs, corticosteroids or bisphosphonates. IL-17, bFGF and ANGIOP-2 were measured in patients' serum with ELISA method according to the manufacturer's instructions. The MCD assessed after immunohistochemical staining using monoclonal antibody to mast cell tryptase. The MCD was measured in three hot spots (maximum vasculature area) x 100 and then we measured mast cells x 400, using a graduated slide which corresponds to an area of 0.0625 mm2. MCD was calculated as mean MCD / HPF.
Results: Statistically significant differences between patients and controls were observed in all measured parameters, MCD (p <0.001), bFGF (p <0.01) and ANGIOP-2 (p <0.01), and for IL-17 (p <0.04). All parameters were increased in parallel with ISS stages (p <0.001) in all cases. Finally, the MCD and IL-17 correlated significantly with all the measured parameters (p <0.001)
Summary/Conclusions: The mast cells increase in the bone marrow(BM) of patients with MM. They release several transmitters that promote directly and indirectly the development of disease progress of MM also accompanied by increased angiogenesis in BM. In conclusion, mast cells and angiogenic factors seem to be important elements in the development of MM and become potential targets for the treatment and prognosis of the disease.

PB1951

HEALTHCARE RESOURCE UTILIZATION ASSOCIATED WITH DIFFERENT TREATMENT MODALITIES OF RELAPSED/REFRACTORY MULTIPLE MYELOMA PATIENTS IN THE US: FINDINGS FROM PREAMBLE

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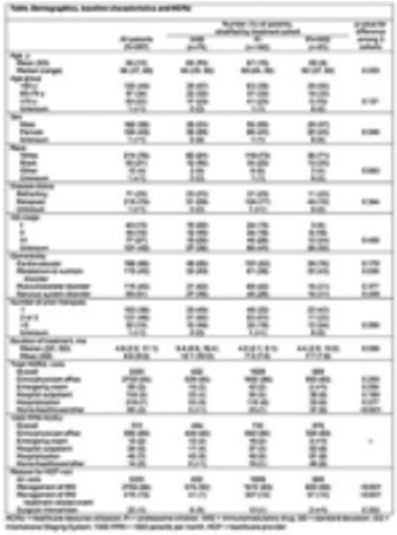
Background: Proteasome inhibitors (PIs), immunomodulatory drugs (IMiDs) and treatments involving both a PI and an IMiD (PI+IMiD) are the principal therapies for treating relapsed/refractory multiple myeloma (RRMM). The widespread adoption of these treatments may come with high healthcare resource utilization (HCRU), of which key drivers are reported in past research. It is important to further understand HCRU by different treatment modalities in real-world practice settings.
Aims: To evaluate HCRU in patients receiving different treatment modalities for RRMM.
Methods: US patients with RRMM, aged ≥18 y, with at least one prior therapy who initiated treatment with a PI, IMiD or IMiD+PI within 90 d before or 30 d after study enrollment (index therapy), were identified from PREAMBLE, an ongoing, prospective, multinational, non-interventional observational study. Patient data collected at each healthcare provider (HCP) visit, over a 3-y period or until the end of patient follow-up, included clinic/physician office visits; home healthcare, hospital outpatient and emergency room visits; and hospitalizations. Demographics and baseline characteristics were summarized using descriptive statistics. HCRU and its associated costs were analyzed using a standard per-1000 patients-per-month metric.
Results: 287 patients (median age 66 y; 56% male) were enrolled in the US. At the time of data cut-off (Sep 2016), 136 (47%) were still in the study and 151 (53%) had withdrawn; 92 (61%) of those withdrawn had died. Median (range) follow-up was 12.7 (0.5–41.0) mo. At study entry, patients were divided into three cohorts based on index therapy: PI (n=162, 56%; carfilzomib n=82/162; bortezomib n=80/162), IMiD (n=74, 26%; pomalidomide n=32/74; lenalidomide/thalidomide n=42/74), and PI+IMiD (n=51, 18%; carfilzomib and/or pomalidomide n=17/51; other n=34/51). The three groups were similar with regard to sex, race, disease status, ISS stage, comorbidities and number of prior therapies (Table 1).

Table 1.



The median duration of treatment (mDoT) was longer for patients on IMiD (6.4 mo), but shorter for those on PI (4.2 mo) or PI+IMiD (4.4 mo). In the PI cohort, carfilzomib had a shorter mDoT than bortezomib (3.5 *vs* 4.7 mo). Of 3220 total HCP visits, the most common type was clinic/physician office (2732, 85%), followed by hospitalization (210, 7%) and hospital outpatient (54, 5%). Mean per-1000 patients-per-month total visits were higher for PI+IMiD (876) than for PI (750) and IMiD (494). This remained true for clinic/physician office, hospital outpatient and home healthcare/other. Patients on PI had more visits for management of MM treatment-related events (16%) than those on PI+IMiD (10%) or IMiD (7%) (Table 1). Notably, among patients on PI, those on carfilzomib had high mean per-1000 patients-per-month total visits (827), with per-1000 patients-per-month emergency room visits (18) and hospitalizations (78) higher than any other treatment; 19% (175) of visits were made for management of treatment-related events.
Summary/Conclusions: Routine management of MM and treatment-related events drive HCRU, which may differ by treatment. Hospitalizations and hospital outpatient visits remain key drivers of HCRU in MM, which highlights an unmet medical need for effective therapy with better safety profiles.

PB1952

ASSOCIATION OF SERUM HEAVY/LIGHT CHAIN PAIR SUPPRESSION WITH RISK FACTORS FOR PROGRESSION IN MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE AND SMOLDERING MYELOMA

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Background: Monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma (SMM) are conditions that usually precede symptomatic multiple myeloma (MM). Risk stratification is crucial, considering the heterogeneous progression rate among these patients and the chemoprevention trials encouraged for high risk individuals. A number of prognostic factors for progression have been identified. In this sense, the novel Hevylite assay now enables us to accurately measure each isotype-specific heavy and light chain (HLC). Recently, isotype-specific uninvolved HLC pair suppression was described as an independent predictor of progression to MM in patients with MGUS. The role of Hevylite as a prognostic factor in SMM is less investigated.
Aims: The aim of the present study was to analyze the impact of HLC pairs in a series of patients with high risk MGUS and SMM and their relationship with other previously described risk factors.
Methods: Forty-four patients diagnosed with high risk MGUS or SMM at a single institution from March 2014 through April 2016 were prospectively included in the present study. Patients were stratified according to the Mayo Clinic and the Spanish PETHEMA group models. Samples at diagnosis were tested for HLC concentrations for the three pairs (IgG, IgM and IgA) by immunonephelometry.
Results: The clinical characteristics and risk stratification of patients are summarized in Table 1.

Table 1. Patient characteristics and risk stratification.

	All patients *N*= 44	MGUS *N*=26	SMM *N*=18
Median age, years (range)	69 (42-88)	69 (42-88)	68 (45-82)
Female gender, *N* (%)	21 (48)	12 (46)	9 (50)
HLC isotype, *N* (%)			
IgGκ	7 (16)	4 (15)	3 (16)
IgGλ	6 (13)	1 (4)	5 (28)
IgAκ	10 (23)	5 (19)	5 (28)
IgAλ	10 (23)	5 (19)	5 (28)
IgMκ	10 (23)	10 (38)	0
IgMλ	1 (2)	1 (4)	0
Serum M- protein, g/L; median (range)	15.2 (0-32.8)	14.1 (0-24.5)	23.9 (10.6-32.8)
Bone marrow plasma cell (%), median (range)	8 (1-97)	4.5 (1-9)	16.5 (10-97)
Proportion of normal plasma cell (%), median (range)	16 (0-100)	38 (0-100)	7.5 (0-88)
Presence of urine light-chain, *N* (%)	25 (58)	14 (56)	11 (61)
Immunoparesis, *N* (%)	20 (45.5)	11 (42)	9 (50)
"Evolving" pattern, *N* (%)	12 (27)	7 (27)	5 (28)
Mayo Clinic risk group (MGUS)*			
2 (High-intermediate)		19 (73)	
3 (High)		7 (27)	
Mayo Clinic risk group (SMM)**			
1			7 (39)
2			8 (44)
3			3 (17)
Spanish PETHEMA risk group (SMM)***			
0			7 (39)
1			2 (11)
2			7 (39)

Risk factors for stratification: (*) serum M-spike > 15 g/L, non IgG isotype, serum free light-chain ratio <0.26->1.65); (**) serum M-spike ≥ 30 g/L, bone marrow plasma cells ≥ 10%, serum free light-chain ratio <0.125->8; (***) ≥95% abnormal bone marrow plasma cells, immunoparesis

An abnormal HLC-pair ratio was detected in 96% of MGUS and 94% of SMM patients, with no differences depending on the heavy chain isotype. A highly abnormal HLC ratio (<0.02 or >45) was present in 9 patients (1 with MGUS and 8 with SMM). HLC-pair suppression (*i.e.*, IgG-κ in patients with IgG-λ gammopathy) was more frequent in patients with SMM (83% *vs* 46%, p=0.02). Severe HLC-pair suppression (>50% below lower level of normal) was present in 12 (27%) patients, the majority of which had a diagnosis of SMM (83%). Severe HLC-pair suppression was significantly associated with a highly abnormal (<0.125 or >8) serum free light chain (FLC) ratio (p=0.004), abnormal/normal bone marrow plasma cell ratio >0.95 (p<0.001) and immunoparesis (p=0.005), being present in 6 (86%) of the 7 patients with high risk SMM. Suppression of the other isotypes (*i.e.*, IgA or IgM HLC pairs in a patient with IgG gammopathy) was identified in 33 (75%) patients, namely in 18 (69%) patients with MGUS and 15 (83%) patients with SMM (p=0.48), and was not significantly

associated with other risk factors for progression. Severe suppression (>50% below lower level of normal) was significantly more frequent in SMM patients (33% *vs* 8%, p=0.04) and was associated with highly abnormal FLC ratio (p=0.001), abnormal/normal plasma cell ratio >0.95 (p<0.001), severe HLC-pair suppression (p<0.001) and highly abnormal HLC ratio at diagnosis (p=0.005). The "evolving" pattern of the serum M-protein was identified in 12 patients (MGUS 7, SMM 5) and it was not significantly associated with either severe suppression of the HLC-pair or of the other isotypes. After a median follow-up of 18 months (range, 6-35) progression to symptomatic MM was observed in 3 patients. All 3 had a diagnosis of SMM with an "evolving" pattern, highly abnormal HLC-ratio and severe HLC-pair suppression.
Summary/Conclusions: The findings presented in this study indicate that highly abnormal HLC ratio, severe suppression of the HLC-matched pair and other isotype HLC pairs are associated with known risk factors for disease progression in patients with high risk MGUS and SMM. The HLC assay could become a valuable tool in the risk stratification of these patients.

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EXTRAMEDULLARY MYELOMA IN THE "NOVEL AGENTS ERA": OUTCOME, HETEROGENEITIES AND PECULIARITIES OF A COHORT OF 84 PATIENTS RETROSPECTIVELY ANALYSED IN A MONOCENTRIC EXPERIENCE

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Background: Extramedullary disease is an uncommon manifestation in multiple myeloma (MM) and can either accompany newly diagnosed disease or develop with disease progression or relapse. Extramedullary myeloma (EMM) seems to have a different pathogenesis from its much more frequently encountered medullary counterpart, showing often a poor prognosis. EMM clinical situations are extraordinarily heterogeneous and their management is challenging.This includes organ or tissue involvement resulting from hematogenous-spread and/or bone involvement originating from different kind of bones.
Aims: We evaluated the impact of this disease features on patients' outcome in the context of novel-agents.
Methods: We reviewed 84 patients presenting EMM (median age 60, range 30-76)describing clinical and biological features (Figure 1B. Our aim was studying prognosis of bone-related extramedullary-disease (bEMD) and its relationship with soft-tissue related EMD (sEMD) in MM patients in our institution.
Results: 42 bEMD and 42 sEMD patients treated at Our Department between 2007 and 2016 were included in this study. Of the first group 10 presented EMM at diagnosis and 32 at relapse as well as 7 and 35 respectevely of the second series. 31 among sEMD were dead and 11 were alive, 20 of bEMD patients were dead and 22 were still alive. EM was diagnosed using imaging techiniques such as PET-CT (35%) or magnetic resonance MRI (65%). Biopsies were carried out only if the lesion was accessible (62%). The treatment was heterogeneous and all patients had receveid either thalidomide or bortezomib in the first-line of therapy. We showed that sEMD cohort has a significantly poorer survival compared to bEMD patients (median OS from diagnosis of EMD of 13 *versus* 58 months, P<0001). Finally lung, liver (parenchyma-EM) and SNC involvement among sEMD patients has shown a poorer outcome when compared to skin and lymph nodes masses respectively median OS of 12 and 10 months *versus* 18 and 15 months P <0001). Conversely among bEMD group there wasn't a significant advantage of outcome regarding the different bones involved. Kaplan-Meier estimates were used for survival analysis and differences between survival-times in patient subgroups were tested using the log-rank test (Figure 1A). Interestingly extramedullary-spread can be triggered by an invasive-procedures (surgery) or by a bone-fracture.In our population we have a case of breast-plasmocytoma diagnosed accidentally after reconstructive breast-surgery,where Polymerase Chain Reaction of immunoglobulin gene-rearrangement in the breast tissue excised confirmed monoclonal-CD 138/lambda plasma-cells. This patients at first was treated with VTD-regimen followed by tandem-ASCT and after EM-relapse achieved complete remission with haploidentical-bone-marrow-transplantation. Allogeneic transplantation should however be remembered in the therapeutic-armamentarium against EM especially in high-risk-young-patients. Furthemore often it has been described in the literature association between EMD, IgD subtype and FLC (free light chain)escape. In fact in our study we have reviewed 6 cases of IgD and 4 FLC-escape, all of them were observed in relapse-setting and in sEMD group. Finally the mechanism of extramedullary spread are poorly established: maybe a decrease expression of integrins and CD56 is involved. In our population absence of CD56 protein was shown in 56% of sEMD group and in 15%of bEMD case-series.
Summary/Conclusions: Cinical features of MM-patients with bEMD were different from the patients with sEMD. Outcome of this population was significantly better than the patients with sEMD, and was comparable to the patients without EMD. Even in the era of novel drugs extramedullary soft tissue has a poor prognosis expecially in a relapse-setting. This work shows a significant difference in prognosis for different type of extramedullary-disease even between sEMD (better OS of skin and lymph nodes involvement) suggesting a different biological-behavior.

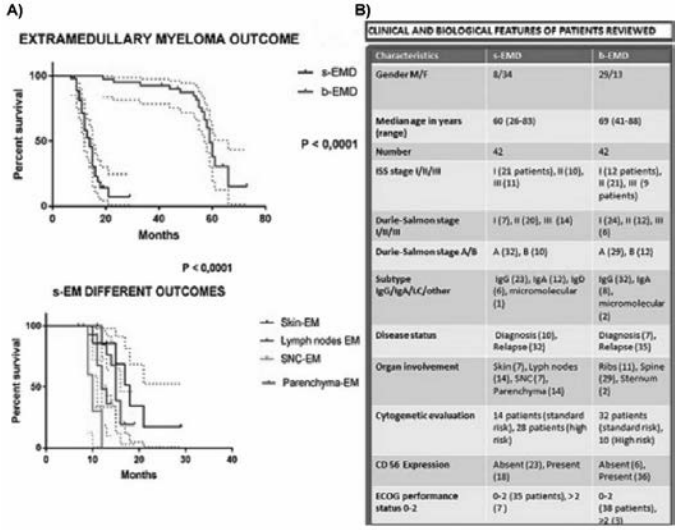

Figure 1.

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DINAMIC PREDICTIVE FACTORS FOR A BETTER STRATIFICATION OF PATIENTS WITH R-ISS II NEWLY DIAGNOSED MULTIPLE MYELOMA

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Background: Revised International Staging System (R-ISS), combining the ISS score with cytogenetics and serum LDH, represents the most recent prognostic model for stratifying newly diagnosed multiple myeloma (MM) patients into three different survival groups. Although data for R-ISS development have been obtained from patients enrolled in clinical trials, this prognostic score has been validated also in real-life scenario (Tandon *et al.*, 2017). In both non-clinical trial setting and IMWG experience, the majority of patients (about 65%) belonged to the intermediate risk group (R-ISS II) that, probably, needs better prognostication.
Aims: The aim of this study was to search for a closer stratification of MM patients with R-ISS II, taking into consideration dynamic aspects, such as therapeutic strategy and response to therapy.
Methods: We investigated the impact of variables, such as initial therapy, response to therapy and maintenance therapy, on PFS and OS in 108 newly diagnosed MM patients classified as R-ISS stage II, diagnosed between 2005 and 2015, who received novel agents such as immunomodulatory drugs and proteasome inhibitors. Score weights of the prognostic factors, found to be significant according to Cox regression model, were determined based on the regression coefficients.
Results: Median age of the 108 patients was 69 years (range 44-93) and 35% of them were older than 75 years. Thalidomide- and lenalidomide-based regimens were administered to 12% and 28% of patients, respectively, whereas 60% of the patients received bortezomib (54%) or carfilzomib-based (6%) regimens as induction therapy. Thirty-eight percent of the study population underwent ASCT and 40% received maintenance therapy. Regarding the response to the therapy, at least CR, VGPR and PR were documented in 35%, 66% and 87% of the patients, respectively. Five-year PFS and OS were 31% and 65%, respectively, similar to those reported by IMWG. Patients who did not achieve a CR, showed a significantly shorter 5yr-PFS (27% *vs* 50%; HR=2.9, 95%CI=1.6-45.0; p<0.0001) and 5yr-OS (53% *vs* 80%; HR=2.8, 95%CI=1.3-5.9; p=0.006) compared to those who did. Moreover, a significant better 5yr-PFS was documented in patients receiving maintenance therapy, compared to those who did not receive maintenance therapy (48% *vs* 20%; HR=1.9, CI95%=1.2-3.3; p=0.010) whereas initial therapy did not affect the outcome. Assigning a value to the variables found to be significantly related to survival measures, according to the above methods, patients were stratified into the following two groups: low-risk (LR), including 38 patients with score 0-1, *i.e.* patients achieving CR and receiving maintenance therapy (score 0) or achieving CR but not receiving maintenance (score 1); high-risk (HR) group, including 70 patients with score 2-3, *i.e.* not achieving CR, who underwent maintenance therapy (score 2) or not achieving CR and not receiving maintenance (score 3). Five year PFS of HR patients was significantly shorter compared to the LR group (20% *vs* 58%; HR=2.5, CI95%=1.6-3.8; p<0.0001), whereas 5-year OS was 57% *vs* 80% (HR=1.9, CI95%=1.1-3.3; p=0.021).
Summary/Conclusions: Our results suggest that in the R-ISS II MM patients,

the outcome of those achieving a CR and undergoing long-term therapy, is comparable with the outcome of the R-ISS I group. On the other hand, patients not achieving CR have a poor outcome, similar to those in the R-ISS III group. Therefore, these patients should require personalized therapy, aimed to achieve CR and to maintain therapy continuously.

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THE IMPACT OF THE UPDATED IMWG DIAGNOSTIC CRITERIA IN A REAL-LIFE SMM COHORT: A SINGLE CENTER EXPERIENCE

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Background: Recently, an update of the diagnostic criteria for smoldering multiple myeloma (SMM) & multiple myeloma (MM) was published by the International Myeloma Working Group (IMWG). In addition to CRAB criteria, 3 biomarkers of disease were introduced being (i) the presence of >60% clonal bone marrow plasma cells (BMPC), (ii) a serum free light chain ratio (FLC-ratio) >100 & (iii) the presence of >1 focal lesion on whole-body MRI (WBMRI). The introduction of these biomarkers has been shown to identify patients having a 70-80% risk of progression to MM over a 2-year time period.
Aims: To evaluate the impact of IMWG criteria in routine practice, focussing on (i) the prevalence of these biomarkers, (ii) the diagnostic strength of BMPC estimation using bone marrow aspirate *versus* biopsy & (iii) the added role of dynamic contrast-enhanced WBMRI (DCEMRI) in the evaluation of SMM patients.
Methods: We retrospectively identified 28 SMM cases diagnosed between 01/01/09-31/12/14. Sufficient data for analysis was available for 25 patients. All patients underwent standard clinical & laboratory evaluation, bone marrow examination & WBMRI (T1- (+/-Gd) & T2-weighted sequences, diffusion-weighted sequences & additional DCEMRI sequences using time intensity curves). Time to progression (TTP) is defined as time from diagnosis until MM development. Overall survival (OS) is defined as time from diagnosis until death from any cause. Survival analysis was performed using the Kaplan-Meier method & significance was tested using the log-rank algorithm. Intergroup analysis was performed using non-parametric rank-based analysis & correlation was calculated using the Pearson coefficient. Reported p-values are 2-sided with a significance level of 5%.
Results: Median follow-up was 64,1 months (analysis performed on 01/02/2017). No patients had a FLC-ratio >100 at time of diagnosis. Also, no patients with >60% of clonal BMPCs were seen. In 20 patients BMPC counts using both aspirate & biopsy were available. Analysis showed a significant higher estimate of BMPC levels using biopsy (14,8%, SD 4,99) *versus* aspirate (8,45%, SD 6,59) (p=0,002). Sensitivity of bone marrow aspirate was calculated to be 30% considering the 10% BMPC cut-off. Correlation between bone marrow aspirate & biopsy was found in 26,6% of cases. WBMRI-positivity was seen in 9 patients (36%). Progression was seen in 7/9 patients (78%) where only 1/16 WBMRI-negative patients (6,3%) developed MM (p<0,001). Median TTP was 19,9 months *versus* not-reached, respectively (p<0,001). No OS difference was seen between both groups (p=0,453). DCEMRI was positive in 14 patients (56%) thus identifying 5 additional WBMRI-negative patients with measurable bone marrow involvement. No significant difference concerning progression risk was however seen between WBMRI-negative patients being DCEMRI-positive (5/19, 26,3%) or -negative (14/19, 73,7%) (p=0,317). Analysis of baseline data revealed no significant difference concerning age, sex, genetic aberrations or the type of the monoclonal protein between both groups. In patients developing MM, progression was seen based on the development of anemia (5/8, 62,5%), bone pain (3/8, 37,5%), hypercalcemia (1/8, 12,5%) & the development of punched-out lesions (4/8, 50%). No renal insufficiency was seen.
Summary/Conclusions: Our data shows that WBMRI-positivity was the most frequent biomarker in a routine clinical setting. WBMRI-positivity, according to IMWG-criteria, clearly identifies patients with an increased risk of progression as was already shown previously. Although increasing the sensitivity of WBMRI, addition of DCEMRI-sequences didn't have an added benefit. Our sample size was however relatively small. And although IMWG-guidelines do not state clear requirements concerning the preferred type of bone marrow evaluation, our data shows that a bone marrow biopsy can never be ommited in suspected cases of SMM, as an aspirate alone clearly lacks diagnostic strength.

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RISK FACTORS FOR VENOUS THROMBOEMBOLISM IN 401 MULTIPLE MYELOMA PATIENTS: OBSERVATION OVER A 25-YEARS PERIOD IN A SINGLE INSTITUTION

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Background: Patients with multiple myeloma (MM) have shown an incidence of 3-10% of venous thromboembolic events (VTE). The introduction of immunomodulatory drugs (IMIDs) in the treatment regimen has further increased the risk of VTE, especially when combined with steroids or chemotherapy (20-30%). Actual guidelines recommend thromboprophylaxis measures, but the proposed strategies are the results of expertise consensus or derived from the extrapolation of data from many studies.
Aims: The aim of this study is to analyze the development of VTE in a large cohort of MM patients, treated for 25 years in a single institution, to assess risk factors suggested in general population, also to confirm the VTE risk of IMIDs based-regimens and the relevance of anticoagulant thromboprophylaxis.
Methods: Four hundred and one consecutive patients diagnosed with MM in a tertiary University Hospital between 1991 to 2015 were included. Data about VTE development, patient characteristics, myeloma-related factors, treatment and thromboprophylatic measures were retrospectively recorded. Multivariable correlates of VTE were assessed using Cox proportional hazards analysis.
Results: The median age at diagnosis was 66 years (range 24-90 years), and 47% were males. The results concerning treatment are extracted from 374 patients who were symptomatic and received myeloma treatment. Among the 164 patients that received IMIDs-based regimen, 27% did not receive any antithrombotic treatment, due to the lack of strong recommendations at the beginning of the use of IMIDs-based regimens. On the other hand, the most common thromboprophylaxis was set with LMWH (54%), followed by low doses of aspirin (13%) and anti-vitamin K (VKA) (6%). Median follow was 40 months (range, 1-293) and VTE occurred in 11% of patients, with a median time from diagnosis of 10 months. IMIDs based-regimen demonstrated to be a risk factor associated on multivariate analysis, and the relevance of thromboprophylaxis has been proved, as the absence of this measure increased significantly the risk. Other factors that have also demonstrated to be independently associated with a higher risk for VTE were: BMI ≥30 kg/m^2, prior Stroke or TIA, prior malignant neoplasm, and the use of high dose of dexamethasone.
Summary/Conclusions: Our data support the actual recommendation of antithrombotic prophylaxis in IMIDs-based regimens, especially in association with high dose of dexamethasone. We recommend the use of a risk factor model including obesity and previous history of thromboembolic disease or cancer, in order to guide the appropriate thromboprophylaxis measures.

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A PHASE III RANDOMIZED, OPEN-LABEL STUDY OF ISATUXIMAB (SAR650984) PLUS POMALIDOMIDE AND DEXAMETHASONE *VERSUS* POM AND DEX IN RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background: Treatment for refractory or relapsed and refractory multiple myeloma (RRMM) remains an unmet need. Isatuximab (ISA), an anti-CD38 monoclonal antibody with multiple mechanisms of tumor killing, has shown efficacy and an acceptable tolerability profile in Phase 1/2 studies in patients with RRMM (Richter *et al.* ASCO 2016; Vij *et al.* ASCO 2016; Richardson *et al.* ASH 2016).
Aims: This Phase III, prospective, multicenter, randomized, open-label study (NCT02990338; ICARIA-MM) is being conducted to evaluate the clinical benefit of ISA in combination with Pom and low-dose dex (Pom/dex) *versus* Pom/dex for the treatment of adult patients with RRMM.
Methods: Eligible patients are those with RRMM and demonstrated disease progression within 60 days of the last therapy. Patients will have received at least 2 prior lines of therapy, including lenalidomide and a proteasome inhibitor (bortezomib, carfilzomib, or ixazomib) alone or in combination. Patients will be randomly assigned in a 1:1 ratio to either ISA (10 mg/kg IV on Days 1, 8, 15, and 22 in the 1st cycle; Days 1 and 15 in subsequent cycles) plus Pom (4 mg on Days 1–21) and dex (at 40 mg for patients <75 years of age and at 20 mg for patients ≥75 years of age, on Days 1, 8, 15, and 22) or Pom and dex. Treatment cycles will be 28 days each. Patients will continue therapy until disease progression, occurrence of unacceptable adverse events (AEs), or their decision to discontinue the study, whichever comes first. All patients will be required to provide informed consent. The primary endpoint is progression-free survival (PFS), *i.e.* time from randomization to progressive disease or death from any cause. Response will be determined by IMWG criteria (2016). Key secondary endpoints include overall response rate and overall survival (OS). Safety evaluations include treatment-emergent AEs/serious AEs (including infusion-associated reactions), laboratory parameters, vital signs, and assessment of physical examination.
Results: Approximately 300 patients (150 in each arm) are expected to be enrolled in this study. Statistical analyses will be conducted according to a prespecified plan. The first patient was recruited in January 2017.
Summary/Conclusions: This Phase III, prospective, multicenter trial will provide a placebo-controlled evaluation of the efficacy and safety of ISA+Pom/dex, a combination which has previously reported preliminary clinical activity and manageable toxicities in heavily pretreated patients with RRMM in a single-arm Phase Ib study.

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LONG TERM SURVIVAL OF IGM MULTIPLE MYELOMA AND WALDENSTRŐM'S MACROGLOBULINEMIA PATIENTS

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Background: IgM multiple myeloma (MM) and Waldenström's macroglobulinemia (WM) are two hematologic malignancies with the common finding of IgM monoclonal gammopathy. IgM MM is a rare and poorly characterized disease.
Aims: The paper presents clinical and laboratory results of long term observations of 15 IgM MM patients selected from a group of 889 MM patients (1.6%) diagnosed and treated for several years at the Institute of Hematology and Transfusion Medicine in Warsaw as well as 15 WM patients investigated and treated at the same period of time at our hospital.
Methods: For analysis of serum proteins new Hevylite and Freelite tests (Binding Site Ltd Birmingham, UK) were applied as well as immunofixation using Sebia (Lisses, France) reagents. Fresh and archived frozen serum samples were used for the study.
Results: The clinical presentation of IgM MM patients is heterogenic starting with typical form for non IgM MM through predominant form with characteristic hyperviscosity syndrome and severe disease course to slow and latent form with survival time up to dozens of years. In 2 patients diagnosis of IgM MM was preceded by a 3-year period of monoclonal gammopathy of undetermined significance (MGUS) while in 4 patients (27%) diagnosis of WM was preceded by a 108, 84, 78, 9 months period of IgM MGUS. Median real overall survival of IgM MM patients was 50 months, 5 patients (33%) survived above 7 years and 2 patients (13%) survived above 12 years. Median overall survival of WM patients was 108 months, 7 patients (47%) survived above 10 years, 3 patients (20%) survived above 15 years. Lytic bone lesions were found in 11 (73%) IgM MM patients and in 3 (20%) WM patients. Urine monoclonal free light chains (FLC) detected by immunofixation was present in 60% of IgM MM patients and increased concentrations of involved monoclonal FLC and abnormal FLC κ/λ ratio in serum (by Freelite) in 75% of IgM MM patients. It was shown that IgM clonality in IgM MM and WM patients can be determined by using immunoglobulin heavy chain /light chain (HLC) immunoassays- Hevylite. Immunofixation and HLC ratios were concordant in all assessed IgM MM and WM patients. In IgM MM patients the suppression of uninvolved polyclonal IgM- detectable by using IgM HLC test - has prognostic significance. The evaluation of IgM HLC in 13 patients with IgM MM at diagnosis revealed a decreased concentration of uninvolved IgM (HLC IgMκ <0.33 g/L, HLC IgMλ <0.20 g/L) in 5 patients and normal values in 8 patients. Median overall survival in patients with a decreased uninvolved IgM was 15 months and in patients with normal polyclonal IgM 55 months ($p<0.01$).
Summary/Conclusions: 33% of IgM MM patients survive above 7 years and 13% above 12 years while 47% of WM patients survive above 10 years and 20% above 15 years. Suppression of uninvolved polyclonal IgM (detectable by using HLC IgM test) at the time of IgM myeloma diagnosis is unfavorable prognostic factor.

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MULTISITE PERFORMANCE EVALUATION STUDY OF THE BD ONEFLOW PLASMA CELL DISORDERS PANEL

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Background: The BD OneFlow solution for plasma cell disorders incorporates a standardized flow cytometry approach based on the EuroFlow (EF) Consortium liquid reagent system. The BD OneFlow solution enables reproducible identification and discrimination of distinct cell populations by combining standardized assays, setup reagents, and protocols. The plasma cell disorders (PCD) panel is composed of the BD OneFlow PCST (Plasma Cell Screening Tube) and BD OneFlow PCD. BD OneFlow PCST helps differentiate normal plasma cell populations from those requiring follow-up. The BD OneFlow PCD classification tube helps differentiate abnormal from normal plasma cell populations. The BD OneFlow PCD tube, when run in parallel with BD OneFlow PCST tube, characterizes the abnormal plasma cell population for identification of plasma cell disorders.
Aims: The objective of this study was to compare the accuracy between the BD OneFlow PCD system and the EF liquid comparator system.
Methods: De-identified remnant human bone marrow specimens (n=48) were collected at two study sites and tested in an unblinded manner within 26 hours of draw. Specimens were simultaneously stained with BD OneFlow PCD and BD OneFlow PCST tubes and EF specified liquid reagents. Acquisition and analysis were performed on a BD FACSCanto II instrument using standardized acquisition and analysis templates in BD FACSDiva software. For qualitative endpoints, overall agreement, negative agreement, and positive agreement, along with their one-sided lower 95% confidence limits, were calculated. For accuracy quantitative endpoints (% positive plasma cell population), the slope, intercept, and 95% confidence limits of the slope from a Deming regression were calculated for the BD OneFlow *vs* EF methods.
Results: The BD OneFlow PCD system is in 100% agreement (26 of 26) with the EF system in classifying patients as having normal plasma cell populations. BD OneFlow PCD system is in 100% agreement (22 of 22) with the EF system in identifying patients with a plasma cell disorder. Furthermore, the BD OneFlow PCD system correctly identified 100% of patients who had a plasma cell disorder based on clinical results.
Summary/Conclusions: The multisite evaluation between the BD OneFlow PCD system (PCST and PCD tubes) and the EF liquid reagent system was fully concordant in identifying patients with abnormal plasma cell populations. Additionally, all subjects identified as having plasma cell disorder based on clinical results were identified as having plasma cell disorder by the BD OneFlow PCD system. The BD OneFlow PCD panel is a fully standardized and validated system for aiding in the diagnosis of plasma cell disorders from bone marrow specimens.
BD OneFlow PCD and BD OneFlow PCST are for in Vitro Diagnostic Use; CE Marked to the European In Vitro Diagnostic Medical Device Directive 98/79/EC. 23-19565-00.

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PRACTICE GAPS AND BARRIERS TO OPTIMAL MANAGEMENT OF MULTIPLE MYELOMA PATIENTS : RESULTS FROM A MIXED-METHODS STUDY IN 8 EUROPEAN COUNTRIES

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Background: Previous studies have identified gaps and barriers in Multiple Myeloma (MM) patient care, especially in relation to treatment decision making. However, only few studies have aimed at better understanding the practice gaps, from the healthcare providers' perspectives, with the purpose to investigate the root causes of those gaps and find solutions to alleviate the challenges.
Aims: We conducted a study to identify the practice gaps and challenges in the diagnosis, treatment and management of MM patients, as experienced and reported by medical oncologists, haematologists and hemato-oncologists (HEM) and oncology nurses (NU) in 8 European countries between February 2016 and June 2016.
Methods: This mixed methods ethics-approved study included exploratory semi-structured interviews (phase 1) designed to generate in-depth discussion around challenges in the diagnosis, treatment and management of MM, followed by a quantitative online survey (phase 2) designed to validate the findings from the interviews with a larger sample. Practice gaps were identified through combined analysis of data from the in-depth interviews and online surveys.
Results: A total of 364 participants (HEM=281, NU=83) from France (n= 58), Germany (n=58), Russia (n=41), Spain (n=58), Italy (n=50), the UK (n=58), the Netherlands (n=16), and Belgium (n=25) participated in this study. Thirty-nine (39) interviews were conducted (HEM=28, NU=11) and 325 participants completed the online survey (HEM=253, NU=72). A majority (79%) of the sample had more than 10 years of clinical practice experience and over a third (39%) had over 20% of MM patients in their patient caseload. Three key findings were identified in the management of MM patients: 1) challenges in managing treatment side-effects. Forty percent (40%) of HEM reported lack of skills in managing cardiovascular side effects or symptoms. Over a third of HEM reported difficulties in managing fatigue (40%), skin toxicities (35%) or peripheral neuropathy (34%). NU reported being challenged by the management of renal insufficiency as a side effect or symptom (46%), peripheral neuropathy (36%), thrombosis (37%), and skin toxicities (33%). Additionally, 2) NU reported challenges in communicating with patients and educating them around their disease, especially in relation to treatment outcomes and long term side effects. For example, 51% of NU reported a lack of skills discussing sexual issues as consequences of the disease/treatment. Finally 3) there was a large variability across countries in the guidelines followed by HEM and NU for the treatment and management of MM patients. Detailed results, including country-specific analyses and investigation of the practice gaps' causalities, will be presented.
Summary/Conclusions: These findings provide real-life recent evidence of the challenges of HEM and NU in relation to specific aspects of the management of patients with MM with 3 main areas, challenges in managing side effects, communication with patients and leverage of guidelines which show differences between HEM and NU but also between countries. The findings support the needs for the development of tailored clinical tools, educational activities and performance improvement interventions, adapted to the local context at a country level. Efforts should aim to address those current challenges before new therapies, such as immunotherapies, become available.

These new agents, with their own specific safety and side effect profiles, are likely to add to the challenges already experienced by health care providers in their management of patients with MM.

PB1961

THE EXPRESSION OF APRIL BY MULTIPLE MYELOMA CELLS AND THEIR ROLE IN THE EVOLUTION OF MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) is a malignant proliferation of plasma cells and is characterized by the accumulation of monoclonal plasma cells in bone marrow that secrete pathological monoclonal immunoglobulins. Inductive factors secreted by tumor cells and other cells of the marrow microenvironment play an important role in disease progression. APRIL, by initial letters A Proliferation Inducing Ligand, is a member of the family of pro TNF, one of the main factors for the survival of immature and activated B cells. One of the main signal transduction pathways for activation of myeloma cells is NF-κB. APRIL, can directly activate the NF -κB and has been found by studies that are the most important factors for the survival of healthy and myeloma cells.
Aims: Aim of this study was the study of APRIL expression in myeloma cells in the bone marrow of patients with MM and their possible association with cell proliferation markers.
Methods: We studied 42 newly diagnosed patients with MM, 19 women and 23 men, aged 64,1±10,4 years. According to the ISS stage, 14 were stage I, 11 stage II and 17 stage III . Regarding the type of paraprotein that had been found, 23 IgG, 14 IGA and 5 patients with light chains. Serum samples and bone marrow biopsy samples were obtained from all patients prior to the initiation of treatment. Patients with impaired hepatic and renal function, chronic or acute infections, chronic inflammatory or autoimmune disorders or other malignancies were excluded from the study. We also excluded patients who were taking anti-inflammatory drugs, corticosteroids or bisphosphonates. 20 age and sex-matched healthy volunteers, were used as controls. The levels of IL-10 and IL-6 in the serum were measured by ELIZA. Bone marrow infiltration by neoplastic plasma cells was calculated in%. The expression of cell proliferation index was calculated in BM biopsy sections with immunohistochemistry techniques. The expression of APRIL was also calculated with immunohistochemistry. For the control of the process we used positive control. The assessing of the staining was checked in the optical microscope, over the whole surface of each sample and had to do with the cytoplasm of tumor cells. It was dotted with brown ting. Non-specific staining was observed at the other cellular components of BM. The degree of staining expression was evaluated as the percentage of neoplastic plasma cells and according to the intensity of staining in four-grade scale 0: negative,+1 weak, +2 moderate and +3 intense staining. Then the proportion of plasma cells stained for each type of staining separately, was calculated using the H-score method (Histoscore), based on the formula:% *% * 1+2+3% *. Our aim is to prove if the intensity of expression is associated with disease stage.
Results: Statistically significant differences were observed between patients and controls for all parameters measured (p <0.001 in all cases) . All values of the measured parameters increased in parallel with the ISS stages of the disease (APRIL p <0,005, bone marrow infiltration p <0.03 Ki-67p <0,01, IL-10 p <0,001, IL-6, p <0.001) Eventually APRIL correlated significantly with all measured parameters *e.g.* BM infiltration r=0,386, p <0,01, with Ki-67 r=0,390 p <0,01 IL-10 r=0,497 p <0,001, IL-6, r=0,484 p <0,001).
Summary/Conclusions: Increased expression of APRIL ligand plays an important role in development and pathobiology of MM and may be an important therapeutic target in the treatment of MM.

PB1962

DEVELOPMENT OF SECOND PRIMARY MALIGNANCY AFTER TREATMENT WITH LENALIDOMIDE: A SINGLE CENTRE EXPERIENCE

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Background: Lenalidomide is a well-established and effective treatment for haematological malignancies particularly multiple myeloma (MM), but also lymphoma and myelodysplastic syndromes. It can be used as both a single agent and in combination with dexamethasone or other chemotherapeutic agents. A previous study in myeloma patients demonstrated an increased incidence of second primary malignancy (SPM) in patients treated with lenalidomide and dexamethasone (7% - 8%) compared to those treated with dexamethasone and placebo (2%>3%) [1 - 3].
Aims: We reviewed all patients treated with Lenalidomide in a single centre from January 2008 to May 2016 to establish the real-world of SPM.
Methods: A database of patients (n=137) treated with lenalidomide in the specified timeframe was created from pharmacy records. A search of the hospital's patient management system was performed to identify: (1) type and date of primary haematological diagnosis, and (2) type and date of second malignancy based on histology. An analysis of the data was performed to establish: (1) incidence of SPM, (2) latency between primary haematological malignancy and SPM, (3) latency between starting lenalidomide and SPM, (4) types and subtypes of SPM.
Results: The majority of patients were treated for Multiple Myeloma (67%). Other primary haematological diagnoses included myelodysplastic syndrome(MDS), Non-Hodgkin Imphoma(NHL), and Idiopathic myelofibrosis(IMF). The incidence of SPM post-treatment with lenalidomide was 12 patients (8%). 9 patients had a diagnosis of second malignancy prior to starting lenalidomide treatment. The median latency between starting lenalidomide and SPM diagnosis was 24 months.. The median latency between primary haematological diagnosis and SPM diagnosis was 37 months. The most common types of SPMs were haematological; 7 (64%), patients had t-MDS 3 (27%), 3 Acute Myeloid Leukemia(AML) (27%), and 1 patient developed B-cell Lymphoma (9%). Next most common was skin malignancy; 3 (27%), all squamous cell carcinoma (SCC). One patient developed prostate carcinoma (9%).
Summary/Conclusions: Studies show the estimated incidence of SPM in MM to lie between 2%>10% over a 25 year period [5]. This study demonstrates a higher incidence (12%), however it includes patients treated for other primary haematological malignancies. This data demonstrates a similar incidence of SPM to previous studies (8%) post-treatment with lenalidomide [1 - 3]. Haematological malignancy was the commonest SPM however this differs from other studies which showed a majority of solid tumors (including skin malignancy) [4]. We found t-MDS/AML and skin malignancy to be the most significant SPMs. This is in agreement with some published reports [4, 5]. No analysis was made of patient specific risks or disease specific risks. This data supports the conclusion that the risk of SPM must be considered before commencing a patient on lenalidomide therapy.

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PB1963

SOLITARY PLASMACYTOMA. A SINGLE-CENTRE RETROSPECTIVE STUDY

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Background: A solitary plasmacytoma (SP) is a rare neoplastic mass of monoclonal plasma cells that can either be localized in bone (solitary plasmacytoma of bone, SPB), or soft tissue (extramedullary plasmacytoma, EMP), without evidence of multiple myeloma (MM). The median age at diagnosis is 65 years. Some patients present a monoclonal band (MB) at diagnosis, and a proportion progresses to MM. The low incidence of this entity has prevented reaching definite conclusions with regards to prognostic factors and treatment.
Aims: In this study we retrospectively analysed the clinical presentation, treatment and outcome of all patients with SP treated in our centre in order to establish relevant prognostic clinical features and management options.
Methods: Between 1985 and 2016, 27 patients with SP (20 SPB, 7 EMP) were treated in Ramon y Cajal Hospital (Madrid), with a median follow up of 8 years. The time to relapse, progression to MM or death was measured in months. The progression free survival (PFS) and overall survival (OS) were estimated using the Kaplan-Meier method. The comparison of PFS/OS was performed using the Log Rank test. Student´s T test was used to compare the average age at diagnosis. To determine the association between the presence of MB or the subtype of SP, and progression to MM, we used Fisher's exact test. All statistical analysis was performed with the software SPSS 24.0.
Results: The average age at diagnosis was 56 years (±18): 51 (±17) for SPB, and 72 (±5) for EMP (p<0.05), with a male:female ratio of 2.4:1. The most frequent location was the axial skeleton (80%) for SPB, and the airway in the case of EMP (57%). In most cases, the initial symptom of SPB had been pain

(45%), and local discomfort for EMP. 52% of patients presented a MB at diagnosis, without significant differences between subgroups. With regards to treatment, combined therapy was the preferred option in the case of SPB (60%), whereas unimodal treatment strategies were more frequently used in EMP (86%). 11 of the 20 patients with SPB progressed to MM (55%) in a median time of 4 years, while none of the patients with EMP progressed (p<0.05). The 5 year PFS and OS was 61% and 90% respectively, 31% and 74% at 10 years. Although a tendency towards a higher PFE was observed in the EMP group, it was not statistically significant. No differences were found in PFS/OS between age groups (<60 or ≥60 years), axial *vs* appendicular skeleton location in SBP, type of treatment received, or the presence of MB. Furthermore, no association was found between the presence of MB at diagnosis and progression to MM (Figure 1).

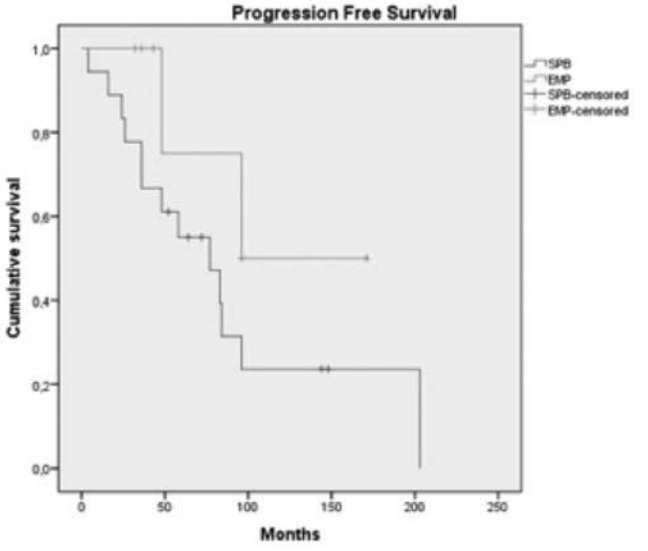

Figure 1.

Summary/Conclusions: The age at diagnosis of SPB is significantly lower than EMP. Moreover, the progression to MM is notably higher in this group of patients. These distinct characteristics in clinical presentation and outcome could suggest a biological difference between both entities.

PB1964

RISK STRATIFICATION ALGORITHM USING REAL-WORLD DATA FROM PATIENTS WITH RELAPSED AND/OR REFRACTORY MULTIPLE MYELOMA: DESCRIPTION OF CLINICAL OUTCOME BY TREATMENT REGIMEN

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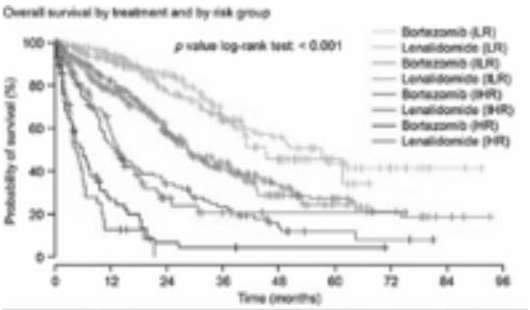
Background: Estimation of survival for patients with RRMM, requires prognostic tools that define the relative risk of death after first relapse. We recently developed a risk stratification algorithm (RSA) using real-world data from the Czech Registry of Monoclonal Gammopathies (RMG). Our RSA uses patient and disease characteristics at diagnosis and at initiation of second-line treatment (2L), and previous treatment outcomes to stratify patients based on their overall survival (OS) expectations from initiation of 2L treatment (Hajek *et al.* Blood 2016). The value of such an algorithm depends on its validation, but also on understanding the evidence that explains these differences in survival expectations.
Aims: To describe 2L treatment patterns by RSA group and to report OS, progression-free survival (PFS) and response by treatment received in 2L per RSA risk group.
Methods: Data were collected from the Czech RMG for patients aged ≥18 years who were diagnosed with symptomatic MM between May 2007 and April 2016 and in whom 2L treatment had been initiated. Predictors of OS from the start of 2L were identified using Cox regression analyses. Hazard ratios for each OS predictor were multiplied to obtain an overall score for each patient. Risk groups were defined based on the overall score. To provide optimal patient stratification, cut-offs of the score were estimated using K-adaptive partitioning for survival (KAPS) analysis.
Results: Data from 1418 patients were analysed. KAPS analysis defined four groups based on risk of death: low (LR; score ≤ 4.1; n=403), intermediate-low (ILR; score 4.2–10.3; n=635), intermediate-high (IHR; score 10.4–20.1; n=237) and high (HR; score ≥20.2; n=143) risk. Median OS (months) was 57, 29, 13 and 5 for the LR, ILR, IHR and HR groups, respectively. Following stratification, compared with patients in the lower risk groups, a higher proportion of those in the HR group had LDH levels above 360 U/L and an Eastern Cooperative Oncology Group Performance Status of 3–4 at initiation of 2L. Treatments received at 2L were similar across all risk groups, with bortezomib and lenalidomide being the most common 2L treatments. Patients who received bortezomib at 1L were often given lenalidomide or thalidomide at 2L and those who received thalidomide at 1L were frequently given bortezomib at 2L. This suggests that 2L treatment choice was not defined by the underlying risk of death for each patient, but rather by the type of previous treatment. For patients receiving bortezomib at 2L, median OS (months) from start of 2L was 57, 29, 13 and 6 (Figure 1), and median PFS (months) was 18, 12, 8 and 3 in the LR, ILR, IHR and HR groups, respectively. A very good partial response or better (VGPR+) was reported for 29.3%, 31.0%, 18.7% and 19.6% of patients in the LR, ILR, IHR and HR groups, respectively. For patients receiving lenalidomide at 2L, median OS (months) was 45, 29, 14 and 5, and median PFS (months) was 20, 12, 10 and 3 for patients in the LR, ILR, IHR and HR groups, respectively. A VGPR+ was reported for 33.6%, 22.9%, 26.0% and 7.1% of patients in the LR, ILR, IHR and HR groups, respectively.



Treatment at second line	Overall survival	Low risk	Intermediate-low risk	Intermediate-high risk	High risk
Bortezomib	Median (95% CI)	57 (47–68)	29 (25–32)	13 (10–17)	6 (4–8)
	Hazard ratio (95% CI)	Reference	2.1 (1.5–2.8)	4.1 (2.9–5.8)	10.2 (6.9–15)
	p value		< 0.001	< 0.001	< 0.001
Lenalidomide	Median (95% CI)	45 (33–58)	29 (22–35)	14 (10–17)	5 (3–7)
	Hazard ratio (95% CI)	Reference	2.0 (1.4–2.8)	4.1 (2.7–6.4)	14.6 (9.1–24)
	p value		< 0.001	< 0.001	< 0.001

CI, confidence interval; HR, high risk; IHR, intermediate-high risk; ILR, intermediate-low risk; LR, low risk.

Figure 1.

Summary/Conclusions: The RSA effectively stratifies patients according to OS from initiation of 2L. However, these results must be validated in an external dataset. The outcomes of each risk group are mainly driven by the underlying risk of death at initiation of 2L; treatment with bortezomib or lenalidomide provided similar outcomes independent of risk group. Use of our RSA at 2L would support physician decision making to improve patient specific care.

PB1965

LACK OF CD56 EXPRESSION IN MULTIPLE MYELOMA PATIENTS WITH RISS 2 DISEASE IS ASSOCIATED WITH WORSE PROGNOSIS AND ABOLISHED WITH AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background: Multiple myeloma (MM) is a hematologic disease in which accumulation of malignant plasma cells and high levels of monoclonal protein and free light chains lead to bone marrow failure, hypercalcemia, lytic bone lesions and renal failure. Myeloma cells are distinguished from normal plasma cells by an aberrant immunophenotype.They express CD56, which is present in 70-80% and can be used to distinguish myeloma cells by flow cytometry. The expression of CD56 is constant throughout the course of the disease. The lack of CD56 expression in myeloma cells decreases the adherence of myeloma cells to the cell matrix and is associated with higher levels of bone marrow infiltration and peripheral blood involvement, higher incidence of extramedullary disease, renal insufficiency, Bence Jones protein, plasma cell leukemia and t(11;14). The lack of CD117 expression is associated with higher levels of bone marrow infiltration, renal impairment, elevated β2-microglobulin and cytogenetic

aberrations including t(11;14), t(4;14) and del(13q). CD28 expression is present in 15 – 45% of patients and is associated with unfavorably cytogenetic changes including t(4;14) and del(17p) and shorter PFS and OS despite aHSCT.
Aims: Aim of our retrospective study was to evaluate the impact of CD56, CD117 and CD28 expression on clinical characteristics and PFS in newly diagnosed MM patients treated with bortezomib based induction therapy.
Methods: We retrospectively analyzed 110 newly diagnosed MM patients from our national registry that had data available at the time of diagnosis. Immunophenotype was determined using a panel consisting of CD19/CD38/CD45/CD56/CD138 to distinguish and to enumerate MM cells. Monoclonal antibodies directed against CD20, CD28, and CD117 were used additionally. All samples were routinely tested for the presence of recurrent chromosomal aberrations, *i.e.* del 1p, amp1q, del6q, amp15q, del13, del17, t(4;14), t(14;16) and t(11;14) using commercially available DNA probes.
Results: We found no association between CD56 expression and age, gender, elevated LDH, cytogenetic risk or RISS stage. We found a strong association between lack of CD56 expression and light-chain only or asecretory myeloma. There was an association between CD28 expression and female gender (Table 1). In multivariate analysis including age, elevated creatinine, RISS, aHSCT , CD28, CD56 and CD117 expression, CD56 expression was associated with a 47% reduced hazard for progression (Exp(B)=0.527, p=0.03). Other factors with statistically significant impact on progression were aHSCT and age. In patients not undergoing aHSCT lacking CD56 expression in comparison to those with an aberrant CD56 expression, the difference in PFS was statistically significant with a PFS of 8 *vs* 18 Month (Log Rank p=0.088, Breslow p=0.046). When stratified according to RISS stage, only patients in stage 2 disease had a significant reduction in PFS with lack of CD56 expression.

Table 1.

Association of CD56, CD28 and CD117 expression with clinical factors.

	CD56		CD28		CD117	
	X^2	p	X^2	p	X^2	p
Age> 70 years	0.001	0.975	0.018	0.894	0.407	0.523
Gender (M)	1.459	0.227	3.909	0.048	0.970	0.325
LDH elevated	1.061	0.303	0.130	0.718	2.551	0.110
Cytogenetic risk	0.11	0.991	0.093	0.993	5.902	0.117
t(4;14)	1.4	0.237	0.098	0.952	1.585	0.453
Creatinine, elevated	3.239	0.072	2.417	0.120	4.341	0.037
Light chain/asecretory myeloma	7.307	0.007	0.541	0.763	1.421	0.491
RISS	0.411	0.814	1.645	0.439	2.641	0.267

Summary/Conclusions: CD56 expression was prognostic for PFS only in the patient cohort not undergoing aHSCT. As previously reported aHSCT seems to abrogate the negative impact of CD56 negativity. We propose CD56 expression to be used as a prognostic marker in patients with RISS 2 stage disease and when possible these patients should undergo aHSCT.

PB1966

AUTOLOGOUS TRANSPLANTATION FOR MULTIPLE MYELOMA IN GERMANY – REAL-WORLD DATA FROM A NATIONWIDE, MULTI-INSTITUTIONAL SURVEY IN 2015-2016

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Background: A nationwide, multi-institutional survey was performed in 2015 and 2016 to analyse routine practice for myeloma patients outside clinical trials in Germany.
Aims: We aimed to investigate implementation of autologous stem cell transplantation (ASCT) into treatment of patients with newly diagnosed or relapsed multiple myeloma (MM) in Germany.
Methods: The analysis is based on a database built from university hospitals (UH), community hospitals (CH), office-based hematologists (OBH). Anonymized data were collected online based on retrospective chart review. The completeness and the plausibility of the data were verified in real-time and by online personal checks. We investigated which institutions initiated treatment in patients with ASCT, which were the characteristics for patients not-considered eligible for transplantation, how stem cell mobilization was performed, how many patients dropped out before planned transplantation and what were the frequencies of tandem ASCT and ASCT for relapsed disease.
Results: Data from 515 patients from 51 centres were available for the first half of 2015 and from 867 patients from 52 centres for the first half of 2016. There were 40% (2015) and 32% (2016) pts considered as eligible for ASCT in 1st line. Although the proportion of patients older than 69 years was not significantly different between health care providers in 2015 and 2016 (2015: 47%UH, 60%CH, 49%OBP / 2016: 54%UH, 56% CH, 47%OBP), patients were considered more often transplant-eligible in UH (2015: 49% / 2016: 53%) than in CH (2015: 29% / 2016: 21%) or OBH (2015: 45% / 2016: 26%). In first-line treatment, 52% of patients eligible for SCT received mobilization chemotherapy in addition to induction therapy. More than 80% of patients received a cyclophosphamide-based chemotherapy in combination with G-CSF for stem cell mobilization in 2015 and 2016. Most participating institutions aimed at collection of three sufficient stem cell transplants (2015: 48% / 2016: 46%). Once patients completed stem cell mobilization, 92% continued to high-dose chemotherapy and 92% of them received ASCT finally. 25% of transplant patients were treated with tandem ASCT in 1st line. In 2015, 8% of patients and 1% of patients in 2016 were considered eligible and were ultimately treated with ASCT for relapsed disease. The most frequent reason for transplant-eligible patients not receiving ASCT were withdrawal of patients consent (first-line: 15%, second-line: 39%).
Summary/Conclusions: With our current analysis of a nationwide survey performed with different health care providers in Germany we demonstrate that implementation of ASCT is strongly influenced by the institution initiating primary therapy. Age does not seem to impact usage of ASCT compared to concomitant disease or patients´ and doctors´ preferences. Patients predominantly collect three autologous transplants, enabling a possible tandem ASCT and ASCT for relapsed disease.

PB1967

MODIFIED HYPERCVAD *VERSUS* BORTEZOMIB-HYPERCVAD IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) is an incurable plasma cell malignancy, in which aggressive relapses may require salvage cytotoxic infusional chemotherapy. Several clinical trials demonstrating the efficacy of bortezomib led to institutional practice changes where vincristine was replaced with bortezomib in the modified hyperCVAD (mod-CVAD) regimen, creating a new treatment regimen, 'bortezomib-hyperCAD' (bort-CVAD).
Aims: The primary objective is to describe the safety and efficacy of the hyper-CVAD regimen with vincristine or bortezomib in patients with relapsed or refractory MM treated at Oregon Health and Science University.
Methods: IRB approval was obtained to perform this retrospective analysis. We describe the effectiveness and tolerability of the 2 regimens among 33 patients with relapsed and/or refractory multiple myeloma (RRMM). Patients who received ≥1 cycle of mod-CVAD (n= 15) or bort-CVAD (n= 18) from Jan 1 2011 and Dec 31 2015 at the Knight Cancer Institute were included. Most patients were previously treated with/refractory to proteasome inhibitors (97%/76%) or immunomodulatory agents (82%/68%) respectively, 13 received prior autologous stem cell transplant (auto-HCT), the median number of prior lines was 3 (range 1-8). High risk cytogenetic factors t(4;14), t(14;16), or del 17p were present in 8 and extramedullary disease in 13 patients overall.
Regimens contained cyclophosphamide 300 mg/m2 IV every 12 hours for 8 doses; doxorubicin 9 mg/m2/day continuous IV infusion every 24 hours and dexamethasone 40 mg by mouth on days 1-4; vincristine 0.4mg continuous IV infusion every 24 hours on days 1-4 (mod-CVAD) OR bortezomib 1.3mg/m2 SQ on day 1 and 4 (bort-CVAD). All patients received MESNA 350 mg/m2 IV every 24 hours on days 1 through 4; granulocyte colony-stimulating factor 24-48 hours following the completion of chemotherapy; and standard infectious prophylaxis. International Myeloma Working Group uniform response and European Society for Blood and Marrow for minor response (MR) criteria were used.
Results: The median number of cycles given was 2 (range 1-6). Cycles were repeated every 3 to 4 weeks. Median follow up was 48 and 33 months in mod-CVAD and bort-CVAD respectively. The ORR was 40% in the mod-CVAD group: 6 partial (PR), 6 minor (MR), and 3 stable disease (SD) compared to 44.4% in the bort-CVAD group: 1 complete response, 7 PR, 2 MR, 6 SD and 2 progressive disease (Fisher's exact p=0.80). A total of 13 patients proceeded to auto-HCT. Median progression-free and overall survival for all patients were 6 and 11 months respectively, which was comparable between arms (Log rank test p=0.6635 and 0.7369). New or worsening of peripheral neuropathy occurred in 2 and 4 patients in the mod-CVAD and bort-CVAD groups respectively. There was no statistically significant association between treatment and febrile neutropenia, emergency department visits, hospitalizations, or peripheral neuropathy (Fisher's exact test P value >0.05). There were no statistically significant differences in safety and tolerability between treatment arms. Three and 6 patients in the mod-CVAD and bort-CVAD arms discontinued therapy due to toxicity or treatment complications respectively.
Summary/Conclusions: Overall effectiveness and safety outcomes were similar between mod-CVAD and bort-CVAD, with both regimens demonstrating an impressive response rate among heavily pre-treated patients with relapsed/refractory disease. This is a useful salvage strategy to gain rapid dis-

ease control; and as a bridge to other therapies including stem cell transplant and novel therapies.

PB1968

EFFICACY AND SAFETY OF LENALIDOMIDE AND DEXAMETHASONE IN PATIENTS WITH RELAPSED/ REFRACTORY MULTIPLE MYELOMA: A REAL LIFE EXPERIENCE FROM TURKEY

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Background: Lenalidomide, an immunomodulatory drug, was approved for treatment of relapse/refractory multiple myeloma (RR-MM). In Turkey, we have been used the combination of lenalidomide and dexamethasone (RD) for RR-MM patients after 2010. Therefore, we analyzed efficacy and safety of RD in Turkish patients with RR-MM.
Aims: We aimed to evaluate the outcome and the tolerability of the RD in patients with RR-MM who had been treated under the standard clinical practice between October 2010 and June 2016.
Methods: This is a retrospective, single center study. Patients' clinical and laboratory data were collected from patient' files. The overall and progression free survival (OS and PFS) were estimated by Kaplan–Meier methods. Log- rank test was used to evaluate the variables affecting OS and PFS (univariate analysis). Cox proportional hazards regression was used for multivariate analysis to analyze the independent variables affecting PFS and OS.
Results: One-hundred and twenty patients (71 male and 49 female) enrolled in the study. The median age at the start of RD was 64 years (29- 84) and the median number of previous line of treatment was 1 (1- 4). Seventy-two patients (60%) received RD as second-line therapy and 51 of patients (42.5%) treated with autologous stem cell transplantation (ASCT). With regard to the initial dose of lenalidomide, 82 (68.3%) of the patients received the recommended dose of 25 mg per day for 21 days in a cycle of 28 days. Objective response (≥PR) was observed in 87 patients (72.5%); 23 patients (19.2%) achieved CR. The median follow-up was 14 months (range, 1– 72 months), and the median DOR was 19 months (range, 12.4- 25.6 months). Median OS and PFS were 32 months (95% CI, 15.8–48.1 months) and 21 months (95% CI, 15.8- 26.1 months), respectively. In the multivariate analysis, the independent prognostic factors for OS and PFS were treated with previous ASCT, patients who achieved at least PR, patients receiving RD for more than 12 cycles. Adverse events occurred in 69 of patients (57.5%). Hematological and non-hematological adverse events were found at the same rate (n= 47, 39.2%). The treatment discontinuation rate due to AEs was 11.7% (14 patients). The overall incidence rate (IR, events per 100 patient-years) of second primary malignancies (SPMs) was 0.93 (95% CI, 0.04- 4.60). Tha rate of anemia was 12.5% and thrombocytopenia was 9.2% in all grades. Penumania (15.8%), fatigue (14.2%) and herpes infections (0.8%) have been reported as most frequent non-hematological side effects.
Summary/Conclusions: RD is a safe, well tolerated and effective treatment in patients with RR-MM. Good response, previous ASCT and using more than 12 cycles are associated with better survival. Higher OS and PFS and ORR seem to be related to using RD in the first relapse. Adverse events are manageable and lower with prophylaxis.

PB1969

OPTIMIZING THE MANAGEMENT OF NON-HEMATOLOGICAL ADVERSE EFFECTS RELATED TO LENALIDOMIDE IN RELAPSED MULTIPLE MYELOMA PATIENTS. ONE CENTER EXPERIENCE

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Background: During many years, the combination of lenalidomide and dexamethasone (Rd) has been an effective treatment for patients with relapsed or refractory Multiple Myeloma (RRMM). On the basis of the available evidence, treatment with Rd may continue in responding patients until progression or unacceptable toxic effects. The data suggest full dose lenalidomide is important for optimal efficacy and to improve the progression free survival (PFS). Approaches to achieve higher doses of lenalidomide could include continuing therapy in responding patients and proactive adverse effects (AEs) management.
Aims: The main aim was to evaluate the incidence of two of most common non-hematologic AEs related to lenalidomide (rash and dystonia) in patients who received treatment with Rd. The second end points were to evaluate the response of rash after switching the enoxaparin to bemiparin and to evaluate the response of the dystonia after treatment with clonazepam, instead of lenalidomide dose reduction.
Methods: We retrospectively reviewed a consecutive cohort of patients with RRMM receiving Rd (R: 25 mg on days 1 through 21. d: 40 mg on days 1, 8, 15, and 22) in 28-day cycles until progression or unacceptable adverse effects, from 2011-2016. All patients received thromboprophylaxis with low-molecular-weight-heparin (LMWH) (Enoxaparin 40 mg subcutaneous daily) the first 4 cycles; thereafter, patients were switched to aspirin 100 mg in a day prophylaxis. Bemiparin 7500 anti-Xa IU once-daily dose was employed if enoxaparin was suspended. Clonazepam dose to treat dystonia was 0,5 mg twice daily. Data were analyzed with SPSS statistical v 22.0.
Results: Between 2011 and 2016 a total of 65 patients received Rd in our center. Baseline characteristics are shown in Table 1. Patients received a median of 2 previous regimens (range 1-6). 51,5% of the patients had undergone one previous autologous stem-cell transplant (ASCT). Rash occurring in 12,3% of patients (grade 2), all of them were concurrently reciving enoxaparine. All rashes resolved switching the enoxaparine to bemiparine, maintaining same dose of lenalidomide. Neither treatment with esteroids or antihistaminic were administrated. Dystonias were reported in 23,1% of patients (grade 2), all of them dissapeared after treatment with clonazepam without lenalidomide dose reduction.

Table 1.

BASELINE CHARACTERISTICS	
Median age (range) — yr	70,5 (38-90)
Gender (M/F) %	45 / 54
Myeloma type (IgG/IgA/IgD/BJ) %	56 / 23 / 5 / 16
Disease stage according to International Staging System — (%) I / II / III	29 / 37 / 34
Adverse Cytogenetic [delp53, t(4;14), t (14;16)] Yes (%)	17%

Summary/Conclusions: Rash and dystonias are frequent adverse effects of immunomodulatory drugs (IMiDs), particularly lenalidomide, often leading to treatment discontinuation and decreasing the potential benefits to patients. According to our data, the rash could be due to synergism between enoxaparin and lenalidomide. In most cases, switch LWHM letting not to reduce lenalidomide dose in order to optimize the benefit of the treatment. Clonazepam, a benzodiazepine, is useful to treat dystonias related to lenalidomide.

PB1970

PROLONGED THROMBOPROPHYLAXIS IN PATIENTS TREATED WITH LENALIDOMIDE AND DEXAMETHASONE DOES NOT SEEM STRICTLY MANDATORY TO PREVENT LATE THROMBOTIC EVENTS

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Background: Risk of venous thromboembolism (VTE) in general population is 1% annually, significantly higher in oncologic setting, in particular with Multiple Myeloma (MM). Treatment with Lenalidomide plus Dexamethasone represents an additional risk factor for VTE, with most of VTE events observed in the first six months since therapy starting. No definitive data are available on the more appropriate duration of thromboprophylaxis (TP) in patients treated with lenalidomide.
Aims: To explore: I) the incidence of late thrombotic events in a real world population of relapsed MM, addressed to Lenalidomide plus low dose Dexamethasone treatment (Len-dex), and concomitant TP with low molecular weight heparin (LMWH) performed for the first 4-6 months of therapy, without TP maintenance, II) the possible correlation between the presence of thrombotic risk factors and the occurrence of a late VTE.
Methods: We performed a retrospective analysis, after regular approval of local ethic committee, on chart data of 103 patients (pts) with relapsed MM treated with Len-dex according to label indication between January 2003 and December 2016 at our single centre institution. VTE prophylaxis was performed with daily dose of subcutaneous LMWH 4000 IU for 4-6 months, with no further TP, regardless the presence of thrombotic risk factors.
Results: Main features of patients on study were: median age 66.3 years (range 41.9-85.2 years), median previous line of therapy 3 (range 1-7), time from diagnosis to lenalidomide starting 33.3 months (range 0.3-159.9 months), median duration of Lenalidomide treatment 8 months (range 0.4-65.2 months) with the following response: ≥PR 56%, CR 7%. Table 1 shows type and distribution of risk factors for VTE. In details median number of VTE risk factors per patient was 2 (range 0-6), 58.2% of pts had ≥2 risk factors, 41.8% of pts (43 pts) had 0-1 risk factor for VTE. Median duration of TP is 4.8 months (range

0.4-6 months). No hemorrhagic events were observed during LMWH. Cumulative incidence of VTE was 11.7% (12/103 pts), similar to that previously reported in the literature in patients with continuous TP. The median time from lenalidomide starting and VTE occurrence was of 12.2 months (range 1- 88.2 months), with only one patient developing early VTE among our group. In detail we observed 10 deep vein thrombosis (83%), 1 pulmonary embolism (8.5%), 1 myocardial infarction (8.5%). Most of patients developing VTE had good disease control (≥PR 83%, 10 pts). Concomitant adverse events (AE) was registered in 41.7% of pts (5/12). Most common concomitant AE were infections of respiratory tract (3 pts) and gastrointestinal AE (2 pts). The median number of risk factors for VTE in patients developing or not thrombosis was similar (2.5 *vs* 2, p=0.092).

Table 1. Baseline distribution of risk factors for thrombosis in the population on study.

Risk Factor	absolute number	Percentage (%)
Age >65	54	52.4
Obesity	10	9.7
CVC/Pacemaker	2	1.9
Immobilization	8	7.8
Trauma	1	1
Surgery	7	6.8
Erythropoietin	25	24.3
Chronic renal failure	16	15.5
Cardiopathy	44	42.7
Light chain disease	3	2.9
Acute Infection	14	13.6
Diabetes	11	10.7
Previous VTE	11	10.7

Summary/Conclusions: This study shows that LMWH is effective and well tolerate for early VTE prophylaxis during Lenalidomide plus low dose Dexamethasone. Incidence of late VTE without TP maintenance is similar to that reported with long-term antiplatelet therapy. We found no difference in factors predisposing for thrombosis among patients developing or not VTE, with a not negligible proportion of concomitant adverse events observed nearby VTE occurrence.

PB1971

ELDERLY PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA IN THE REAL WORLD

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Background: Many new agents for multiple myeloma (MM) were launched during the last decade, and the clinical trial using such new agents showed promising results for MM patients. However, clinical course of elderly patients with newly diagnosed MM (NDMM) in the real world is different from the results of clinical trial.
Aims: We examined the clinical parameter to assess survival in elderly patients with NDMM in clinical practice.
Methods: We performed a retrospective study involving 125 elderly NDMM patients from April 2012 to September 2015. Patients aged 60 years or older, who were ineligible for autologous stem cell transplantation, were selected. The study included 57 males and 68 females, with median age at diagnosis of 74 years (range, 60-95 years). ECOG performance status at diagnosis were 0-1, 67; 2-4, 58. We collected pretreatment parameter at diagnosis as follows; monoclonal protein type (IgG,60; IgA,32; IgD,1; BJP,30; non-secretary,2), light chain (kappa, 72; lambda, 52; unknown 1), hemoglobin level (mean 8.9 g/dL [range 5.8-15.2]), estimated glomerular filtration rate (eGFR) (mean 49.3 mL/min [range 3.6-114.2]), calcium level (mean 10.0 mg/dL [range 8.7-20.2]), albumin level (mean 3.4 g/dL [range 1.0-5.3]), beta-2-microglobulin (mean 5.1 mg/L [range 1.6-51.5]), involved:uninvolved serum free-light chain (FLC) ratio (mean 143.8 [1.83-21133]), cytogenetic abnormalities by using fluorescence *in situ* hybridization (FISH) [none, 53; t(4;14), 7; del(17p), 14; t(4;14) & del(17p), 5; t(4;14) & t(14;16) & del(17p), 1].
Results: Of 125 patients, 76 patients received bortezomib based therapy (VMP, 49; VD, 21; VCD, 6), 6 patients received lenalidomide based therapy (Ld, 6), 10 patients were received MP therapy, 19 patients received dexamethasone therapy (high dose, 16; low dose, 3), 1 patient received radiation therapy as first line therapy, and 13 patients received only supportive care due to their fragility. After induction therapy, the overall response rate (at least partial response, PR) was 52.7% (stringent complete response (sCR) 0.3%, CR 4.5%, very good PR 16.1%, PR 29.5%). Overall survival (OS) was 74.5% at 1 year, 66.2% at 2 years with median follow-up of 19 months (range 1-52) for patients who were still alive at the date of last contact and 14 months (range 1-52) for entire cohort. Death occurred in 41 patients during the follow-up period. International staging system (ISS), with ISS1, 19; ISS2, 42; ISS3, 60; N/A, 4, can divide elderly patients into three distinct survival groups (P <0.001) (Figure 1A). Univariate and multivariate analysis showed a lower OS was associated with eGFR lower than 40 ml/min (HR 2.279, 95%CI 1.152-4.510) (Figure 1B) and serum calcium level greater than 11 mg/dL (HR 3.036, 95%CI 1.412-6.529) (Figure 1C). Among 80 patients with FISH data, survival of those with t(4;14) or del(17p) or t(14;16) was not statistically different (P=0.394). Survival of patients treated with bortezomib or lenalidomide as an induction therapy was better, while not statistically significant (P=0.066) than those who ware not.

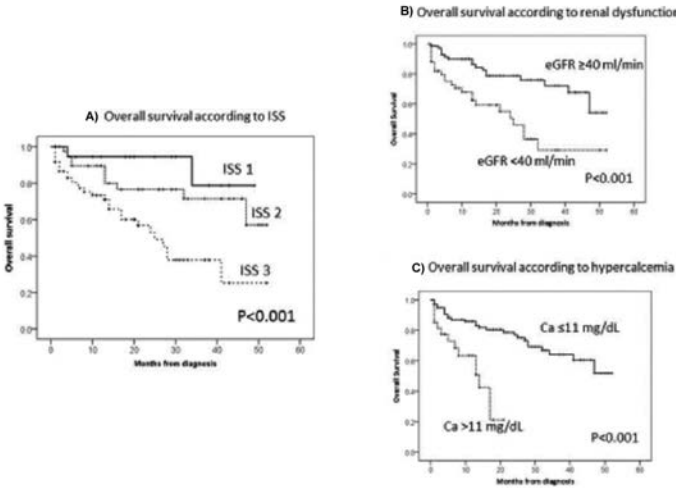

Figure 1.

Summary/Conclusions: Renal dysfunction and hypercalcemia at diagnosis is predictive of poor OS for elderly NDMM patients in real world.

PB1972

RETROSPECTIVE ANALYSIS OF 121 MULTIPLE MYELOMA PATIENTS USING THE R-ISS PROGNOSTIC STAGING SYSTEM AND RESPONSE TO FIRST LINE OF TREATMENT

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Background: The International Myeloma Working Group has developed the R-ISS (Revised International Staging System) as a simple and powerful prognostic staging system. We collected the LDH level and the cytogenetics of a group of patients and studied the difference between the ISS (International Staging System) and the R-ISS (Revised International Staging System) for those patients.
Aims: To evaluate and compare between the ISS and the R-ISS for a group of patients treated in Kuwait Cancer Control Centre.
Methods: A retrospective analysis of the data collected from 121 patients registered as multiple myeloma from 2011-2015, 5 of the patients presented to our centre after initial work up and starting the treatment abroad. The patients were categorised according to age, gender, ISS stage, R-ISS stage, first line therapy and response.
Results: We recognised increase of the number of the yearly diagnosed patients with myeloma 2.48% of patients the actual date of diagnosis was before 2011 but they presented to our centre from2011-2015. Median age of patients at presentation is 56 years old , 3.33% between30-40 years old, 18.33% between 40-50 years old , 35% between 50-60 years old , 31.67% between 60-70 years old and 11.67% between 70-80 years old. Male to female ratio 1.75:1 (Table 1). According to ISS stage patients were categorised into14% stage I, 31% stageII, 47% stage III. Restaging using the RISS revealed10% stage I, 26% stageII, 56%stage III. Almost half of our patients are diagnosed in the third stage, and more patients were shifted from stage I or II were categorised in the third stage due to either high LDH level, high cytogenetic risk or

even both. First line treatment 55% of the patients received Bortezomib based triple therapy, 22% received CTD (Cyclophosphamide, Thalidomide, Dexamethasone), 7% RD (Lenalidomide, Dexamethasone), 3% CyBorD (Cyclophosphamide, Bortezomib, Dexamethasone), 3%RV (Lenalidomide, Bortezomib), 2% Thal-Dex (Thalidomide, Dexamethasone), 2% RT (local Radiotherapy), 2% WatchfulWait,1% MP (Melphalan, Prednisone) and 3% refused for treatment and lost follow up.

Table 1.

ISS stage	% of patients	RISS stage	% of patients
Stage I	14%	Stage I	10%
Stage II	31%	Stage II	26%
Stage III	47%	Stage III	56%
Plasmacytoma	2%	Plasmacytoma	2%
MGUS	2%	MGUS	2%
Unknown stage(diagnosed outside our centre)	4%	Unknown stage(diagnosed outside our centre)	4%

Summary/Conclusions: Applying the RISS system to myeloma patients is very effective and easy method to categorise myeloma patients, a significant number of patients in Kuwait are diagnosed as stage III, with median age of 56 years although the use of novel therapies shows excellent response to most of them.

PB1973

FEASIBILITY/PHASE II STUDY OF MYELOABLATIVE BEAM ALLOGENEIC TRANSPLANTATION FOLLOWED BY ORAL IXAZOMIB MAINTENANCE THERAPY IN PATIENTS WITH HIGH RISK MYELOMA

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Background: While the role of allo-HCT in MM remains controversial several studies have shown encouraging PFS and OS with this treatment even in patients with high-risk myeloma (HRM). HRM manifests with early relapses and refractoriness. Median OS is 2.5 years despite aggressive therapy with novel agents. Post auto-HCT maintenance with lenalidomide is considered standard of care, but post allo-HCT maintenance presents unique challenges and has not been well studied. Ixazomib (Ixa) is a new oral proteasome inhibitor with activity in bortezomib resistant patients, and is a promising agent in the maintenance setting.
Aims: Here we present preliminary results for this trial. The primary objective is safety defined as day 100 transplant related mortality (TRM), and safety of Ixa maintenance (incidence of grade III-IV GvHD and Ixa related toxicity). Other objectives include determination of efficacy (ORR, PFS, MRD for CR), the ability to start Ixa, and quality of life.
Methods: The protocol was approved by a local institutional review board and ethics committee. The study was conducted in accordance with the Declaration of Helsinki. All subjects provided written informed consent prior to treatment. Eligibility criteria include: age ≤65; relapsed MM previously treated with auto-HCT, bortezomib and an immunomodulatory agent; one of the following high-risk criteria: deletion (del)17p, t(4;14), t(14;16), t(14;20), amp1q gain or del1p, del13q by conventional karyotyping, hypodiploidy, high-risk GEP, B2M >5.5mg , plasmablastic morphology (>2%), or relapsed plasma cell leukemia; 8/8 HLA matched unrelated (MUD) or sibling donor. The BEAM conditioning regimen includes: BCNU 300 mg/m^2 on day -6; cytarabine 400 mg/m^2 daily day -5 to day -2; etoposide 200 mg/m^2 daily day -5 to day -2; melphalan 140 mg/m^2 on day -1. Oral Ixa 4mg on days 1 and 14 of a 28-day cycle. Ixa may start between day 100 and 180 post HCT, and continue for up to 24 cycles.
Results: Six subjects were enrolled, 3 at OHSU and 3 at Duke, from Sept 2015 to Dec 2016. Median age of 51 (range 46-57), 2 female, and all of white race. High risk factors: del17p, del17p+t(14;16), del17p+amp1q+del1p, del13q+amp1q, amp1q, plasmablastic morphology (>2%). At study entry 2 subjects were in VGPR, and 4 in PR. Three subjects received 8/8 HLA matched MUD, and 3 received sibling donor HCT. GvHD prophylaxis with tacrolimus and methotrexate was given in 3 subjects with the addition of methylprednisone in 3 subjects. Two subjects started Ixa (at day 139 and 128) and remained on therapy for 198 and 59 days respectively; both discontinued for disease progression. Three subjects remain on study, and have not started Ixa. At day 100 post HCT, 4 of 6 subjects were evaluable for response. All had a deepening of response; 3 VGPR, 1 CR. One subject died from BEAM-allo related complications; namely acute stage 4 GvHD (skin and gut), and disseminated adenovirus infection (day 62). Grade 3/4 adverse events include: febrile neutropenia(3); colitis(1); epididimytis(1); mucositis(4); pancreatitis(1); pulmonary edema(2); diarrhea(3); infection(4); staph bacteremia(2); E coli bacteremia(1); adenovirus viremia(1); neutropenia(4); thrombocytopenia(4); acute GvHD(1). Ixa related toxicities include grade 3 neutropenia(2), thrombocytopenia(1), pneumonia(1), nausea and vomiting(1).
Summary/Conclusions: Although this is very early data, it is the first clinical trial to report the use of BEAM conditioning followed by Ixa maintenance for relapsed HRM. Thus far stopping rules have not been met, with expected toxicities occurring.

PB1974

EPIDEMIOLOGY OF MULTIPLE MYELOMA. THE GRANADA MYELOMA REGISTRY

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Background: The Granada Myeloma Registry is the second largest single-institution population-based registry (Ríos-Tamayo et al, 2015) of multiple myeloma (MM) referenced to date. Here we update and point out the epidemiological variables of interest.
Aims: To highlight the importance of the epidemiological perspective in the knowledge and outcome of MM.
Methods: From January 1985 to February 2017 all consecutive patients diagnosed with MM at our institution have been registered, including clinical, biological and socio-demographic variables, as previously reported. A comprehensive approach to comorbidity was recorded as well as diagnostic and treatment delay. Overall survival (OS) was estimated by the Kaplan-Meier method.
Results: 700 patients have been included in the registry, 343 men (49%) and 357 women. All cases have their place of residence in the Granada province. The median age was 67 years (range: 12-93). The race was Caucasian in 98.9%. In relation to occupation, 18.4% were skilled or elementary agricultural workers. Only 9% had a previously documented precursor disease (solitary plasmacytoma, monoclonal gammopathy of undetermined significance , or smoldering MM), and 14 patients (2%) remain alive with smoldering MM without progression. The subtype of MM is IgG 55.6%, IgA 24.8%, Light chain Only 15.9%, Non-secretory 3%, IgD 0.6% and IgM 0.2%. The International Staging System is known in 378 patients: 1 (25.9%), 2 (25.7%), and 3 (48.4%). Baseline performance status (ECOG) was: 0 (4.7%), 1 (41.1%), 2 (26.7%), 3 (21.7%), and 4 (5.9%). Comorbidity was assessed in 498 patients. 30.6% of patients were obese at the moment of diagnosis. 8.2% had other previously known or synchronous neoplasm. 150 patients (30.1%) had three or more comorbidities. Median diagnostic delay was 4.1 months (0.1-80) and median treatment delay was 13 days. 44 patients (6.3%) were very unfit and they did not receive active treatment. Information about stem cell transplant is available in 606 cases: 151 of them (24.9%) received a first autologous transplant. Median OS for the whole cohort was 43.1 and 22.4 months for patients younger than 65 years or 65 years and older, respectively (p <0.001). For patients diagnosed in 2010 or later, median OS is not reached for younger than 65 and 40.4 months for the elderly (p=0.001). Information about the main cause of dead is available in 230 patients: 101 (43.9%) of them died by infection.
Summary/Conclusions: MM is a very heterogeneous disease from a clinical, biological and epidemiological perspective. The distribution by sex is identical. Farmer is the most frequent occupation. Almost one in three patients are obese, and one in ten had another prior or associated neoplasm. Infection is the leading cause of death. Information derived from population-based registries may help to complement data from clinical trials.

PB1975

REAL WORLD USE OF IXAZOMIB WITH LENALIDOMIDE AND DEXAMETHASONE FOR PATIENTS WITH RELAPSED AND RELAPSED REFRACTORY MULTIPLE MYELOMA

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Background: Ixazomib (Ixa) is a novel oral proteasome inhibitor (PI) approved in combination with lenalidomide and dexamethasone (IRD) for the treatment of relapsed/refractory multiple myeloma (MM). This was based on the TOURMALINE-MM1 trial which demonstrated a progression free survival benefit over RD. However real world use often differs to clinical trials due to heterogeneous patient selection, more flexibility with dosing intensity and country specific prescribing practices/funding restrictions.
Aims: To characterise real word use of IRD by demographics, response rate (RR) and progression free survival.
Methods: This was a retrospective review of patients sequentially treated with IRD at a large UK Haematology Centre. Patients received Ixa 4mg D1, 8, 15 with lenalidomide (dose as per label) days 1-21 and dexamethasone 40mg weekly or as tolerated every 28 days until disease progression or intolerance. In some cases, Ixa was added later to RD. RR and PFS were assessed according to IMW criteria and haematological toxicities graded by CTCAE 4.0 criteria.
Results: Up to 31st October 2016, 30 patients were treated with the IRD schedule. Median age was 65 years (32-75), male (57%), ISS: stage I 18 (60%), stage II 4 (13%), stage III 8 (27%). Patients had a median of 2 (2-5) prior lines of therapy. All patients had previous treatment with a proteasome inhibitor (PI) (29 bortezomib, 5 carfilzomib) and 8 (27%) were refractory to a PI. 3 (10%) had prior lenalidomide and all remained sensitive. 23 (77%) had a prior autol-

ogous stem cell transplant. Out of those with results, 18 (69%) had adverse cytogenetics including 6 (23%) with TP53 loss. The median number of treatment cycles completed was 6 (2-35) with a median time on treatment currently of 5.5 months (1.6-40) for a median follow-up of 6.8 months (1.6-40). 24 patients were evaluable for efficacy analysis. 7 discontinued therapy, 6 due to disease progression of which 5 (83.3%) were refractory to a PI. 1 patient discontinued due to personal choice. The overall response rate (ORR) was 70.8% (PR 13 (54.1%), VGPR 3 (12.5%), CR 1 (4.2%)). For those refractory to prior PI, the ORR was 37.5% (PR 2 (25%), VGPR 1 (12.5%)). 7 (29%) had Ixa added for sub-optimal response (<PR) or PD. 2 had an improvement in response (VGPR and MR); 1 stabilised disease and the rest remained refractory. 5 (21%) had Ixa added whilst responding to RD, with the intention of deepening response and prolonging PFS. All continued to maintain their response. The median overall PFS was 19.23 months. The PFS for those refractory to prior PI was 11.6 months *vs* not reached for those sensitive (p=0.0159). Those with TP53 loss had a median PFS of 7.5 months. IRD was well tolerated with 5 (20.8%) patients experiencing grade 3-4 neutropenia and thrombocytopenia and 1 patient experiencing grade 4 anaemia. This resulted in Ixa dose reductions in 4 (16.7%) patients. Ixa was stopped in 1 patient due to adverse events.

Summary/Conclusions: This real world dataset highlights differences in patients treated in routine practice to trials. No patients were treated at first relapse due to funding restrictions, whereas most in the trial were. Patients had up to 5 prior lines, all had prior PI exposure and a higher proportion were PI refractory (33% *vs* 2%) which correlated with a worse outcome. Nevertheless the overall efficacy of our study (ORR 70.8%; median PFS 19.23 months) was comparable to the TOURMALINE-MM1 trial which had an ORR of 78.3% and median PFS of 20.6 months in the Ixa group.

PB1976

EFFICACY AND TOLERABILITY OF LENOLIDOMIDE AND POMALIDOMIDE IN RELAPSED/REFRACTORY MYELOMA PATIENTS IN A REAL WORLD STUDY

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Background: New agents have revolutionised the treatment of multiple myeloma. Immunomodulatory drugs (IMiD) such as lenalidomide and pomalidomide are potent re-induction drugs leading to improved Progression Free Survival (PFS) and Overall survival (OS). Published studies include exclusion criteria such as cytopenias, renal dysfunction and poor performance status (common in multi-relapsed patients), raising the question regarding the benefit of IMiD therapy in the real-world setting.

Aims: In our study we aimed to describe the real-world experience of the use of lenalidamide followed by pomalidamide rescue in a relatively elderly co-morbid cohort over a 4 year period and compare this to national averages. We reviewed IMiD efficacy, including sequential lenalidomide followed by pomalidomide, together with tolerance.

Methods: Records of delivered chemotherapy cycles were retrieved from local pharmacy data and national averages from Celgene ePAF data. Outcome data collected from clinical notes and laboratory results.

Results: We collected data on 46 patients treated between 2011-2014 with lenalidomide, 17 whom progressed to recieve pomalidomide. The median age at initial myeloma diagnosis was 71 years, with median age at starting lenalidomide 77years (range 36-94). This gave an average of 5 years from diagnosis to commencing lenalidomide (range 1-15 years). Myeloma subtypes included IgG 28/46, IgA 11/46, light chain disease 4/46 and 3 with IgD and non-secretory myeloma. High risk cytogenetics [17p-, t(4:16), t(4:20), hypodiploidy, chromosome 1 abnormalities] were identified in 9/46 and 16/46 were high-risk based on biomarker staging (ISS). All patients had at least 1 preceding line of therapy before starting lenalidomide, average 2 lines (range 1-6). Prior treatment included alkylating agents/steroid duplets, thalidomide combinations, bortezomib-based therapy and autograft. National average for the% of patients reaching cycle 26 was 16% comparing to the local average of 31%. This included patients receiving 5mg due to severe renal impairment or cytopenias. In the patient group between 65-75 years of age, 50% reached cycle 26 compared to the national average of 16%. Average duration on treatment was 15 months. (Local-cohort). Lenalidomide-treatment breaks occurred in 16 patients with a median of 5 months (infection, cytopenias, liver dysfunction, foreign travel, other). Cytopenias or infections were seen in 45% of local patients with 28% of patients having subsequent dose reductions. Based on performance status, renal function and prior drug tolerability, only 30% of patients were prescribed lenalidomide 25mg od. Despite this 17 patients (36%) achieved a prolonged PFS of >20 months and 13/46 (30%) a PFS of >30 months. The longest observed PFS in the local cohort was 53 months. The average number of cycles in those who progressed to pomalidomide was 12.8 (n=17), which is double that of the national average reported in seminal trials. These patients had few treatment breaks and treatment was well tolerated (pomalidomide duplets or triplets).

Summary/Conclusions: We conclude from this real-world retrospective review of 2nd and 3rd line IMiD therapy that these salvage regimes are highly effective. Patients on lenalidomide monotherapy post triplet/duplet induction were often re-escalated back onto dexamethasone and alkylator (IV/oral) based regimes with successful salvage, contributing to the observed long duration of local therapy compared to national averages. Pomalidomide was highly effective at rescuing patients failing lenalidomide-based regimes and well tolerated.

PB1977

APPLICATION OF CONDITIONING REGIMEN WITH BUSULFAN AND CYCLOPHOSPHAMIDE IN AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA

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Background: Busulfan is the most commonly used drug in conditioning regimens for hematopoietic stem cell transplantation and high-dose melphalan (MEL)is the standard conditioning regimen in autologous stem cell transplantation(ASCT)for multiple myeloma.Studies have shown that in ASCT for multiple myeloma,conditioning regimens containing busulfan is equally effective as HDM.

Aims: Evaluate the safety and efficacy of BUCY(busulfan and cyclophosphamide)conditioning regimen for autologous hematopoietic stem cell transplantation (ASCT) in patients with multiple myeloma (MM).

Methods: We retrospectively analyzed the clinical data of 72 MM patients who received transplantation in the Hematology Department of the First People's Hospital of Soochow University from May 2012 to June 2015. Among them, 36 patients underwent BUCY regimen while the others received high dose melphalan.Those were compared between the two groups including the complication, the hematopoietic reconstitution and the post-transplantation efficacy.

Results: There were no significant differences in age, stage, induction therapy, mobilization method between the two groups.The transplantation-related adverse events were similar in both groups but the incidence of pulmonary infection and bloodstream infection were slightly higher in BUCY group .The median time to neutrophil engraftment in the BUCY and HDM groups were 10(8-17) days *versus* 10(9-13)days, taking the same time on average(P=0.046). On the other hand, the median time to platelet engraftment was 10(8-18) *versus* 11(9-47)days accordingly(P=0.017) The TRM in both group was 2.7%.The SCR/CR rates after ASCT (47.2% and 50.0%) were higher than those before it (38.9% and 26.6%), in both groups.In the BUCY group, the median follow-up was 12.5 (0-26) months. Six patients (16.7%) underwent disease progression.The 2-year progression-free survival(PFS) rate was 68%.Correspondingly,in the HDM group,the median follow-up time was 23 (0-38) months. Fifteen patients (41.7%) developed disease progression and the 2-year PFS rate was 55%.

Summary/Conclusions: The BUCY regimen is a safe and effective therapy for ASCT in patients with multiple myeloma.Besides, BUCY regimen is not inferior to HDM regimen.In conclusion, BUCY regimen may replace HDM regimen as a standard conditioning regimen for ASCT in multiple myeloma.

PB1978

MULTIPLE MYELOMA WITH CENTRAL NERVOUS SYSTEM INVOLVEMENT, 12 CASES AND REVIEW OF THE LITERATURE

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Background: Central nervous system (CNS) propagation is a rare event in multiple myeloma (MM), but may become more prevalent as newer treatment options allow patients to have a prolonged life expectancy and with this comes the selection of increasingly aggressive clones.

Aims: We reviewed 12 MM cases with CNS involvement treated in two hospitals.

Methods: Statistical analyses were performed using the SPSS (version 20.0) software package.

Results: Between 2008 and 2015 twelve MM patient developed CNS involvement which presented in all cases at relapse. The median age at diagnosis and at CNS presentation were 55.5 and 57.4 years. At first presentation nine had ISS 3, one ISS 2 and two ISS 1 stage disease, two patient presented originally as plasma cell leukaemia. FISH showed 1q amplification in 4, 13q deletion in 4, translocation (4;14) in 1, t(11;14) together with 17p deletion in 1, hyperdiploidity in 1 and complex karyotype in 2 cases. In 2 cases we demonstrated the development of new karyotypic abnormalities (one 1q amplification, one 17p deletion) at CNS progression. The median number of treatment lines prior to CNS progression was 2 which included bortezomib in all and thalidomide in all but one cases, two patients had lenalidomide. Six patients had ASCT before the CNS progression from which one had a second ASCT and one a reduced intensity allogeneic transplantation. The median time from diagnosis to CNS

progression was 23.9 (3-65) months. Eight patients presented with cerebral nerve palsy, 2 with paraplegia, 1 with hypacusis and 1 with headache. CSF cytospin or flow cytometry was positive in 7, MRI or CT supported the diagnosis in 4 patients. Treatment consisted of combination chemotherapy, intrathecal chemotherapy, cranio-caudal radiotherapy and imids with various success. The PFS and OS from CNS progression was 63 and 125 days. Two patients survived for over a year (427 and 776 days), both responded in terms of CNS symptoms to imid-based combination therapy and one had cranio-caudal radiotherapy (Figure 1).

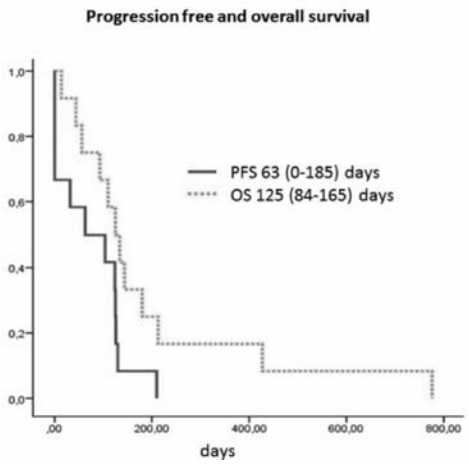

Figure 1.

Summary/Conclusions: CNS progression in MM has a particularly poor prognosis as it represents a late stage of an aggressive relapse which often shows chemo-refractoriness. The differential diagnosis includes infection, autoimmune or vascular diseases of the CNS as well as paraneoplasia and drug toxicity. The CNS penetration of the effective myeloma drugs is poor except for the imids, and drugs with CNS availability are usually not very effective in refractory MM.

PB1979

DARATUMUMAB; CHALLENGES OF INTEGRATING THIS NEW THERAPY INTO STANDARD CARE

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Background: Daratumumab (Darzalex) is the first anti-CD38 human Monoclonal Antibody approved for Multiple Myeloma (MM). Targeting the CD38 antigen on the surface of MM cells it causes apoptosis, and has an immune modulated tumour lysis effect. Success in Clinical trials meant that this drug, administered as single agent, or in combination with other novel therapies (Lenalidomide or Bortezomib), received accelerated FDA Approval in the US. It is now being introduced into standard hospital care.
Aims: Daratumumab presents unique challenges to the delivery of risk managed care, due to effects on some blood and bone marrow testing, and to the Infusion Related Reactions (IRRs) seen at the outset of treatment. This poster will highlight important aspects of the treatment pathway for this new therapy, from a single centre perspective.
Methods: We outline the pathways integrated at MDT level; patient characteristics and adverse event profiles of the 15 myeloma patients we have treated with Daratumumab, in a standard service setting.
Results: Daratumumab (Dara) affects certain pathology tests so samples should be clearly identified. Relevant laboratory teams need to be aware of the methods used to process samples. Daratumumab binds to CD38 on Red Blood Cells, and therefore with Cross Match Compatibility testing and Antibody Screening. Obtaining RBC Products for patients receiving Dara will take longer, requiring up to 48 hours' notice. Cross match samples taken prior to treatment provide the National Blood Service Laboratory with a baseline antigen profile to aid selection of suitable blood products. Dara is detected during Paraprotein Electrophoresis; Pre and Post baseline samples help to identify the Darzalex Band in the serum; our lab use a Sebia capillarys 2 analyser to separate the Dara band for accurate reporting. Bone Marrow Testing: Daratumumab affects Immunophenotyping by masking the CD38 epitope used to identify plasma cells by flow cytometry; special kits are available using a different CD38 epitope thus dealing with this issue. Infusion Related Reactions (IRRs) have been reported in over half of patients receiving Daratumumab: 95% of these were seen at the first dose. Typically involving the upper respiratory tract and include rhinitis, cough, wheeze, bronchospasm, laryngospasm and chest pain. More rarely they include rash, fever, and nausea. Reactions can be grade 1-4 so it's important that the patient is closely monitored where there is quick access to specialist staff, resuscitation equipment and respiratory support in a high dependency setting. Staff training is important, and patients made aware that they report all new symptoms so the infusion is interrupted immediately and the IRRS treated and re-started at a lower rate when the symptoms have resolved. Premedication is given one hour prior to infusion and patients with a history of COPD receive extra support. Patient characteristics. Total:15. (Table 1).

Table 1.

Age	Gender	Number of prior treatments	Regimens	Disease outcome
Range:36-81years Mean: 61 years	Male: 8 Female: 7	Range: 2-14 Median: 4	Single agent Dara: 1 Dara with Lenalidomide: 4 Dara with Bortezomib: 1	Response: 14 Progression: 1
1st dose dates	**IRRs Log**	**IRRs seen**	**Grades**	**Infusion duration**
27.7.2016-15.11.2016	IRRs: 14/15 (no IRRs 1). Total completed: 13 Total aborted: 2 (*both successfully re-challenged at second dose).*	Common: Nasal congestion. Rhinitis. Throat irritation. Cough. bronchospasm. Chest pain. dyspnoea. Rare: Rash, nausea. Fever, hypotension.	1-3	Range: 6hr30 - 10hr55. Mean: 8hr39 1 aborted at 65mins 1 aborted at 10hrs

Summary/Conclusions: Education, to include Blood Transfusion, Protein and Histopathology laboratory, and High Dependency Unit staff, in the key aspects of monitoring and risk management are an important part of integrating this new therapy to the treatment pathway for myeloma patients. Daratumumab is likely to become an important treatment for improving both Outcomes and Quality of Life for Myeloma patients going forward.

PB1980

MULTIPLE MYELOMA IN HIV+ PATIENTS LITERATURE REVIEW AND OWN CASE

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Background: Multipe myeloma (MM) and HIV infection in AIDS stage until now its considered not to be associated. Recently new ideas appear in the literature such as influence of HAART on the treatment outcomes of MM in HIV negative patiens.
Aims: To find literature sources on multiple myeloma in HIV positive patients and elucidate the problem of this association. evaluate the impact of HAART in multiple myeloma.
Methods: Patients were retrospectively identified out of 39 cases of MM and HIV from Pubmed/Medline from 1983 to 2017, and own case reported.
Results: Patients with MM and HIV infection did not differ significantly from the MM in HIV-negative with respect to age, gender, stages and renal function. Effects of HAART on levels of serum M-protein HAART itself has been reported to decrease M-protein in an HIV+ patient with MM. We determined whether HAART alone, in the absence of MM treatment, had any effects on the level of serum M-protein in HIV+MM patients. Depending on the interval between the discovery of the HIV infection HAART treatment initiation, and the diagnosis of MM and initiation of its treatment. The overall and progression-free survival of HIV+ MM patients on HAART appeared to be superior to that of HIV-negative MM patients. The survival of the HIV+ MM patients were also superior to that of non-HIV MM patients reported in the literature. The majority of HIV+ MM patients who had long-term follow-up in our study did not show clinical symptoms of MM and were free of serum-M protein after primary MM therapy in the presence or absence of HAART and maintained treatment with HAART alone. Although MM is not an AIDS-defining illness, meta-analyses of large population studies reveal an increased risk of MM in HIV/AIDS patients. HIV infection is commonly associated with B cell hyperproliferation, as indicated by polyclonal hyperglobulinemia and the development of various autoantibodies. This is presumed to be usually due to these CD4 deficient patients' inability to control Epstein-Barr virus infections, which immortalize B cells. This may help to explain the increased incidence of MM in HIV+ patients. However, HIV can neither infect B lymphocytes or plasma cells, nor drive their malignant transformation. Some authors are going to treat multiple myeloma in HIV seronegative patients with HAART in combination with chemotherapy (Geling Lia and co-authors, Leukemia Research, 2014). A 38 year-old Russian male presented at the Moscow clinical Center in 2015 with pronounced ossalgya and inability to move. Total protein 135 g/l with 81.7 g/l of IgG-k M-protein and no presence of Bence Jones protein. Bone skeletal survey showed multiple generalized lytic lesions. Bone marrow aspirate and biopsy showed 46% plasma cells. Serum creatinine – 104 mkmol/l. HIV and hepatitis C (genotype 1a) screening test were positive, confirmed with Western blot analysis. The CD4 count was 290 cells, HIV viral load 1500 copies/ml, hepatitis C viral load 14,2 mln copies. He was started on HAART, combined with chemotherapy 5 courses of CP+CVP+MP and 7 V-MP. In 2017 total serum protein– 97,3 g/l, M-protein 31,2 g/l, serum creatinine 63,0 mkmol/l. Now he is active without any bone pain receives Pegasis and lamivudine (Table 1).
Summary/Conclusions: Patients with MM and HIV infection did not differ significantly from the MM in HIV-negative with respect to age, gender, stages and renal function, and treatment with addition of HAART.Recently was reported that HAART itself may reduce and even remove m-gradient in HIV positive

patients. It is considered to include HAART in HIV negative patients with MM.The problem of MM and HIV/AIDS association remains unclear and needs to be elucidated.

Table 1.

Characteristics of patients with HIV infection and MM

Age	mean 46 yrs (26-65)					
Sex	28 M (71%)			11 W (29%)		
M-Gradient	IgAκ 4 (3%)	IgAλ 0 (0%)	IgGκ 22 (58 %)	IgGλ 8 (21%)	IgMκ 1(3%)	Ig-non-sec. 3 (8%)
Plasma cells in BM	23% (10-90)					
Bone lesions	63%- 24 pts					
CRF	21% - 8 pts					
Total serum protein (>80g/l)	16% - 4 pts					
Chemotherapy	34% - 13 pts					
	VAD /MACOP-B/CHOP/Thalidomide, clarithromycin, dexamethasone/VTD-PACE /VCd regimen/lenalidomide + dexamethasone					
Radiation therapy	16%- 6 pts					
Auto-SCT	8% - 3 pts					
Outcome	Remission 24%-9 pts		Alive 8%- 3 pts		Died 71%- 27 pts	
	Duration of HIV infection in the years before the development of MM		CD 4 before HAART		Viral load	
Characteristics of HIV infection in MM patients	3 (1-15 yrs)		125 (3-300 cells)		54000 (150-35000 cop/ml)	

PB1981

OPTIMIZATION OF APPROACHES FOR STEM CELL MOBILIZATION FOR AUTOLOGOUS STEM CELL TRANSPLANT FOR MULTIPLE MYELOMA: PRACTICAL CONSIDERATIONS

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Background: Autologous stem cell transplant (ASCT) is a well-established treatment for myeloma. However, the optimal strategy for stem cell mobilization remains undefined. The goal of mobilization is to collect adequate stem cells for at least 2 ASCT ($4x10^6$/kg), with the minimum apheresis sessions and toxicities such as febrile neutropenia.
Aims: We aim to compare stem cell mobilization using granulocyte colony stem cell factor (GCSF) only (steady state), high dose cyclophosphamide (4 g/m2) with GCSF or low dose cyclophosphamide (2 g/m2) with GCSF.
Methods: We performed a retrospective analysis of 79 patients mobilized with GCSF only from mid-2014 to Aug 2016 with 32 patients mobilized using high dose cyclophosphamide and 23 patients with low dose cyclophosphamide during a similar period.
Results: Patients undergoing steady state collection required a median of 2 days for adequate collection, in comparison to 1 day for both high and low dose cyclophosphamide. Addition of pleraxifor was required in 27.8% of patients on steady state collection, in contrast to 3.1% and 13% of patients on high and low dose cyclophosphamide respectively. The mean yield of CD34+ x 10^6/kg cells collected was 5.39, 9.14 and 8.5 for steady state, high and low dose. There was no significant difference in time to engraftment despite a lower dose of CD34+ cells reinfused for the steady state cohort. Admission for febrile episodes was observed in 50% of patients mobilized with high dose cyclophosphamide, as compared to 13% of patients on the lower dose regime and none in the steady state cohort. Patients mobilized with cyclophosphamide had a longer interval between stem cell collection and transplant (median of 20, 42 and 34 days respectively for steady state, high dose and low dose). However, we observed that 60.7% patients with steady state mobilization had increases in their myeloma markers during this period, in contrast to biochemical improvement in 50% of patients mobilized with high dose cyclophosphamide and 26% with low dose cyclophosphamide.
Summary/Conclusions: All 3 strategies for stem cell mobilization have their own merit. Steady state mobilization is safe and yields sufficient stem cells; however, patients require more apheresis sessions. Moreover, more than a quarter require additional therapy with plerixafor. Of concern, greater than half of these patients have increased myeloma markers during the interval between stopping chemotherapy and mobilization which may potentially affect outcomes. Mobilization with high dose cyclophosphamide yield more CD34+ cells but with increased toxicities- 50% of patients required admission for febrile episodes. Conversely, half of these patients had improvement in their myeloma markers. The use of low dose cyclophosphamide for mobilization resulted in lower admission rates (13%), however, pleraxifor is required in a fraction.
In light of these findings, we propose that patients who have not achieved at least VGPR should be mobilized with cyclophosphamide, the dosage dependent on their individual risks.

PB1982

MINIMAL RESIDUAL DISEASE MONITORING IN MULTIPLE MYELOMA PATIENTS BY FLOW CYTOMETRY: A SINGLE CENTER EXPERIENCE

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Background: Multiple myeloma (MM) is a malignant disease characterized by an increased number of clonal (abnormal) plasma cells in the bone marrow (BM). High-dose chemotherapy followed by autologous peripheral blood stem cell transplantation (SCT) is used for the treatment of young MM patients and produces a high rate of complete remissions (CR). Recent trials with novel agent combinations alone have also resulted in high CR rates, even among old patients, high-risk patients and relapse/refractory MM. Unfortunately, most patients have a recurrences of the disease. This is due to the persistence of residual tumor cells, known as minimal residual disease (MRD), responsible for tumor relapse.
Aims: BM samples from 51 MM patients who had achieved partial or complete response or were resistant after chemotherapy, including autologous SCT, were evaluated by multiparameter flow cytometry (MFC). The study was conducted to assess the quality of remission, the correlation between the number of abnormal plasma cells of BM and other signs of disease activity, readiness of patients for autologous SCT.
Methods: The study included 51 patients MM, average age - 54 years (36-70 years), who underwent assessment of MRD from November 2014 to February 2017. According to the classification Durie-Salmon the vast majority of patients (n=40) had III stage of disease, 8 patients – II and 2 patients – I. Response to treatment was assessed according to standard EBMT criteria At the time of MRD assessment 20 patients were in CR, 8 had a partial response (PR) and 15 had a resistant disease; 5 patients had a primary MM, 3 patients were in the first relapse. Most of the patients were underwent high-dose chemotherapy with autologous SCT (n=42). Re-evaluation of MRD after therapy was managed to hold in 36 patients at a mean of 3,1 months (1,9-5,7, min-max). Analysis was performed using a FACSCantoII flow cytometer (BD) and FACSDiva software (BD). Instrument performance was checked daily by recording fluorescence intensity with calibrating beads (Cytometer Setup and Tracking from BD Biosciences). Whole BM was estimated using combination of surface and intracellular staining CD38/CD56/CD27/CD117/CD81/CD19/CD45/cytLambda/CD138/cytKappa. The sensitivity of our panel MRD is 0.01% (*i.e.* 10^{-4}).
Results: Among patients in CR (n=20) confirmed the absence of MRD in 6 patients, but 14 CR patients were MRD positive. MRD was detected in all patients with PR and resistant disease (n=31). The relative content of abnormal plasma cells in CR patients with MRD positive (n=14) was significantly lower than that in PR/resistant patients (n=31): 0.095% (0,026-0,271%) *versus* 1,3% (0,203 -5,9%), pU=0,000092. PR patients (n=8) had a lower relative content of abnormal plasma cells (as expressed tendency), than patients with resistant disease (n=15): 0,286% (0,177-1,129%) *versus* 1,48% (0 , 90-8,0%), pU=0,053. Besides the relative content of abnormal plasma cells in PR/resistant patients (n=31) correlated with the serum M-gradient concentration (r_s=0,42; p=0,019) and bone marrow plasma cells (r_s=0,54, p=0,0017).
Summary/Conclusions: Currently, we can conclude that MFC could be considered as the method of choice for MRD monitoring in MM. If the disease is measured, then, indeed, enough to evaluate only the M-gradient level of serum. If the M-gradient is not defined, it is necessary to assess the number of abnormal plasma cells in the BM and strive for the high-quality responses at the time of transplantation. And also it can help us to regulate duration of maintenance therapy.

PB1983

AUTOLOGOUS STEM CELL TRANSPLANTATION IN ELDERLY MULTIPLE MYELOMA PATIENTS

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Background: Autologous stem cell transplantation (ASCT) is currently approved as a "gold standard" first line treatment for multiple myeloma (MM) patients (pts) under 65 year old but the procedure could also be considered feasible in fit elderly patients based on several retrospective studies.
Aims: The aim of our study was to retrospectively evaluate the tolerability and the efficacy of high dose chemotherapy followed by ASCT in selected ≥65 year old MM population.
Methods: We retrospectively analyzed consecutive MM pts aged 65 or older who underwent upfront ASCT at our institution from January 2009 to November 2016. Each patient received induction therapy including proteasome inhibitors and/or immunomodulatory drugs (bortezomib and/or thalidomide based), followed by high-dose cyclophosphamide plus G-CSF and subsequently underwent peripheral blood stem cells (PBSC) collection.

Results: Overall we analized 35 pts: 21 males and 14 females (median age 66, range 65-70); 23 had IgG MM, 4 had IgA MM and 8 had light chain MM. Induction therapy was bortezomib-based (bortezomib in combination with dexamethasone, VD, in 7, or VD plus thalidomide in 26 pts) for a median of 4 cycles (range 3-6), 2 patients received thalidomide plus dexamethasone (6-12 cycles). PBSC were collected after high-dose cyclophosphamide (2 g/sqm in 2 pts, 3 g/sqm in 11 pts, 4 g/sqm in 22 pts) plus G-CSF; plerixafor was administered in 4 pts. Three pts also received lenalidomide and dexamethasone to improve the depth of response before ASCT. At the time of conditioning, among 34 evaluable pts, 8/34 pts were in complete response/stringent complete response (CR/sCR), 19/34 in very good partial response (VGPR), 5/34 in partial response (PR) and 2/34 in stable disease (SD). The conditioning regimen consisted of melphalan 140 mg/sqm in 11 pts or 200 mg/sqm in 24 pts. A median number of 4.11 x10^6 CD34+ cells/Kg was reinfused (range 2.09-10.44). The most frequent complication was fever (9 pts) with gram negative bacteremia documented in 3/9 and gram positive bacteremia in 1/9. Other complications were represented by 1 case of atrial fibrillation and 3 cases of pneumonia and 1 case of VZV reactivation. All 35 pts achieved neutrophils recovery after a median of 12 days (range 8-25) and platelets recovery after a median of 13 days (range 8-45) after transplant. No grade 3-4 toxicities were recorded. No transplant-related mortality was recorded within 100 days post transplantation. Three months after ASCT, among 28 evaluable pts, 10/28 pts were in CR, 14/28 pts in VGPR and 4/28 pts in PR. Three pts underwent tandem ASCT. After a median follow-up of 32 months (range 3-96) among 33 evaluable pts, 20 experienced disease relapse and 7 deaths occurred. Median PFS and OS were 21 and 40 months.
Summary/Conclusions: Our data support the use of ASCT as an effective and safe first-line treatment approach also in elderly MM pts. A careful patient selection is needed to reduce the toxicity of the procedure.

PB1984

EVOLUTION IN THE INCIDENCE OF MONOCLONAL GAMMOPATHIES IN A SOUTHERN SPAIN TERTIARY HOSPITAL IN THE LAST THIRTEEN YEARS

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Background: Monoclonal gammopathy (MG) is the most common plasma cells disorder. It affects around 3% of the population older than 50 years. The great majority of MG are monoclonal gammopathies of undetermined significance (MGUS), which is a premalignant disorder defined to present less than 3 g/dL of serum monoclonal protein, less than 10% of clonal bone marrow cells and absence of end-organ damage. MGUS is easily detected in laboratory tests and should be monitored because 1% of MGUS per year progress to Multiple Myeloma (MM). Incidence of MGUS and MM is not always easy to determine, but there is a general perception of an increasing incidence that can be attributed to different causes. One is the aging of the population. Another reason is the contribution of clinical laboratories, which count on new determinations (free light chains) or improved techniques in electrophoresis, nephelometry or immunofixation, allowing them to support the diagnose of MGUS that years before remained undiagnosed.
Aims: The aim of this study is to determine the incidence of MGUS, MM and its different types in the reference population of a tertiary hospital in southern Spain between 2003 and 2015.
Methods: In a retrospective study, we determined the total number of MG and its different types diagnosed in our hospital between 2003 and 2015. We calculated the incidence per 100.000/year of MGUS and MM, with 95% confidence intervals. Our reference population, in 2015, was 480.851.
Results: Results in Figure 1.

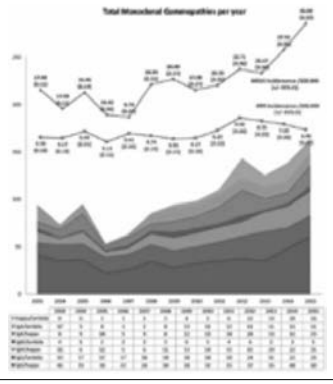

Figure 1.

Summary/Conclusions: The aging of population and the higher sensitivity of laboratory techniques for diagnosing of MG is reflected in the incidence of MGUS, which increased from 17.04 cases per 100.000 in 2003 to 35.00. MM incidence in our area did not increased in parallel.

PB1985

CHARACTERIZATION OF A SERIES OF PATIENTS WITH PLASMA CELL LEUKEMIA

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Background: Plasma cell leukemia (PCL) is a rare malignancy characterized by the proliferation of monoclonal plasma cells in the bone marrow and ≥2x10^{9}or ≥20% plasma cells in the peripheral blood. It is an aggressive disease, with a median survival of 7 to 11 months. Due to its rarity, it is difficult to design prospective studies or randomized trials in PCL, so collecting and publishing data from the largest number of cases is essential for the understanding of PCL's pathophysiology and outcome.
Aims: To characterize a series of PCL patients, in order to obtain data with the potential to be used as prognostic factors and to improve clinical outcomes.
Methods: Single-center, observational, retrospective study including all PCL cases admitted in our hospital between 2007 and 2016. Data regarding demography, clinical characteristics, laboratory results, treatment, *follow-up* and mortality were collected and analyzed using Statistical Package for Social Sciences (21stversion), searching for significant associations ($p<0.05$) with overall survival (OS) and progression free survival (PFS).
Results: 15 patients were included, with a median age of 58 years. Most patients were male (60%) and had PS ECOG 0-1 (93,3%) at presentation and primary PCL (80%). Median hemoglobin (Hb) and platelets values were 8,5 g/dl and 74x10^9/L, respectively. Median plasma cell percentage was 37.3% (peripheral blood) and 60% (bone marrow). IgG heavy chain was present in 33.3% and lambda light chains in 53.3% of cases. Most patients had total serum calcium ≥4.5mmol/L (60%), total proteins ≥65g/L (66.7%), monoclonal component ≤ 30g/L (53.3%), albumin ≥35g/L (60%), creatinine clearance ≥50ml/min (66.7%), elevated β-2 microglobulin (93.3%), ISS III (80%), R-ISS III (73.3%) and at least 1 cytogenetic change associated with poor prognosis in multiple myeloma (86.7%). Ten (66.7%) patients received bortezomib-based chemotherapy and nine patients (60%) were submitted, at least, to one autologous stem cell transplant (ASCT). Complete response (CR) or very good partial response (VGPR) were achieved, after chemotherapy, in 53.3% and, after ASCT, in 88.9% of patients. Mortality rate was 66,7%, with median PFS of 5 months and median OS of 4 months. In univariate analysis, OS was significantly associated with albumin ≤ 35g/L, splenomegaly and R-ISS III; PFS was significantly associated with platelets ≤100x10^9/L, splenomegaly and lambda light chains. In multivariate analysis, only the presence of splenomegaly kept its association with OS; none of the characteristics associated with PFS kept their significance. Chemotherapy followed by ASCT and the achievement of, at least, VGPR after chemotherapy and ASCT were associated with longer OS and PFS.
Summary/Conclusions: This study's retrospective design and the small sample limit the strength of our data and our conclusions. Interesting results were obtained regarding pre-treatment prognostic characteristics and the association of improved OS and PFS with treatment response and ASCT execution. More studies are necessary to determine the clinical relevance of this findings and the best treatment strategies in PCL.

PB1986

OPTIMIZATION OF POMALIDOMIDE PLUS LOW DOSE DEXAMETHASONE IN REFRACTORY/RELAPSED MYELOMA MULTIPLE PATIENTS

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Background: MM-003 study has presented a median PFS of 4.0 months and median OS was 13.1 months overall for Pomalidomide and low doses of dexamethasone in RRMM patients. Those results were better when a third drug was added (Poma-Dexa , Poma-Cyclophosphamide-dexa, and Poma-Bortezomib-dexa, ORR 38.9, 64.7 and 85%; PFS 4.4, 9.5, 10.7 months respectively).
Aims: To evaluate the response at therapy with pomalidomide plus dexamethasone in RRMM, and to analyze the efficacy of another drug in high risk MM.
Methods: we reported the clinical experience of the 8 patients treated with pomalidomide and dexamethasone. In patients with high risk MM (cytogenetic, extramedullary myeloma or plasmatic cell leukemia) pomalidomide and dexamethasone have had poor response. In those cases, we have added a third drug (cyclophosphamide or Bortezomib) and we have obtained the best results.
Results: we have used pomalidomide and dexamethasone in 4 patients and poma-dexa-cyclophosphamide in 3 patients (extramedullary myeloma) and

poma-bortezomid-dexa in 1 PCL patient. Table1. Demographic characteristic`s patients. Figure1. Response of monoclonal spike.

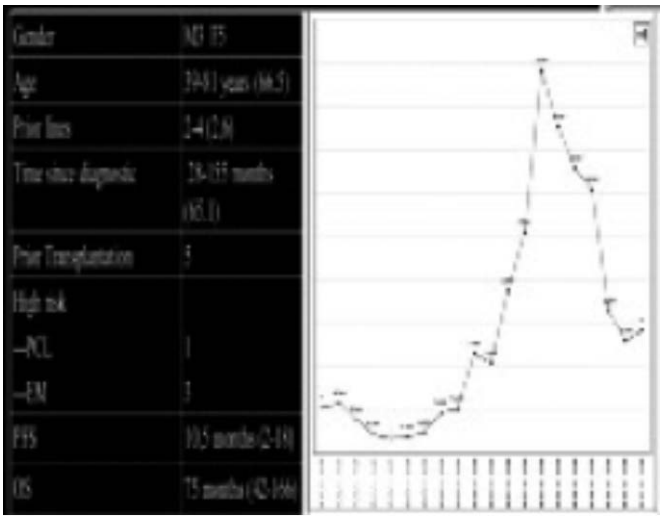

Figure 1.

Summary/Conclusions: pomalidomide, dexamethasone and a third drug (cyclophosphamide or Bortezomib) obtain best results (PFS and OS) in high risk RRMM patients. We have not reported more toxicity adding a third drug. In our experience, the response of the extramedullary myeloma with pomalidomide´s triplets is a great option.

PB1987

PROGNOSTIC SIGNIFICANCE OF PLASMABLASTIC PLASMA CELLS IN THE ERA OF NOVEL AGENTS IN MULTIPLE MYELOMA

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Background: Plasmablastic (PB) feature of plasma cells in multiple myeloma (MM) has long been identified as poor prognosis. Interestingly it does not take part of International Revised Scoring System (R-ISS). Similarly, the prognostic impact in the era of novel agents and novel classes in MM is unknown. Finally, the percentage of PB in the bone marrow to which a poor prognosis develop is unclear.
Aims: To assess which modality of treatment of plasmablastic MM was associated with longer progression free survival (PFS) and overall survival (OS).
Methods: We have performed a retrospective analysis of all MM in our center from May 2005 to November 2016, and sought for MM with plasmablastic features, characterized by immature cells with high proliferative index rate. The PFS and OS were calculated since the first time the PB morphology was observed in the bone marrow aspiration, at the outset in newly diagnosed patients or in relapsed patients.
Results: 65 patients with PB were included. Adverse cytogenetic per IMWG criteria was reported in 6 patients, del17p x3, t(4;14) x3, and one with both. 33,8% were ISS 3, and 23,1% R-ISS 3. Extramedullary disease (EMD) was reported in 40%. 35 patients (53,8%) were in first-line therapy. The overall response rate with any triplet-based treatment containing always a proteasome inhibition and IMIds or alkylator was 49,2%, with 29,2% VGPR and 4,6% CR. The median PFS and OS were 6,9 and 14,9 months as a whole, respectively. The median PFS was greater when treatment combined bortezomib, lenalidomide and dexamethasone: 36,1 months ([0-99] *vs* 5,5 [0,59-10,4] otherwise; p=0,014). However, no difference in OS was demonstrated. Importantly, high dose therapy with ASCT was associated with longer PFS and OS, respectively 21,4 months ([12,8-30,1] *vs* 2,83 [0-5,7] p=0,003) and 50,4 months ([29,9-70,7] *vs* 6,27 [1,1-11,4] p=0,001). In multivariate analysis, poor OS was associated to acute renal failure at disease entry, presence of EMD, of del(17p), of hypercalcemia, and elevated lactate dehydrogenase. We then sought to demonstrate that use of a direct anti proliferative-based agent such as anthracycline would participate to rapid disease reduction and PB clone control. It turns out that there was no significant difference in terms of survival in patients treated with an anthracycline-based regimen.
Summary/Conclusions: this study confirms the poor prognosis of PB feature in MM. A triple-based association with a proteasome inhibitor (PI) and an immunomodulatory drug (IMiD) seems to provide the best PFS in MM with PB, ideally complemented by ASCT. The role of anthracycline remains to be demonstrated. This data will be validated in a large cohort.

PB1988

INTERNATIONAL OPPORTUNITIES TO COMPARE 'REAL WORLD' DATA FROM MYELOMA REGISTRIES: BASELINE CHARACTERISTICS, FIRST-LINE THERAPIES AND EARLY OUTCOMES FROM AUSTRIA AND AUSTRALIA/NEW ZEALAND

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Background: Most outcome data for multiple myeloma (MM) come from clinical trials which can not necessarily be extrapolated to 'real world' patients. More information is needed on patients treated in the 'real world' and in a wider range of settings.
Aims: To compare and contrast baseline characteristics, investigations, and initial therapies in different geographical regions, Australia/New Zealand (ANZ) and Austria, through first analysis of data from two established MM registries on behalf of the steering committees of the Australian and New Zealand Myeloma and Related Diseases Registry and the Austrian Myeloma Registry.
Methods: Analysis of data from newly diagnosed MM patients enrolled on the Austrian Myeloma Registry (AMR) and the ANZ Myeloma and Related Diseases Registry (MRDR) from 2012-2016.
Results: Available data from 250 and 691 patients from the AMR and ANZ MRDR, respectively, were included. DEMOGRAPHICS: The AMR cohort was younger (median age m:f 63.5 yrs:64 years *vs* 65 yrs:66 yrs on the AMR and MRDR, respectively). The proportion of male/female patients was similar between the AMR and MRDR (m:f 56%:44% and 61%:39% respectively). PRESENTATION: IgG myeloma was the most common sub-type of disease in both registries (m:f 64%:55% and 55%:58%, respectively) with more light chain only disease on the AMR (m:f 26%:33% *vs* 20%:19%). Presence of documented preceding plasma cell dyscrasias was similar (m:f 21%:19% *vs* 15%:20%, on the AMR and MRDR, respectively). INVESTIGATIONS: A higher proportion of patients underwent MRI (m:f 51%:58% *vs* 25%:27%) and skeletal survey (SS) (78% *vs* 60%) at diagnosis on the AMR than the MRDR, respectively. Baseline laboratory investigations were similar, however, patients on the MRDR demonstrated higher median LDH (m:f 176:178 *vs* 187:186 units/L) and serum calcium (m:f 2.34:2.28 *vs* 2.41:2.45 mmol/L) but decreased serum albumin (m:f 39:39g/L *vs* 35:35g/L) when compared to the AMR. STAGE: ISS staging was similar on both registries with ISS stage 2 being most common in both cohorts (m:f 42%:37% *vs* 40%:40%, on the AMR and MRDR, respectively) while ECOG performance status at diagnosis was lower in the MRDR cohort (ECOG≤1 m:f 43%:44% *vs* 81%:78%, on the AMR and MRDR, respectively). FIRST LINE THERAPY: First line therapy was predominantly bortezomib (Velcade - V) based on both registries (81% *vs* 85%). V/dexamethasone (D) was the most common on the AMR (29%) followed by V/thalidomide/D (VTD) (25%) with V/cyclophosphamide/D (VCD) (79%) most common on the MRDR. V was predominantly administered subcutaneously on both registries (79% *vs* 88%) but more commonly weekly on the MRDR (51% *vs* 67%) *versus* twice weekly on the AMR (40% *vs* 27%). RESPONSE TO THERAPY: Overall response rates were similar between the two cohorts but with higher CR rates on the AMR (CR 21% *vs* 11%, VGPR 27% *vs* 31%, PR 31% *vs* 43%, SD 12% *vs* 14% and PD 8% *vs* 2%, on the AMR and MRDR, respectively).
Summary/Conclusions: This pilot study between the AMR and ANZ MRDR demonstrates many similarities but also highlights significant differences, particularly in first line therapy and depth of response. Future studies between the AMR and MRDR will provide a platform for ongoing international benchmarking.

PB1989

DETECTING EARLY RELAPSE IN MULTIPLE MYELOMA AFTER ASCT: USEFULNESS OF IMMUNEASSAYS

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Background: The Free Light chain immunoassay (FLC) (Bindingsite, Birmingham, UK) is part of the mandatory response assessment for MM, the role of the Heavy/Light Chain immunoassay (HLC), is under investigation. Also relapses in MM patients are frequent, autologous stem cell transplantation (ASCT) is the standard consolidation therapy and there is an interest to detect early relapses and optimize therapy. We hypothesized that the combination of these techniques could permit to detect early biological (non-symptomatic) relapses (EBR) in this setting.
Aims: To analyze the usefulness of HLC and FLC to detect EBR in MM after ASCT.
Methods: A retrospective study was performed following these criteria: all patients diagnosed of secretory MM, in our center, and treated (including ASCT), between May 2011-August 2015; the protocol for follow-up included FLC, HLC, serum and urine electrophoresis (SPE, UPE) with immunofixation (IFX), pre-ASCT, after 12

weeks and every 3 months later (minimum follow-up: 6 months). EBR was defined as 25% on M-protein increase (any amount for patients on CR/SR) and/or ≥20mg/dl FLC increase, and/or 25% involved HLC increase with abnormal ratios. For urine, an increase >500mg/24 hrs of involved free-chain protein.
Results: Fifty-five patients were registered. Median follow-up 47 months. MF ratio: 29/26, mean age 59.5 y (33-71). Immunoglobulin subtype: IgG-Kappa: 41.8% (23), IgG-Lambda: 23.6% (13), IgA-Kappa: 16.4% (9), IgA-Lambda: 7.3% (4), Bence-Jones-Kappa: 3.6% (2), Bence-Jones-Lambda: 7.3% (4). Durie-Salmon Stage: IA: 13.5% (7), II-A: 32.7% (17), III-A: 44.2% (23), III-B: 9.6% (5), missing-data 3 case. All patients received Bortezomib based therapy and MEL200 as ASCT conditioning. Status pre-ASCT: minimal response: 12%, Partial Response (PR): 50.0%, very-good-PR (VGPR): 28.0%, complete response (CR): 6% and string response (SR): 4.0%. After ASCT, evaluation reveals that 13.0% achieved SR, 13.0% CR, 30.4% VGPR and 39.1% PR. During follow-up, 34/50 (68%) patients who achieved at least PR after ASCT, had a clinical relapse/progress, median PFS 41 months (31.5-50.5). EBR were detected in 28 patients, of them 22/34 (64.7%) clinically relapsed patients at median time 8.0 (2-22) months before symptomatic relapse. The EBR were detected by FLCr (36.7%), HLCr (22.7%), FLC+SPE (4.5%), FLC+IFX (9.1%), FLC+HLC+SPE (13.6%), FLC+HLC+SPE+UPE (13.6%).
Summary/Conclusions: Both FLC and HLC are useful tools to detect EBR in more than 50% of patients in our cohort ahead other techniques.

PB1990

EARLY MORTALITY (<6 M) IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS: COMPREHENSIVE INTERVENTION
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Background: Early mortality in the first 6 to 12 months from diagnosis is well recognized in newly diagnosed multiple myeloma (NDMM) patients, with rates in the real-world setting of around 20-30%.
Aims: In a retrospective analysis of the causes of death performed by the end of 2012 we identify 2 different causes in the 2 consecutive periods analyzed. In the first period (1998-2006) the main cause was MM progression and in the second (2006-12) was secondary to serious infectious complications. Additional analysis were done after it an can identify a patient and a infectious profiles. Main risk factors from the patient were: age (over 75), suboptimal treatment and renal failure (calculated ClCr<50 ml/min). The infectious ocurred mainly in the first 3 months from diagnosis and principally polimicrobial and multiresistent infections.
Methods: After this analysis several measures were taken to reduce this high early mortality: 1) To promote the ambulatory regime both in diagnosis and for the rapid assessment of complications to avoid or shorten income and to reduce these nosocomial-behaviour infection complications. 2) Early initiation of "optimal" anti-myeloma treatment. 3) Get infectious prophylaxis in patients over 75 years and / or renal failure with Septrim ©.
Results: 343 pac NDMM were treated between 1998 and 2015 (127 in the 1st period, 115 in the 2nd: 242 pts before 2013; and 101 in the 3rd period: 2013-15). The median age at dx was 74 years (39-100). The number of patients died <6m was 77 years. 60 died before 6 months: 55 before 2013 (29 in the 1st period (22.8%) and 26 in the 2nd (22.6) and 5 after 2013 (5.0%). Of these 60, 37 had a severe infectious complication. The main cause of mortality before 2013 was infectious complications, (14 of 28 early death in the first period and 22 of 26 in the second). Severe pneumococcal infections were infrequent (11%) In the 3rd period, mortality <6m was reduced by 77% (22% *vs* 5%) (p.001); There was only 1 severe infection (G5) in this period (CMV reactivation, probably Pneumocystis pulmonary infection, E. Coli bacteriemia and a intestinal necrosis after an atrial fibrilation embolism) Figure 1 (upper corner). Improvement in early mortality increases significatly overall survival: 32.5 monts vs not reached pre and post-2013 (p.0034).

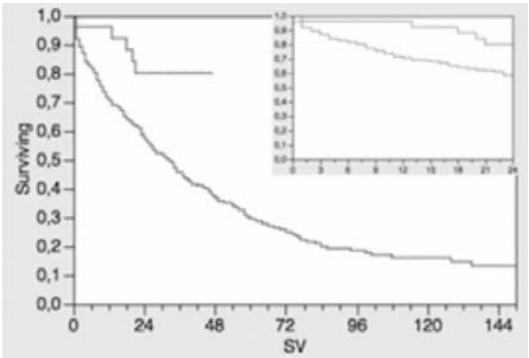

Figure 1 (large graphic; legends: red: pre2013; blue: post2013).

Summary/Conclusions: Infectious complications and progression of MM have been the main cause of early mortality in patients with NDMM. Identifying potential "modifiable" variables and acting on them improves the short-term prognosis of patients with NDMM like: Supportive treatment to prevent infectious complications (avoid unnecesary hospitalization, antibiotical prophylaxis) and rapid access to optimal antiMM treatments. These improvemente of short-term

PB1991

FIRST LINE USE OF NOVEL AGENTS BEFORE AUTOLOGOUS SCT HAS A POSITIVE IMPACT ON TIME TO SECOND PROGRESSION AND SURVIVAL IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA UNDER 70 YEARS
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Background: Most clinical trials for multiple myeloma (MM) patients using novel agent-based regimens before autologous stem cell transplantation have shown improvement in response rates and progression-free survival, however they have failed to identify a significant overall survival (OS) benefit.
Aims: Our aim is to analyze the potential impact of initial induction in the feasibility and outcome of subsequent treatment lines and other major factors affecting OS in a real clinical practice setting.
Methods: Newly MM patients less than 70 years of age diagnosed between December 1999 and December 2009 were prospectively registered. Patients were assigned to a first cohort if they received conventional chemotherapy (CC) induction regimens with new agents available at relapse or to a second cohort if received novel agents based first line treatment (NA).
Results: The overall response rate after completing first line treatment for all the 154 eligible patients was 85%, 79% in CC compared to 94% in NA (*P*=0.012). Very good partial response or better for NA was significantly higher than for CC (39% *vs* 59%, *P*=0.012). Patients in NA demonstrated not only a superior median progression-free survival (2.8 years *vs* 1.6 years, *P*=0.03) but also superior median progression-free survival from diagnosis to second progression – PFS2 (5.2 years *vs* 2.7 years, *P*=0.003). In both cohorts PFS1 and PFS2 represented more than 50% and 80% of life expectancy respectively. It could be hypothesized that CC patients would obtain more benefit than NA patients of second-line therapy, as they would be naïve to the novel agents used at relapse, but this is not the case. The use of thalidomide and/or bortezomib induction did not reduce the efficacy of these same agents second line. Indeed, these patients also had the best second responses that also contributed to longer PFS2 periods. After a median follow-up of 6.97 years, clear differences in OS were observed (7.97 years for NA compared to 3.35 years in CC, *P*<001). Despite the fact that better risk patients in the NA group were more likely to remain in first or second response, relapsed and refractory patients in this group still presented longer survivals beyond second relapse than patients in the CC group (Figure 1).

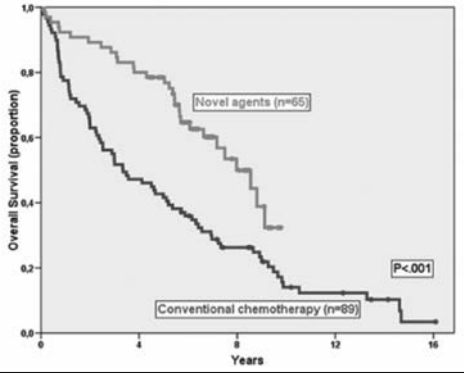

Figure 1.

Summary/Conclusions: New agent based first line induction treatments in newly diagnosed MM patients provide benefits beyond first progression free survival that contribute to a significant improvement in OS.

PB1992

SAFETY AND EFFICACY OF NOVEL AGENTS IN VERY ELDERLY MULTIPLE MYELOMA PATIENTS (AGED 80 YEARS OR MORE): A REPORT BY THE RETE EMATOLOGICA PUGLIESE (REP)
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Background: Multiple Myeloma (MM) is mainly a disease of the elderly and the very elderly patients (80 years of age or more) comprise one third about of all MM patients. This subset of patients suffer from concomitant disabilities and/or comorbidities and require a different and a more individualized therapeutic approach, including the novel agents.
Aims: The aim of our study is to verify safety and efficacy of novel agents with the reliability to maintain a good quality of life and obtain a maximal disease control.
Methods: Patients from 8 Hematology Centers of the "Rete Ematologica Pugliese (REP)" were included in this study. Between January 2011 and December 2016, 71 patients (M/F: 42/29) with a median age of 82 years (range 80-91) were diagnosed as newly symptomatic MM. Of the entire study population, 40 (56%) patients showed an ECOG score lower than 2. According to immunoglobulin heavy and light chain isotypes, patients had IgG-k (n=23), IgG-λ (n=16), IgA-k (n=14), IgA-λ (n=6), micromolecular k (n=8) and λ (n=4) chains. On the basis of ISS, patients were classified as I (n=4) score, II (n=23) and III (n=44) score, respectively. When CRAB features were considered, bone lesions represented the most frequent (n=43, 60.6%) clinical manifestations, while anemia, hypercalcemia and renal failure were found in 35 (49.3%), 2 (2.8%) and 2 (2.8%) patients, respectively. Majority of patients (n=49, 69%) showed at least 1 comorbidity requiring specific treatments, and 11 patients (15,5%) showed more than 3 comorbidities. Patients were treated according to Bortezomib-based regimens (VMP, VCD and VD) (n=45; 63.4%), Lenalidomide-based regimen (RD) (n=8; 11,3%) and Thalidomide-based regimen (MPT) (n=5; 7%). Only 13 patients (18.3%) did not receive any novel agent.
Results: Based on IMWG criteria, 15 patients (21.1%) achieved a CR, 15 patients (21.1%) a VGPR and 15 patients (21.1%) a PR. Fourteen patients (19.7%) and 12 (17%) patients experienced a SD and a PD, respectively. As second line of treatment, Bortezomib was used in 14 (33.3%) patients, Lenalidomide in 17 (40.5%) patients and Thalidomide in 3 (7,2%) patients. Height patients (19%) were treated with old drugs (Melphalan, Cyclophosphamide or Bendamustine). Pomalidomide was used as third line-therapy in 3 patients. After 72 months (median 32.5 months) of follow-up, 33 (46.5%) patients remained alive with a median survival of 36 months and 25 (28.2%) died. Last follow-up from 13 patients was unavailable. Hematological and extra-hematological toxicities were similarly distributed (18,3% and 18,3%, respectively) and usually weak/moderate. Neuropathy was the most common toxicity reported (n=5, 7%). Of patients treated with only novel agents (n=58), hematological and extra-hematological toxicity was observed in 14% and 16% patients, respectively.
Summary/Conclusions: We showed that all MM patients can be treated by novel agents independently of the age. Results from our study show that particularly very elderly and frail patients can benefit from these drugs by prolonging their life expectancy and maintaining a good quality of life.

PB1993

BORTEZOMIB-MELPHALAN-PREDNISONE *VERSUS* MP AS INITIAL TREATMENT FOR VERY ELDERLY PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA

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Background: Although bortezomib-melphalan-prednisone (VMP) therapy is a well-established standard treatment for patients with multiple myeloma (MM) who are ineligible for high-dose therapy, it is not clear whether very elderly patients should be treated with VMP in clinical practice, considering the toxicities.
Aims: The purpose of this case-control study was to compare the efficacy of VMP *versus* melphalan-prednisone or cyclophosphamide-prednisone (MP/CP) as initial therapy for very elderly patients.
Methods: We retrospectively studied 233 patients aged 75 years or older with newly diagnosed multiple myeloma between March 2007 and February 2015. One-hundred thirty one patients received VMP and 102 patients received MP/CP regimen were enrolled from 15 institutions throughout Korea.
Results: Patient characteristics were comparable in these two groups. Overall response rate was 70.2% in VMP patients and 48.0% in MP/CP patients (P=0.001). Complete response rate was 22.9% in VMP patients and 7.8% in MP/CP patients (P=0.002). After a median follow-up for survivors of 28.5 months, progression-free survival (PFS) and overall survival (OS) were significantly different between the two groups (PFS, median 21.3 *vs* 11.8 months in VMP and MP/CP group, respectively, P=0.018; OS, median 34.9 *vs* 22.8 months in VMP and MP/CP group, respectively, P=0.006). Nonetheless, for 61 patients who were aged ≥80 years, PFS and OS was not significantly different between the two groups (PFS, median 19.6 *vs* 13.2 months in VMP and MP/CP group, respectively, P=0.378; OS, median 27.8 *vs.*17.8 months in VMP and MP/CP group, respectively, P=0.443).
Summary/Conclusions: Although VMP therapy was associated with a significant improvement in overall survival among patients ≥75 years, there is no differences for patients aged 80 or older. Frailty and comprehensive geriatric assessment should be incorporated to guide treatment decisions for this population.

PB1994

EFFICACY OF AUTOLOGOUS STEM CELL TRANSPLANTATION FOR THE TREATMENT OF MULTIPLE MYELOMA IN HIV-POSITIVE PATIENTS – A CASE-SERIES

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Background: While hematopoietic malignancies are found at increased rates in individuals with acquired immunodeficiency syndrome (AIDS), coincidence of multiple myeloma (MM) and human immunodeficiency virus (HIV) is less common, leading to a paucity of expertise in the treatment of individuals with these co-morbid conditions. Prior to the advent of highly active anti-retroviral therapy (HAART), autologous stem cell transplant (ASCT) was relatively contraindicated for MM patients with HIV due to issues associated with stem cell harvest and the risk of opportunistic infections. With the widespread use of HAART for control of HIV, high dose chemotherapy and ASCT is now the preferred treatment for relapsed lymphoma, the leading hematopoietic malignancy associated with HIV/AIDS. It stands to reason that MM patients with HIV on HAART may benefit equally from aggressive combination treatment of chemotherapy and ASCT.
Aims: This study seeks to evaluate the clinical course and treatment outcomes of patients with HIV and MM treated with high dose therapy and ASCT.
Methods: A single center retrospective case-series study was performed. Data from patients who were HIV-positive and on HAART undergoing ASCT for treatment of MM between January 2000 and June 2016 were collected and analyzed.
Results: The following Table 1 lists patient characteristics. All were male with average age 53.2 years. All were diagnosed with HIV prior to diagnosis of MM and were appropriately treated with HAART prior to ASCT. All patients had undetectable HIV viral titer prior to ASCT, and most remained undetectable after ASCT. Four of five patients had CD4 >200/uL and one patient had CD4 <50/uL prior to ASCT; however all patients recovered CD4 counts after ASCT (and most with improved CD4 count). Adequate CD34(+) stem cells were collected. Patients received high dose melphalan (200 mg/m^2) followed by ASCT. HAART was continued during ASCT. Patients experienced usual ASCT toxicities including diarrhea, mucositis, and neutropenic fever. One patient developed sepsis and small bowel obstruction, which resolved with antibiotics and conservative management. All patients had normal neutrophil and platelet engraftments. Post ASCT responses were complete remission (2 patients), very good partial remission (1), partial remission (1) and minimal response (1). All patients are currently alive without relapse or progression 1-4 years from ASCT and receiving post ASCT maintenance with lenalidomide.

Table 1.

Case	Patient #1	Patient #2	Patient #3	Patient #4	Patient #5
Sex	Male	Male	Male	Male	Male
Race	Caucasian	Caucasian	Caucasian	Caucasian	African-American
Age at ASCT	58	48	52	58	50
ISS Stage	Stage I	Stage I	Stage II	Stage III	Stage I
Marrow cellularity	30%	40%	70%	65%	not determined
Cytogenetics	del(13q)	+11q	46XY	46XY	abnormal
CD34 cells collected (10^6 cells/kg)	11.95	21.34	10.81	10.91	9.47
Date ASCT	7/18/2013	7/20/2013	12/10/2015	5/18/2016	7/20/2016
Length of hospital stay (days)	16	17	27	18	13
Days to neutrophil engraftment	11	12	13	10	9
Days to platelet engraftment	11	13	16	11	10
Disease Status	CR	CR	PR	VGPR	MR
Overall Survival (days)	1295	1314	375	279	173
HIV viral load before ASCT (copies/mL)	undetectable	undetectable	undetectable	undetectable	undetectable
CD4 before ASCT (cells/μL)	248	44	370	564	321
HIV viral load after ASCT (copies/mL)	undetectable	76073	undetectable	undetectable	undetectable
CD4 after ASCT (cells/μL)	314	198	1028	221	278

Summary/Conclusions: Multiple myeloma patients with concurrent HIV infection that is controlled on HAART tolerate ASCT for treatment of myeloma as well as myeloma patients without HIV infection and have generally good outcomes.

PB1995

FEASIBILITY OF USING GLOBAL FDG UPTAKE IN BONE MARROW TO ASSESS TREATMENT RESPONSE IN MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) is characterized by plasma cell proliferation and expansion primarily in the bone marrow. Modern assessment of MM using FDG-PET has so far mostly been limited to the analysis of focal lesions, requiring subjective interpretation to determine overall disease activity.
Aims: A novel method using CT segmentation to determine global bone marrow activity portrayed by FDG uptake was used to achieve a comprehensive understanding of disease burden in patients with MM before and after therapy.
Methods: Prospective FDG-PET/CT data of 23 MM patients between ages of 50 and 76 (mean=64.3, males=21, females=2) were collected from Odense University Hospital (NCT02187731) and included scans before initiation of treatment and at end of treatment (EOT) two months after high dose chemotherapy with stem cell support. . All scans were conducted 60 min after intravenous injection of 4 MBq/kg of FDG. Images were analyzed using an iterative thresholding algorithm that delineates a continuous region based on Hounsfield units from the CT data (OsiriX software; Pixmeo SARL; Bernex, Switzerland), allowing for segmentation of the total skeleton on a fused PET/CT image. This enabled the quantification of FDG uptake representing the entire skeleton, providing a global SUVmean that considers all bone marrow involvement. Global SUVmean scores were compared before and at EOT using a two-tailed paired t test.
Results: A decrease in marrow FDG uptake was observed at EOT compared to baseline in most patients. The calculated global SUVmean uptake decreased after initiation of treatment in 17 (73.9%) of the cases and increased in 6 (26.1%) of the cases, supported by the observed statistical difference of the dependent means before and after treatment (P=0.0053).
Summary/Conclusions: We assessed the effects of treatment in MM patients using a novel technique for global quantification of FDG uptake in the bone marrow and skeleton and found lower global uptake at EOT. However, a limitation of bone segmentation is present in cases with extramedullary disease. Global assessment rather than focal analysis of discrete lesions represents a robust and straightforward method of determining total disease activity that potentially will be of value in treatment evaluation, disease monitoring and prognostication in multiple myeloma.

PB1996

VALUE OF MYELOMA PROGNOSTIC INDICES IN ERA OF NOVEL DRUGS IN TRANSPLANT SETTING

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Background: Despite the era of emerging novel agents, autologous peripheral blood stem cell transplantation remains backbone of myeloma treatment.
Aims: The main aim of our study was to evaluate the role of tandem transplantation in myeloma treatment as well as prognostic indices in era of novel drugs.
Methods: We consecutively included all patients transplanted due to myeloma at our center from 2012 to the end of 2016. Patients were treated with either VAD or bortezomib based therapy. After induction treatment, all patients proceeded to mobilization therapy cyclophosphamide 3g/m^2 and received pegfilgrastim. Preparative regimen was either MEL 200 for fit patients or MEL 140 for frail and those with severe renal function impairment. Patients treated with VAD who had poor response after autologous transplantation were subsequently treated with bortezomib based therapy. We examined following baseline characteristics: age, proportion of plasma cells in bone marrow biopsy or aspirate, FISH and lactate dehydrogenase (LDH). Additionally, for each patient International Staging System (ISS), Revised International Staging System (ISS-R) and Durie Salmon staging were calculated. Patients with other malignant diseases prior to myeloma diagnosis were excluded. The main outcomes were overall survival (OS) defined as death from myeloma or any other cause and time to next treatment (TNT), defined as time from transplant to next new therapy or death of any cause.
Results: From January 2012 to December 2016 hundred and one patient with MM (49 male, 52 female), median age 55 (range 22-71), were transplanted. Bortezomib based induction therapy was used in 55 (54,5%) and VAD induction was used in 46 (45.5%) patients. Median OS of all treated patients was 73 months; median OS of VAD group was 73 months while in bortezomib group median OS was not reached, but this difference was not statistically significant (p=0,13). TNT was significantly longer in bortezomib group than in VAD one (27,8 *vs* 17,5 months respectively; p=0,02). Interestingly prognostic indices could not discriminate patient groups according to OS (p=0,1), but could discriminate them due to TNT (p=0,008), possibly due to cross-over to bortezomib treatment after treatment failure. TNT had a significant correlation with levels of LDH (p=0,04) and no significant correlation with number of plasma cells in bone marrow. OS was significantly longer in those with longer duration of time to next treatment (p=0,0004). There was no difference in OS or TNT in patients treated with tandem transplant *vs* single transplant (p=0,68 and p=0,57 respectively), possibly due to heterogeneity of tandem group.
Summary/Conclusions: Even though novel drug therapy seems to converge risk groups to lower ones, prognostic indices remain relevant. Due to heterogeneity of patients and myriad of known prognostic factors further studies are needed so they may be translated into risk adapted therapy approach.

PB1997

WHICH ORGAN SHOULD WE BIOPSY TO DIAGNOSE AL AMYLOIDOSIS?

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Background: Light chain (AL) amyloidosis is a deposition disease with can affect many organs and with a variable but usually bad, prognosis. Therapy requires a quick and correct diagnosis. Accurate identification of amyloid deposition and of the amyloid subtype in tissue biopsies is thus, mandatory. Random biopsies of easily accessible tissues such as subcutaneous fat, gingivae or rectum are usually recommended but sensitivity of this approach is low.
Aims: To present our experience with tissue biopsies performed in 62 consecutive patients diagnosed of AL amyloidosis in our center.
Methods: We reviewed all tissue biopsies performed during the study period (2004-2017) in 62 consecutive patients diagnosed of AL amyloidosis at the same center. A bone marrow (BM) biopsy was performed per protocol in all cases. Decisions on biopsies were taken considering organ involvement and accessibility: skin, lymph nodes, lung or tongue biopsies were performed when lesions were seen on clinical or X-ray examinations, cardiac biopsies in the presence of increased NT-proBNP (N-terminal natriuretic peptide) levels and typical echocardiographic findings, kidney biopsies in patients with nephrotic syndromes. Biopsies were stained with Congo Red and read under polarized light with a Texas filter. Subtyping of the amyloid was done using anti-kappa, anti-lambda, anti-TTR and anti-A antisera. If any biopsy was positive for AL amyloid, no further biopsies were performed unless necessary for therapeutic decisions.
Results: A total of 152 biopsies were performed during the study period: see Table 1.

Table 1.

Tissue	Biopsies	AL amyloidosis
Bone marrow	59	25 (42.2%)
Heart	37	33 (89.2%)
Kidney	8	8 (100%)
Intestine/Rectum/ Stomach	10	7 (70%)
Gingivae	13	4 (30.7%)
Subcutaneous fat	11	4 (36.4%)
Liver	2	2 (100%)
Skin	2	2 (100%)
Tongue	3	3 (100%)
Lung	2	2 (100%)
Sural nerve	3	3 (100%)
Lymphnode	1	1 (100%)
Tonsil	1	1 (100%)
Total of biopsies	152	95 (94.7%)

Summary/Conclusions: Prognosis in AL amyloidosis is slowly improving with the use of new anti-myeloma drugs and may improve further with new monoclonal antibodies. Therapy requires an early and accurate diagnosis. We do not perform random biopsies of tissues such as fat or gingivae due to low sensitivity. In our hands biopsies of tissues or organs with clinical, analytical or radiological involvement shows higher sensitivity. A bone marrow biopsy is required for diagnosis of the neoplastic disease underlying AL amyloidosis and may show amyloid in up to 50% of the cases. Cardiac biopsy is also highly sensitive and in centers with a high degree of expertise such as ours, has no complications. Our data allow us to recommend a different approach to AL amyloidosis of what is usually published. Biopsies of clinically involved organs yields almost 100% sensitivity. Random biopsies of gingivae, subcutaneous fat or rectum should be discouraged.

PB1998

A COMPARISON OF CYCLOPHOSPHAMIDE-GLUCOCORTICOIDS AND LENALIDOMIDE-DEXAMETHASONE AS TREATMENT FOR MULTIPLE MYELOMA IN FIRST RELAPSE AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background: The optimal management of relapsed Multiple Myeloma (MM) with respect to therapeutic combinations and sequence remains controversial and is actively evolving. Many commonly used regimens have not been directly compared. These agents vary widely in cost, and knowledge of their relative efficacy is of particular importance in regions where cancer medicines are publicly funded.
Aims: We sought to compare the efficacy and safety of two commonly used regimens for relapsed MM using historical cohorts from a single transplant center.
Methods: A retrospective observational study was performed between January 1991 and November 2016 to compare the efficacy of cyclophosphamide and dexamethasone/prednisone (Cyclo), or lenalidomide and dexamethasone (Len-Dex) for relapsed MM post autologous stem cell transplant (auto SCT). The primary outcome was Time to Next Treatment 2 (TTNT2), defined as time from first relapse requiring therapy after auto SCT to second relapse requiring therapy. The secondary outcome was overall survival, defined as time of diagnosis to death from any cause. Outcomes were assessed by Kaplan Meier methods and overall differences determined by log rank test. Hazard ratios were calculated for individual treatment groups and compared by univariate and multivariate logistic regression.
Results: A total of 243 patients underwent treatment for MM at first relapse post autologous transplant. Of these, 139 were included in this analysis: 88 Cyclo and 51 Len-Dex. Patient demographics and disease characteristics were similar between each group for age, sex, subtype of MM and ISS Stage (p>0.05). Vincristine, Doxorubicin and Dexamethasone (VAD) was the most common treatment at diagnosis for the Cyclo group (68%), whereas bortezomib-based therapy was the most common for the Len-dex group (76%) (p<0.0001). No differences were observed in overall response rate or depth of response based on induction therapy between both groups. Median time to first relapse requiring treatment after auto SCT was longer in the Cyclo group, 36 months *vs* 25 months (p=0.0008). The median TTNT2 was similar for the two groups: 12.2 months (IQR, 4.56-27.96) for Cyclo and 12.1 months (IQR, 4.80-29.16) for Len-dex (p=0.52). However, after adjusting for standard patient and disease related factors in a multivariate model, TTNT2 was shorter for Cyclo compared to Len-dex (HR 2.29; 95% CI, 1.17 – 4.51; p=0.016). The median overall survival was 84 months for Cyclo and 75.6 months for Len-dex (p=0.31). In the multivariate analysis, overall survival was not different for Cyclo compared to Len-dex (HR 0.99; CI 0.42 – 2.34; p=0.99). There was no significant difference in rates of hospitalization, infection, or grade 3 adverse events between the two groups (Figure 1).

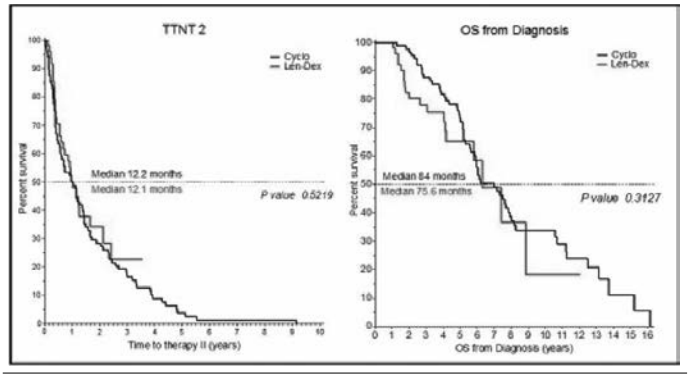

Figure 1. Survival curves.

Summary/Conclusions: In this observational study of patients with relapsed multiple myeloma post autologous stem cell transplantation, Lenalidomide-dexamethasone was associated with longer TTNT2 compared with Cylophosphamide-glucocorticoids. However, there was no difference in overall survival. Cyclophosphamide is considerably less expensive than the novel agents. In an era when fiscally sustainable care for MM remains a challenge, further prospective studies are required to compare cyclophosphamide with novel agents in the management of relapsed multiple myeloma.

PB1999

CLINICAL IMPACT OF THE PLASMA LENALIDOMIDE CONCENTRATION AND THE ANALYSIS OF ANTI-TUMOR IMMUNE RESPONSE IN NEWLY DIAGNOSED MULTIPLE MYELOMA TREATED WITH LENALIDOMIDE AND DEXAMETHASONE THERAPY

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Background: Lenalidomide (Len) and dexamethasone (DEX) combination therapy (Ld) is now the standard treatment of multiple myeloma (MM). Len has both a direct effect on MM cells and an immunomodulatory effect and recently many drugs are combined with Ld therapy to expect the synergistic anti-tumor immune response. However, adverse events (AEs) make continuation of Ld therapy difficult for some patients especially for elderly patients.
Aims: To investigate the safe and effective plasma concentration of Len and the anti-tumor immune response change in MM patients treated by Ld therapy.
Methods: Forty patients (18 men and 22 women) were enrolled in this study. Median age was 75.5 years old (range 61-86). Len was administered on days 1–21 of a 28-day cycle; and DEX, on days 1, 8, 15, and 22. The plasma concentrations of Len just before oral administration and 1, 2, and 4 hr thereafter were analyzed by using liquid chromatography-tandem mass spectrometry. Before and after Ld therapy, Peripheral blood mononuclear cells (PBMCs) of MM patients were isolated from whole blood by Ficoll-Hypaque density-gradient centrifugation. PBMCs were stained with the fluorescent dye-conjugated antibodies against surface and intracellular antigens and evaluated by multicolor flow cytometry. Intracellular cytokine production of IFN-γ, TNF-α, IL-2 and CD107a molecule was detected after stimulation with PMA/ionomycin for 5 hours in the presence of protein transport inhibitor Golgi stop (BD Bioscience). Analysis was performed using LSR Fortessa (BD Bioscience) and Flowjo version 10.2 software (TreeStar). This study protocol was approved by the Ethics Committee of Akita University Hospital, and all recipients gave written informed consent.
Results: 21 patients showed renal impairment (RI) necessary to adjust initial Len dosage. Adverse cytogenetics of del17p and t(4;14), detected by using fluorescence *in situ* hybridization, were found in 2 and 4 patients, respectively. The median initial dosage of Len was 15 mg and DEX 20 mg. The overall response rates were 68.6% and the 2-year progression-free survival was 70.8% at a median follow-up of 26.5 month. Grade 3 to 4 nonhematologic AEs were observed only in 8 patients. We estimated the $AUC_{0\text{-}24}$ of Len by using formula as we previously reported (Ther Drug Monit 2014) and the cut-off value of the hematologic AEs was 2613.5ng•hr/ml (sensitivity 81.8%, specificity 80%) and non-hematologic AEs 3023.6ng•hr/ml (sensitivity 78.9%, specificity 62.5%). After Ld therapy, naïve subset of CD4 and CD8 T cells and monocytic MDSC reduced significantly. On the other hand, effector memory subset and intracellular cytokine productions of IFN-γ, TNF-α, IL-2, and CD107a of CD4 and CD8 T cells increased significantly (Figure 1).

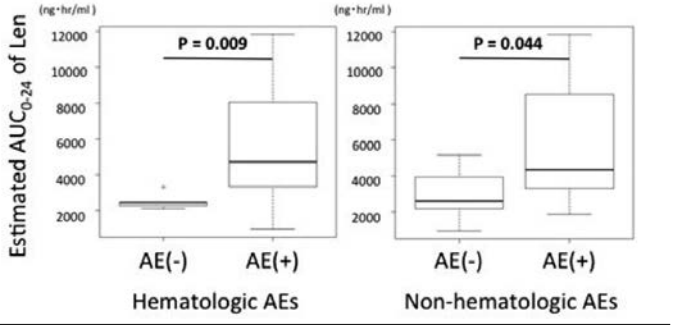

Figure 1.

Summary/Conclusions: Len can be administered safely even in elderly patients with RI by using the estimated $AUC_{0\text{-}24}$ of Len as a prediction marker of AEs. Enhanced cytokine production and increased memory subset of T cells were observed after Ld treatment.

PB2000

THE ROLE OF EXPRESSION CD56 ON BONE MARROW PLASMA CELLS AND EXTRAMEDULLARY PLASMA CELLS IN PATIENTS WITH MULTIPLE MYELOMA

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Background: The myeloma cells interact with the bone marrow microenvironment by several adhesion molecules. One of them is CD56 (a neural cell-adhesion molecule N-CAM) – a membrane glycoprotein, a member of the immunoglobulin superfamily, expressed on the surface of malignant plasma cells of patients with multiple myeloma (MM). Decreased expression of CD56 is considered as one of the possible factors, that help tumor cells to spread outside the bone marrow.
Aims: To evaluate the impact of CD56 expression on the rate of overall survival (OS) in MM patients with extramedullary disease (EMD).
Methods: The study included 32 patients with primary MM (17 males, 15 females) 23-77 years old (median value: 52 years old). The disease was diagnosed in accordance with the IMWG criteria (2014). 17 patients had EMD including 14 patients with soft-tissue plasmacytomas associated with bone and 3 patients with extramedullary foci in the neck area, in the stomach, in the liver. In all cases a tumour biopsy and bone marrow trephine biopsy were performed, that confirmed the presence of malignant plasma cell infiltration. Paraffin block slices from trephine biopsy material and tumour biopsy material were used to perform an immunohistochemistry (IHC) analysis with an antibody to CD56. Kaplan-Meier survival curves were generated, statistical analysis was done using the program «Statistica» ver.10.
Results: In patients with plasmacytomas the IHC analysis of trephine biopsy material showed $CD56^+$ in 59% cases *vs* 73,4% in patients without EMD. Five-year OS in patients with $CD56^+$ in the bone marrow was 90%, which was significantly higher (p=0,04) than that of the patients with $CD56^-$ - 0% with follow-up of 5 to 61 months (median 20 months, Figure1). Expression of CD56 on the surface of extramedullary MM cells was found in 76,5% patients. OS in the group of patients with $CD56^+$ in extramedullary MM cells and in bone marrow cells (n=9) was 67% which was significantly higher (p=0,04) than that in the group of patients (n=4) with $CD56^+$ in extramedullary MM cells and $CD56^-$ in bone marrow cells – 50%. Simultaneous lack of CD56 in extramedullary MM cells and in bone marrow cells was observed in 3 patients with 2 of them died of progression in 31 and 51 months. However simultaneous expression of CD56 in extramedullary MM cells and in bone marrow cells was observed in 9 patients with median follow-up of 40 months and 1 patient died of progression after 47 months.

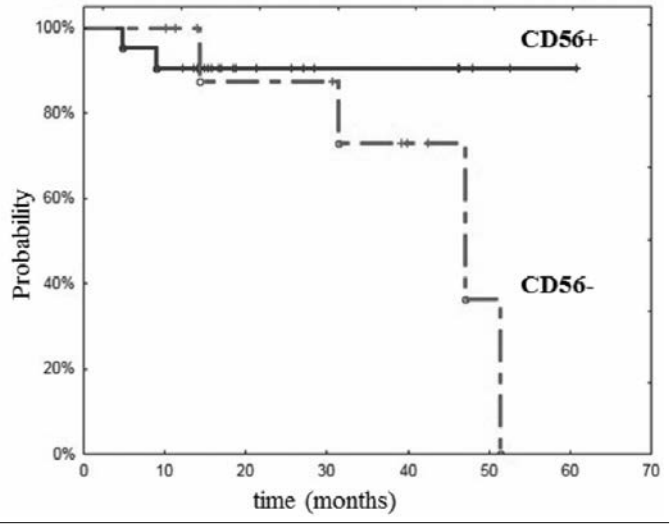

Figure 1. Probability of overall survival in patients depending on CD56 expression in bone marrow.

Summary/Conclusions: CD56 expression in bone marrow plasma cells significantly increases the OS rate in MM patients regardless the presence or absence of plasmacytomas. Double CD56 negativity both in extramedullary and bone marrow MM cells is a poor prognostic factor with high risk of early relapse and death.

PB2001

BENDAMUSTINE-BORTEZOMIB-DESAMETASONE IN THE MANAGEMENT OF RELAPSED AND REFRACTORY MULTIPLE MYELOMA

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Background: Bendamustine is a bifunctional alkylating agent, with low toxicity, proved to be effective in relapsed, refractory and in new diagnosed Multiple Myeloma (MM).
Aims: It has been evaluated efficacy and tolerance of Bendamustine, in combination with bortezomib-dexamethasone (BVD) in patients with relapsed and refractory MM (rrMM), whose prognosis is particularly severe. A regional retrospective real-life analysis of patients with rrMM who had been treated with BVD as salvage therapy has been performed.
Methods:
56 patients (31 M/25 F, Table 1), with rrMM, median age at diagnosis 57.3 years (r. 36-82), median age at start of treatment 61.8 years (r.37-83) treated with several lines of treatments (median 6, r. 2-11), every refractory to all the drugs previously received (also Bortezomib), received BVD (Bendamustine 90 mg/sqm days 1,2; Bortezomib 1.3 mg/sqm days 1,4,8,11, Dexamethasone 20 mg days 1,2,4,5,8,9,11,12, Pegfilgrastim day +4) every 28 days, until progression. ISS was equally distributed, and cytogenetic was evaluable in 12 patients, and in particular one del13q and one t(11;14). All the patients had previously been treated with schedule containing bortezomib and IMIDs, and 30% had also received radiotherapy. 67% of them had undergone at least to a single auSCT. All patients were relapsed and refractory to last therapies received before BVD, including bortezomib.
Results: According to IMWG, after a median follow-up of 14 months (r.2-36), ORR was 64% (36/56 : 4 CR, 7 VGPR, 16 PR, 9 MR) with 8 PD and 12 patients in SD, which can be considered as an impressive result in this subset of rrMM patients. In particular, for 11 patients, BVD was, after having achieved at least a PR, a bridge to second auSCT, and for two patients a bridge to alloSCT. Median time to response was 1.2 months (r.1-3), median OS from diagnosis was 62.7 months (range 6-151), median OS from start of Bendamustine was 9.8 months (range 2-36).

Table 1.

Total patients	**56**
Male	31
Female	25
Median age, years	
at diagnosis, (range)	57.3 (36-82)
at start of BVD, (range)	61.8 (37-83)
Previous regimens	
median no. (range)	6 (2-11)
FISH analysis	**12/56**
negative	10
del13q	1
t(11;14)	1
Previous therapies : no. of patients/(%)	
Bortezomib	56 (100%)
IMIDs	56 (100%)
Autologous SCT	38 (67%)

Summary/Conclusions: BVD has shown significant efficacy in a particularly severe setting of patients, relapsed and refractory to all available therapeutic resources, and, in particular cases, it could be considered as a bridge to a second autologous or allogenic SCT.

PB2002

VE-CADHERIN IN MULTIPLE MYELOMA: AN INDEPENDENT PROGNOSTIC FACTOR FOR PROGRESSION-FREE SURVIVAL

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Background: Endothelial damage and perivascular infiltrates are vital in the development of multiple myeloma. Recent studies have found that endothelial dysfunction might be result in multiple myeloma progression and adverse effects of drug implementation. On the other hand, there is a direct correlation between microvessel density in multiple myeloma and parameters of disease progression. Endothelial cells participate in inflammatory events leading to atherogenesis by regulating endothelial cell permeability via the expression vascular endothelial-cadherin (VE-Cadherin) on their surface. VE-cadherin is cell adhesion molecules localized at the endothelial junction, which plays critical roles in angiogenesis, neovascularization, neoplasm development, stem cells mobbing, and endothelial integrity. Indeed, VE-cadherin chiefly organizes the opening and closing of the endothelial barrier. It has found that VE-cadherin as a transmembrane protein probably modulates intensity of angiogenesis in multiple myeloma and may be useful in prognosis. However, the predictive role of VE-cadherin as a prognostic factor for survival of patients after treatment of multiple myeloma is not still clear.
Aims: We aimed to evaluate the prognostic value of circulating VE-catherin for progression-free survival in patients with multiple myeloma in complete or partial remission.
Methods: One hundred twelve out subjects with multiple myeloma were

enrolled in the study. Diagnosis and staging of multiple myeloma were defined by current clinical practice guidelines. To be achieving remission chemotherapy with bortezomib, thalidomide, dexamethasone, cyclophosphamide, melphalan, anthracyclines was used accordingly contemporary clinical guidelines. All subjects were at complete or partial remission at baseline. Observation period was up to 12 months. ELISA method for measurements of circulating level of VE-catherin was used.
Results: Medians of circulating levels of VE-catherin in subjects without progression of multiple myeloma (n=89) and subjects with progression (n=23) during 12 months were 0.92 ng/ml (95% confidence interval [CI]=0.66-1.19 ng/ml) and 1.77 ng/ml (95% CI=1.47-2.07 ng/ml) (p=0.0002). The best VE-catherin cutoff value for predicting disease progression risk was 1.31 ng/mL with AUC value 0.839 (p=0.0001), the sensitivity and specificity were 77.8% and 61.5% respectively. The presence of high levels of serum VE-cadherin was significantly correlated to a shorter progression-free survival (PFS). In a multivariate analysis along with clinical and biologic prognostic parameters, high serum VE-cadherin level (>1.31 ng/ml) was an independent adverse prognostic variable for PFS (median PFS 9.93 (IC=8.16-11.71) months *vs* 7.35 (IC=5.75-8.95) months (p=0.02).
Summary/Conclusions: The serum VE-cadherin level is a valuable biomarker for predicting treatment response and an independent prognostic factor for progression-free survival for patients with multiple myeloma.

PB2003

THE UTILITY OF FACS PURIFICATION OF PLASMA CELLS FOR FISH ANALYSIS IN MONOCLONAL GAMMOPATHIES

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Background: Despite the prognostic value of chromosomal aberrations, conventional metaphase karyotyping in monoclonal gammopathies (MG) is often uninformative due to the inherent difficulty of obtaining proliferating plasma cells (PC). Interphase fluorescence *in situ* hybridization (FISH) is a simple, quick and effective technique for the detection of cytogenetic aberrations that can overcome this limitation. However, the signal of interest is frequently diluted by the noise of the mixed cellularity of the sample, originating both false negatives and false positives. Fluorescence-activated cell sorting (FACS) of the target cells enables a focused application of FISH on pathologically significant cells – such as the PC in MG – reducing the confounding noise. This is particularly relevant when the percentage of pathologic cells in the sample is low, such as in monoclonal gammopathy of undetermined significance (MGUS) where, by definition, there are less than 10% PC in the bone marrow.
Aims: This study aims to analyze the utility and effectiveness of FACS purification of PC for the cytogenetic workup of MG by FISH.
Methods: We analyzed all FISH studies performed in our laboratory, in individual patients, on clonal interphase FACS-separated bone marrow PC, between the 1st June 2015 and the 15th September 2016. The probes used in our standard MG panel were del(1p32), amp(1q21), t(4;14) and del(17p13.1) (TP53 gene) and, starting in April 2016, t(14;16). We had previously established 20 000 cells per sample as the minimum (and sufficient) number of cells needed to guarantee the confident application of all 5 probes in our lab.
Results: After the exclusion of samples diluted with peripheral blood, we identified 102 patients with FACS separated purified PC. An average of 165 393±270 516 PC were separated per patient, and 98 of the cohort (96.1%) had a sufficient number of cells for the hybridization of at least one FISH probe; all 5 probes were applied in 30% of patients, 4 in 50%, 3 in 12% and 2 in 8%; the motives underlying the selection of fewer than all 5 probes in samples with a sufficient number (>20 000) of cells included the individual decision of the assisting physician and, for t(14;16), the date of the study. Considering only those studies performed after the introduction of t(14;16), all 5 probes were used in 67.5% of patients; we were able to apply four or more probes in 80% of patients with 1% or less bone marrow PC according to flow cytometry. The median age of the 98 patients with a FISH result was 63.6 years old (37.8 to 87.3), and 56.1% were male; 41.8% eventually received a diagnosis of MGUS and 58.2% of multiple myeloma (MM), with an identical median age (64.2±6.9 *vs* 63.0±10.8 years old, p=NS). We found that 16.3% (of 92) were positive for t(4;14), 12.2% (of 90) for del(17p13.1), 5.6% (of 90) for del(1p32) and 41.1% (of 90) for amp(1q21); t(14:16) was not identified in any of the 30 patients in whom the probe was used. The t(4;14) translocation was present in 22.4% of MM and 7.7% of MGUS patients (p=0.055), and del(17p13.1) was found in 18.5% *vs* 2.8% (p=0.026); on the other hand, both del(1p32) (5.6% *vs* 5.6%, p=NS) and amp(1q21) (46.3% *vs* 33.3%, p=NS) were identically distributed across diagnoses. We observed that 40.4% of MM and 65.8% of MGUS patients were positive for 20% of less of the tested aberrations, while 54.4% *vs* 34.2% were positive for 20 to 50%, and 5.3% *vs* 0% were positive for over 50% of the aberrations (p=0.026).
Summary/Conclusions: We have found that the application of FISH probes in FACS-separated PC is highly efficient with a robust yield, providing a large enough sample for the application of at least two probes in over 95% of patients, irrespective of bone marrow plasmacytosis; in fact, we obtained an average of 165 000 pure PC per patient, which is more than 8-fold higher than the number we consider invariably sufficient to apply 5 probes, which we achieved in at least 80% of patients.

PB2004

CLINICAL SPECTRUM AND EVOLUTION OF MONOCLONAL GAMMOPATHY ASSOCIATED NEUROPATHY *VERSUS* CHRONIC INFLAMMATORY DEMYELINATING POLYNEUROPATHY PATIENTS

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Background: Paraproteinemic neuropathy (PPN) refers to a disorder of the peripheral nervous system associated with a monoclonal gammopathy (MG). It is known that about 10% of idiopathic peripheral neuropathies are of this type. Unfortunately, PPN is often underdiagnosed or confused with chronic inflammatory demyelinating polyneuropathy (CIDP), subsequently leading to inappropriate management. Since progression of neuropathy is associated with possible malignant conversion of underlying monoclonal gammopathy, it is important to recognize underlying hematological conditions.
Aims: We aimed to determine whether the clinical characteristics and course differed in patients with PPN compared to those with CIDP in order to identify factors useful for differential diagnosis.
Methods: This study was carried out at Seoul National University Hospital, which is a tertiary academic center. During the period between January 2005 and December 2016, patients with 1) monoclonal gammopathy of undetermined significance (MGUS), and 2 CIDP were identified. Those with previous history of cancer or autoimmune disease requiring treatment with immunodulatory agents were excluded from analyses. In the end, a total of 18 MGUS patients and 34 CIDP patients, with complete set of data including clinical physical examinations, electrodiagnostic studies, and laboratory test results, were enrolled.
Results: In both groups, males were predominant. IgG MG was most common (55.6%) in our cohort. PPN appeared to be mainly sensory regardless of heavy chain or light chain. Compared to PPN patients, CIDP patients were associated with motor symptoms manifesting as motor weakness (50.0% *versus* 91.2%, P=0.001) and ataxia (44.4% *versus* 61.8%, P=0.043) (Table 1). There were equal number of axonal type neuropathy and demyelinating type neuropathy in patients with PPN, and there were no differences in type of neuropathy between various immunoglobulin subclasses. However, demyelinating type PPN was associated with more severe clinical presentations, including more dysethesia, pain and sensory symptoms. During median follow-up of 49 months, 2 PPN patients developed overt hematologic malignancies: 1 case of Waldenstrom macroglobulinemia and 1 case of AL amyloidosis. Both of them showed malignant transformation within 8 months of neuropathy development, and were associated with worsening neuropathic symptom at the diagnosis of hematologic malignancy. There were no differences between the two groups with regards to overall survival.

Table 1. Clinical characteristics of all enrolled patients.

	MGUS (n, %)	CIDP (n, %)	P
Total	18	34	NA
Age (mean, years [±SD])	67.6 (10.3)	57.8 (16.5)	0.027
Sex (male)	13 (72.2)	24 (70.6)	0.902
Ig type			
IgG	10 (55.6)	NA	NA
IgA	3 (16.7)	NA	
IgM	3 (16.7)	NA	
Light chain	2 (11.1)	NA	
Light chain			
Kappa	7 (38.9)	NA	NA
Lambda	11 (61.1)	NA	
Clinical presentation			
Motor weakness	9 (50.0)	31 (91.2)	0.001
Sensory changes	16 (88.9)	33 (97.1)	0.229
Pain	4 (22.2)	6 (17.6)	0.690
Atrophy	4 (22.2)	14 (41.2)	0.172
Ataxia	8 (44.4)	21 (61.8)	0.043
MRC scale (mean ±SD)	55.5 (7.2)	54.4 (5.3)	0.532
Pattern			
Axonal	9 (50.0)	0	<0.001
Demyelinating	9 (50.0)	34 (100.0)	
Treatment			
Steroids	11 (26.2)	31 (91.2)	0.009
IVIg	7 (38.3)	23 (76.7)	0.046
Rituximab	4 (22.2)	4 (11.8)	0.320
AZA/MMF	4 (22.2)	8 (23.5)	0.915
Treatment response			
Worsen	2 (11.1)	6 (17.6)	0.268
Stable	10 (55.6)	11 (32.4)	
Improved	6 (33.3)	17 (50.0)	

MGUS, monoclonal gammopathy of undetermined significance; CIDP, chronic inflammatory demyelinating polyneuropathy; NA, not applicable; SD, standard deviation; MRC scale, Medical Research Council scale for muscle strength; IVIg, intravenous immunoglobulin; AZA, azathioprine; MMF, mycophenolate mofetil.

Summary/Conclusions: Although both PPN and CIDP patients suffer from sensory symptoms, CIDP patients were more often associated with superimposed motor symptoms. Among PPN patients, demyelinating type neuropathy seems to be associated with more severe clinical presentations. Worsening of neuropathic symptoms in PPN patients warrants a high level of suspicion of malignant transformation of underlying disease.

PB2005

MOLECULAR GENETIC CRITERIA PREDICTING THE EFFICIENCY OF PERIPHERAL BLOOD HEMATOPOIETIC STEM CELLS TRANSPLANTATION IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Global gains in treatment of MM using auto-PBHSCT testify to heterogeneity of long-term outcomes of transplantation - different term of the achievement and duration of complete remission, progression-free survival (PFS), overall survival (OS). These facts determine individual approach to the application of PBHSCT.
Aims: Finding molecular genetic criteria of predicting the effectiveness of autologous peripheral blood hematopoietic stem cell transplantation (auto-PBHSCT) for improving of algorithm of multiple myeloma (MM) patients cure at various stages of treatment.
Methods: The study involved 61 patients with MM with relapse and primary therapy resistant patients. Molecular cytogenetic, immunogenetic, hematological and statistical methods were used.
Results: Since appearance of genetic abnormalities in the malignant plasma cells is one of the pathogenic mechanisms of the disease, genetic support of patients is essential. It was determined that the carriage of the allele HLA-DQB1*03: 02 in MM patients is associated with a high risk of high-dose chemotherapy resistance (F=4,83, p=0,028; OR=1,75, p=0.038), and achieving remission after auto-PBSCT is associated with carriage of haplotype HLA-C *06 - HLA-DQA1*01: 01 (F=4,87, p=0,028; OR=7,34, p=0,05). Abnormalities of chromosomes 4, 11, 13, 14, 16 and 17 were determined in 35 of 61 (57%) MM patients with complicated disease course and minimal therapy response. Significant alterations were revealed in the presence of two or more abnormal clones (23 patients (37,7%), Ro Spirman=0,42, p <0,05), deletion of chromosome 17 (17 patients (27,9%), Ro Spirman=0,41, p <0,05) , deletion/monosomy of chromosome 13 (10 of 15 patients surveyed, Ro Spirman=0,33, p <0,05), the translocation t(4;14) (4 patients (6,6%), Ro Spirman=0,50 , p <0,02).
Summary/Conclusions: The results indicate the necessity of introducing the molecular genetic support into protocol of examination MM patients on various stages of treatment with auto-PBHSCT.

PB2006

THE INFLUENCE OF MINIMAL RESIDUAL DISEASE AND TUMOR LOAD ON THE PROGRESSION FREE SURVIVAL IN MULTIPLE MYELOMA PATIENTS

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Background: Use of modern drugs and their combinations in the complex antimyeloma therapy (induction, high-dose therapy (HDT) with autologous stem cells transplantation (ASCT), consolidation and maintenance therapy) to improve efficacy of treatment and duration of responses. Despite the achievement of complete response (CR) many patients has a relapse which is caused by activation of residual clonal plasma cells.
Aims: To define influence of induction therapy regimens, HDT with ASCT to the frequency of Minimal Residual Disease (MRD) negative status and estimate a role MRD in duration of Progression Free Survival (PFS) in multiple myeloma (MM) patients.
Methods: We analyzed 52 patients with MM (median age 55 years, male/female – 2.1:1). The induction therapy with Bortezomib-based regimens (VD, CVD, VMP, PAD) was used in 36/52 (69%) patients, Immunomodulator-based regimens (Thal+D, RD, VRD, PomD) – in 14/52 (27%), chemotherapy – in 2/52 (4%). ASCT is carried out 31 (59.6%) patients. Primary tumor cells phenotype and MRD were detected by 5-color flow cytometry. Clonal plasmatic cells were detected by markers: CD38, CD138, CD45, CD19, CD20, CD27, CD56 and CD117. MRD-negative status considered in identifying less than 1 tumor cell in 10000 (0.01%).
Results: MRD-negative CR was reached in 23.8% (10/42) patients after 4-6 cycles of therapy. The frequency of MRD-negative status in the "Bortezomib group" was 31% (9/29), in the "Immunomodulator group" - 7.7% (1/13) (Chi-square =0.1; *p >.05*). The general frequency of MRD-negative CR after HTD with ASCT was 33.3% (7/21). The carrying out HTD with ASCT allowed to MRD eradication in 36.4% (4/11) patients. One patient with a "light chain" myeloma lost MRD-negative CR after HTD with ASCT that led to development of a clinical relapse after 6 months. Carrying out a maintenance therapy with bortezomib or lenalidomide didn't allow to achieve MRD-negative status. Carrying out a maintenance therapy with bortezomib or lenalidomide didn't allow to achieve MRD-negative status in patients with MRD-positive response. On the contrary, achievement MRD-negative status promoted to increase of PFS. The PFS median in MRD-positive group of patients (n=36: 21 CR, 6 VGPR, 9 PR) was 21 months, in the MRD-negative group (n=16) – 66 months (p=.0068). The PFS median patients with CR was higher in the MRD-negative group than in the MRD-positive group (66 and 48 months, respectively, p=.0045). The tumor load is also a strong prognostic factor like MRD status. Patients who attained low-level MRD had of benefit in the duration of PFS: <0.01% - 66 months, 0.01%>0.1% - 48 months at, 0.1%>1% - 22 months, >1% - 10 months (p=.0009) (Figure 1).

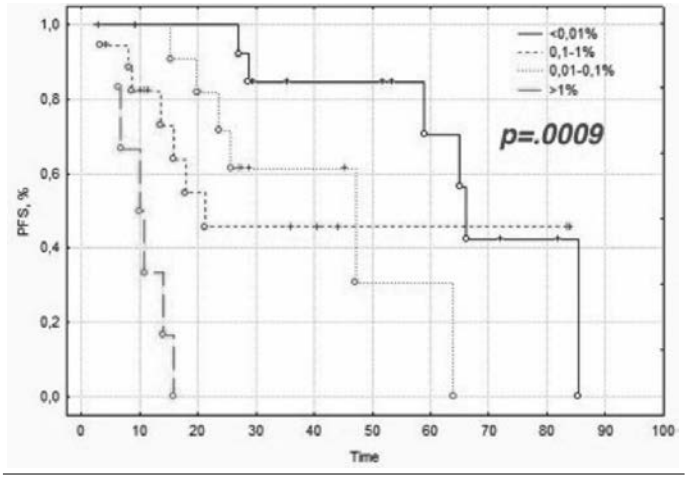

Figure 1. The influence of tumor load on progression free survival.

Summary/Conclusions: The frequency of achievement MRD-negative doesn't depend from program of induction therapy, HDT with ASCT and maintenance therapy. Negative prognostic role of MRD status independent from clinical response. Presence of MRD after treatment to associated with decrease of PFS and early relapse. Control of MRD allows to increase of PFS and can be done by means of modern drugs and its combinations, HDT with ASCT and maintenance therapy. Impact of MRD requires further studies, especially after HDT with ASCT.

PB2007

QUALITY OF RESPONSE AS PREDICTOR OF SURVIVAL AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN REAL LIFE MULTIPLE MYELOMA PATIENTS IN A SINGLE INSTITUTION

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Background: High dose chemotherapy (HDC) followed by autologous stem cell transplantation (ASCT) is the standard treatment approach for younger patients with multiple myeloma (MM). Since the introduction of proteasome inhibitors and immunomodulatory drugs in MM treatment more patients achieve deep and durable responses and better disease control before ASCT.
Aims: To evaluate the association between the depth of response before ASCT and survival outcomes in a cohort of patients with MM.
Methods: Retrospective analysis of patients with MM treated with HDT and ASCT between 2007 and 2016 in a single institution. All patients received peripheral blood stem cell support after conditioning with high dose melphalan (200 mg/m^2 and 140mg/m^2 for patients with renal insufficiency). Response was assessed 100 days after ASCT according to the International Myeloma Working Group response criteria. The Kaplan-Meier method was used to estimate progression free survival (PFS) and overall survival (OS) and comparison between risk groups was performed by using the log-rank test. The prognostic factors of survival were analyzed by Cox regression univariate and multivariate analysis.
Results: We included 195 MM patients, mainly males (57.9%) with a median age at ASCT of 61 years (28-71). The most prevalent subtype was IgG k (44.1%). The median number of previous therapeutic lines was 1 (1-4) and the majority of patients (61%) received bortezomib as part of first-line regimen. Patients undergone ASCT within a median of 10 months after diagnosis. With a median follow-up time from ASCT of 28.55 months (2.8-121.4), OS at 2 and

5 years was 83.8% and 68.9% and PFS was 74.8% and 37.3%, respectively. Before ASCT, 101 patients (51.8%) achieved very good partial response (VGPR) or better (≥VGPR) and 94 patients (48.2%) a partial response (PR). The patients in ≥VGPR presented significantly longer OS (median OS not reached *vs* 96.9 months, p=0.023) and PFS (58.5 *vs* 41.2 months, p=0.003) compared with those in PR. At 100 days after ASCT, 107 patients (54.9%) presented ≥VGPR, 79 (40.5%) PR and 7 (3.6%) progressive disease. Two patients were not assessed due to loss of follow-up. The group of ≥VGPR showed superior OS (median OS not reached *vs* 72.4 months, p=0.023) and PFS (58.5 *vs* 34.79 months, p=0.007) compared to the PR group. We did not found statistically significant differences in survival of patients who achieved ≥VGPR before or after ASCT. Univariate analysis indicates that depth of response before and after ASCT (≥VGPR *vs* PR) are significant predictors of OS (HR 0.49; 95% CI 0.31-0.80, p=0.004 and HR 0.49; 95% CI 0.30-0.81, p=0.005) and PFS (HR 0.50; 95% CI 0.27-0.92, p=0.026 and HR 0.49; 95% CI 0.27-0.90, p=0.021). Multivariate Cox regression model showed that these factors retain their prognostic value after adjustment for age, International Staging System stage and number of previous lines of treatment.

Summary/Conclusions: These findings provide evidence for quality of response as a predictor of OS and PFS after ASCT in patients with MM. Outcome after ASCT seems to be better for MM patients who achieve deep responses (at least VGPR) before or after transplant. Our results support the use of more effective induction regimens in order to improve initial response as this may correlate with higher response rates and survival post-ACST.

PB2008

LEPTOMENINGEAL INFILTRATION SCREENING SHOULD BE PERFORMED IN PATIENTS DIAGNOSED WITH PLASMA CELL LEUKAEMIA

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Background: Plasma cell leukaemia (PCL) is a rare and aggressive plasma cell (PC) disorder characterized by the presence of circulating plasma cells. PCL can either originate *de novo* (pPCL) or as secondary PCL (sPCL) in patients with relapsed/refractory multiple myeloma (MM). PCL has a more aggressive clinical presentation than MM with a more frequent extramedullary involvement, such as leptomeningeal infiltration. However, because of the low incidence of this entity, most clinical data come from small retrospective studies. Classical diagnosis criteria of PCL are today under review and the incidence of leptomeningeal infiltration is unknown.

Aims: We aimed to study the clinical features with special emphasis in the incidence leptomeningeal infiltration in patients diagnosed with PCL in our centre.

Methods: Seventeen patients were diagnosed of PCL between 2008 to 2016 in our centre. PCL was defined based on criteria from the Chronic Leukaemia Myeloma Task Force, by the presence of 2x109/L peripheral blood PC or plasmacytosis accounting for more than 20% of the differential white cell count. Medical records were retrospectively reviewed. Clinical response was evaluating per IWMG criteria. Clinical and biological features, progression free survival (PFS) and overall survival (OS) were analyzed. Survival curves were estimated using the Kaplan-Meier method and compared using the Log-Rank test.

Results: Seventeen patients with PCL were included. Six (35.3%) were pPCL and eleven (64.7%) sPCL. Median age at diagnosis was 57 years (range 35-78) and 8 (47.1%) were males. Clinical and analytical features at the moment of diagnosis are recorded in Table 1.

Table 1.

Clinical characteristics:	
Durie-Salmon stage	
I	2 (11.1%)
II	2 (11.1%)
III	13 (76.1%)
ISS stage	
I	3 (17.6%)
II	4 (23.5%)
III	10 (58.5%)
Extramedullary plasmacytomas	
Yes	4 (23.5%)
Bone disease	
Yes	8 (47.1%)
Leptomeningeal infiltration	
Yes	5 (29.4%)

Laboratory characteristics:	
Anaemia	
Hb<120g/L	17 (100%)
Thrombocytopenia	
<150 x 109/L	16 (94.1%)
Neutropenia	
<1x 109/L	3 (17.6%)
Heavy chain	
None	10 (58.8%)
IgG	5 (29.4%)
IgA	2 (11.8%)
Light chain	
Kappa	9 (52.9%)
Lambda	8 (47.1%)
CD56 expression on PCs	
Yes	14 (82.4%)
Karyotype	
Normal	11 (64.7%)
t(11;14)	1 (5.9%)
Polyploidy	3 (11.8%)
None documented	3 (17.6%)
FISH	
Normal	8 (47.1%)
CCDN1	3 (17.6%)
IGH	2 (11.8%)
RB	2 (11.8%)
None documented	2 (10%)
Renal insufficiency	
Yes	12 (70.6%)
Hypercalcemia	
Yes	8 (47.1%)
Elevated LDH	
Yes	10 (58.8%)
Elevated B2M	
Yes	15 (88.2%)
Hyponatremia	
Yes	9 (52.9%)
Hypocalcitoemia	
Yes	5 (29.4%)

Five (29.4%) patients presented with leptomeningeal infiltration; in three of them it was diagnosed at the time of the diagnosis of PCL. All the patients had neurological features. Thirteen (76.4%) patients were able to start a curative treatment: VD in 7 (53.8%) patients, VTD in 2 (15.4%), VAD in 1 (7.7%), D-PACE in 1, MTX-ARAC in 1 patient and RD in the remaining one. Three patients received intrathecal treatment. The intention-to-treat response was: 2 (15.4%) CR, 2 PR, 7 (53.9%) refractory disease/progression and 2 non-evaluable. Only 2 (15.4%) patients achieved enough response (2 CR) to undergo an autologous stem cell transplant (ACST) and only 1 to undergo an allogenic-SCT. With a median follow up of 4 months for all the patients inclosed, median of PFS was 3 (CI 95% 0.47-4.76) months and median of OS was 4 (IC 95% 0.47-7.53) months.

Summary/Conclusions: Prospective multicenter studies are required to provide a better understanding of the pathogenesis of PCL.Staging procedures should include lumbar puncture or magnetic resonance at diagnosis when extramedullary involvement is suspected. Intrathecal prophylaxis with cytarabin, metotrexate and dexamethasone is not today a standard of care for patients with PCL.

PB2009

MANAGEMENT AND OUTCOMES OF PATIENTS WITH MULTIPLE MYELOMA IN REAL-WORLD SETTINGS IN BULGARIA, CROATIA AND SLOVAKIA

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Background: The multiple myeloma (MM) treatment (Tx) landscape is rapidly evolving, with varying Tx practice patterns and access schemes across countries. However real-world (RW) data describing patient (pt) management, MM Tx use and outcomes in some Eastern European Countries are limited.

Aims: To understand the characteristics, management, Tx patterns and outcomes of pts with symptomatic MM in a RW setting in Bulgaria (BG), Croatia (HR) and Slovakia (SK).

Methods: Data were collected within a cross-sectional (X) and retrospective (R) phase of a chart review in 6 countries between June/15 and June/16 by (onco-)hematologists who managed at least 15 pts with MM per month (mo) and were responsible for initiating MM Tx. Data from 3 countries with limited access to MM Tx are shown. In the X-phase, data included characteristics and current Tx by line of therapy for all pts with MM seen during a 3-week observation period, regardless of pts' Tx status and strategy. In the R-phase, data included pt and disease characteristics at diagnosis, Tx response, comorbidities and clinical outcomes by Tx line. Pts were selected in reverse chronological order and those who had completed specific lines of active Tx within the past 3 mo were included as follows: 3 pts in first line (1L), 4 pts in second-line (2L) and 7 pts in third or higher lines. Analyses were descriptive.

Results: In the X-phase, 7 physicians from BG, 6 from HR and 5 from SK included 84, 89 and 56 pts respectively. In BG, HR and SK respectively, 45%, 51% and 52% of pts were <65 years; 41%, 35% and 38% were 65–75 years. Only 4 pts (from BG) were enrolled in clinical trials. Median time since diagnosis was 24, 31 and 54 mo in BG, HR and SK respectively. In BG, fewer pts received stem cell transplantation (SCT) than in HR and SK (8% *vs* 24% and 36%). The proportion of pts that had received SCT at any point increased from 1L to 2L (3% to 19%, 7% to 35% and 9% to 54% in BG, HR and SK respectively). 82% of pts in BG, and 70% both in HR and SK were currently receiving Tx (Table 1), while 17%, 30% and 25% of pts respectively, were treated previously. Only 4 pts (1 in BG and 3 in SK) had never been treated. In the R-phase, 6 physicians from BG, and 5 from each of HR and SK included 43, 39 and 44 pts respectively. In BG, HR and SK respectively, 44%, 41% and 41% of pts were <65 years; 49%, 36% and 39% were 65–75 years. Depth of response, as assessed by physicians, decreased in BG with each additional line of Tx, but remained stable or increased in HR and SK: 43%, 55% and 50% of pts achieved at least a very good partial response (≥VGPR) in 1L, while 13%, 54% and 69% of pts achieved ≥VGPR in 2L. The most common (≥20%) adverse events (AEs) and comorbidities in 1L were anemia (23% in HR, 43% in SK) and neutropenia (43%) and thrombocytopenia in SK (21%). Mostly, these AEs did not impact on Tx.

Summary/Conclusions: These findings suggest a high unmet need for access to more effective and innovative Tx options with manageable safety profiles in these countries. In particular, in BG where bortezomib- and chemotherapy-based regimens are the only treatments used, pts might be re-treated with the same agents, which may explain why most do not achieve ≥VGPR from 2L. In HR and SK, sustained or increased rates of ≥VGPR in 2L may be due to the use of newer or different agents from those used in 1L and to the fact that most pts had previously received a SCT. These RW data provide useful input for economic evaluations of new MM agents to include in earlier Tx lines in these countries.

Table 1.

Multiple Myeloma Treatments in 1L to 3L*	Bulgaria	Croatia	Slovakia
1L MM therapies, % (n)	**(39)**	**(15)**	**(11)**
- Bortezomib-based regimen	80 (31)	20 (3)	64 (7)
- Chemotherapy-based regimen	20 (8)	47 (7)	27 (3)
- Thalidomide-based regimen**	-	27 (4)	9 (1)
- Other	-	7 (1)	-
2L MM therapies, % (n)	**(21)**	**(26)**	**(13)**
- Bortezomib-based regimen	43 (9)	65 (17)	31 (4)
- Chemotherapy-based regimen	29 (6)	15 (4)	8 (1)
- Thalidomide-based regimen	19 (4)	4 (1)	-
- Lenalidomide-based regimen	-	4 (1)	54 (7)
- Bortezomib/lenalidomide-based regimen	10 (2)	-	-
- Other	-	12 (3)	8 (1)
3L MM therapies, % (n)	**(6)**	**(14)**	**(9)**
- Bortezomib-based regimen	-	21 (3)	22 (2)
- Chemotherapy-based regimen	83 (5)	14 (2)	22 (2)
- Thalidomide-based regimen	17 (1)	7 (1)	-
- Lenalidomide-based regimen	-	57 (8)	56 (5)

* owing to rounding, percentages may not total 100%

**bortezomib-thalidomide-dexamethasone

PB2010

SINGLE SHOT MEDIUM DOSE MELPHALAN IN RELAPSED MM PATIENTS: A RETROSPECTIVE, SINGLE CENTER EXPERIENCE

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Background: Multiple myeloma (MM) patients refractory to proteasome inhibitors, IMIDs or both, have an extremely poor prognosis. Moreover, they frequently fail to respond to further therapies, and represent a major challenge in everyday clinical practice.
Aims: With this in mind, we treated 12 patient with relapsed MM with a single shot of medium dose melphalan (60 mg/m2) between October 2010 and January 2016.
Methods: The median age was 72 years (range, 62 – 79) and the median time from initial diagnosis until melphalan treatment was 51 months (range, 24 – 144). Patients were heavily pretreated with a median number of 3 prior lines of therapy. All patients were refractory to the previous therapeutic regimens and had failed to respond or were refractory to regimens containing bortezomib. Seven patients (84%) had previously received at least one IMiD, 8 (67%) autologous stem cell transplantation (ASCT) and 1 allogeneic stem cell transplantation. The patients included in the series were not eligible for any clinical trial available at the Institution. All patients gave informed consent.
Results: All patients had cytopenia (anemia, neutropenia and thrombocytopenia). We observed 3 cases of gastrointestinal toxicity (1 bleeding, 1 subocclusion, 1 mucositis grade IV sec. WHO), 3 cases of clinically documented infection (1 Escherichia coli bacteremia, 1 fever of unknown origin, 1 erysipela) and 2 deep vein thrombosis. Response was assessed between six and eight weeks after melphalan therapy. Overall, 10 out of 12 patients had a response (1 complete response, 3 very good partial response, 2 partial response and 4 stable disease); only 2 had progressive disease. Median overall survival was 11 months (range, 2 -37). 10 of 12 patients relapsed after a median time of 5 months (range: 2-12). Concerning two patients not relapsed, 1 patient died in partial response 8 months after therapy of other causes; 1 patient is still alive, in complete remission 18 months after melphalan. He underwent ASCT and maintenance with lenalidomide.
Summary/Conclusions: Many patients refractory to proteasome inhibitors and IMiDs are probably still sensitive to alkylating agents and could be rescued with medium dose melphalan. We suggest therefore melphalan as a "bridge" strategy for further therapy, particularly in patients needing immediate disease control. Even in this era in which several novel drugs became available, single shot medium dose melphalan could be an affordable and safe therapy, able to control aggressive relapse, and to reduce disease burden prior to targeted therapy.

PB2011

LENALIDOMIDE AT THE DOSE OF TWENTY-FIVE MG EVERY OTHER DAY IN PATIENTS AFFECTED BY MULTIPLE MYELOMA AND RENAL FAILURE: A REAL-LIFE EXPERIENCE

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Background: Lenalidomide, available as oral compound, is an IMiD with both antiproliferative and immunomodulatory activity which is largely used in the management of newly diagnosed, relapsed or refractory MM and as maintenance therapy after autologous stem-cell transplantation. Due to its renal route of excretion, it is mandatory to adjust lenalidomide dose in patients with RI, guided by Creatinine Clearance (ClCr), in order to impede a systemic prolonged exposure that could boost myelosuppression. With normal renal function, lenalidomide reaches its maximal plasma concentration after a median time of 0.6-1.5 h, and it is cleared by glomerular filtration and active tubular secretion in 3 to 4 hours. Serum half-life increases up to 9 hours if moderate/severe renal impairment is present (creatinine clearance <50 or <30 mL/min, respectively). In the latter cases a reduction of the daily dose is recommended. Dose adjustment based on RI severity decreases the daily amount of lenalidomide from 15 up to 5 mg (in patients undergoing dialysis); other studies include a schedule with 10 or 15 mg every other days. However, there is no theoretical assumption against the possibility that protracting the time of full standard doses can be equally effective and tolerated by patients requiring reduced doses.
Aims: In this report, we describe our retrospective experience on the administration of lenalidomide 25 mg every other day for patients with MM and RI.
Methods: From March 2014 to February 2016, 19 consecutive patients, 11 female and 8 male, with a median age of 63.3 years (range: 49-81) affected by advanced, resistant and progressive MM (median number of previous treatment lines: 3, range : 1-5, all including bortezomib) with concomitant renal failure not in dyalitic support (median calculated ClCr 36.4 ml/min, range : 18-66) were treated, after informed consent, with monthly 21-day courses of 25 mg lenalidomide every other day and dexamethasone (20-40 mg on days 1-8-15-22, every 28 days).
Results: Disappearance of urinary light chain and reduction of serum creatinine (complete response) were detected in 7 patients (36.8%); 3 patients (15.7%) had a very good partial response, 3 (15,7%) had a partial response, 4 of them (21.0%) were in stable disease, whereas 2 patients (10.5%) had signs of progressive disease. Overall response ratio was 68.2%. More than half of the patients (11/19, 57.8%) had a renal response (median calculated ClCr 51.5ml/min, range 20-148). Median progression free survival was 8 months (range 3-18 months). No patient experienced grade 4 myelotoxicity; four patients required red cell transfusions for grade 3 anemia. No SAE occurred during treatment.
Summary/Conclusions: Dose adjustment RI-related of Lenalidomide is recommended in most guidelines, but there is not a leading scheme with a proven effectiveness more than others.These preliminary observations point to a significant therapeutic effect of lenalidomide, at the dose of 25 mg every other day for 21 days, in more than half of a small population of patients with advanced MM and renal impairment, with not negligible logistic and economic advantages. However, these results should be validated by controlled studies involving larger number of patients.

PB2012

A FEASIBILITY-STUDY ON IMPLEMENTATION OF THE INTERNATIONAL MYELOMA WORKING GROUP RECCOMENDATIONS FOR MULTIPLE MYELOMA PATIENTS IN ROUTINE CLINICAL PRACTICE: A PERIPHERAL CENTER EXPERIENCE

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Background: Renal impairment (RI), defined as serum creatinine above upper normal limit or >2 mg/dl or a estimated glomerular filtration rate (eGFR) <60 ml/min/1,73m2, is one of the most common complications of MM, and it is associated with an increased risk of early death. The incidence of RI at MM diagnosis ranges from 20% to 50%, while its comparison occurred in 60% MM patients (pts). In this scenario tempestive diagnosis of RI in MM pts and exclusion of possible alternative causes of RI (like amiloydosis, diabetes or MIDD) are essential.
Aims: We applied a diagnostic algorithm obtained from the International Myeloma Working Group Reccomendations in pts admitted to our department for RI (with known and unknown MM, or suspected cast nephropathy, CN), in order to investigate if this diagnostic workflow could positively impact on MM pt management.
Methods: We enrolled adult pts, known or unknown MM, admitted to our hospital for RI or suspected CN, with or withouth monoclonal component. Primarly, we performed complete blood analysis, with eGFR (CKD-EPI and MDRD methods), serum and urine electrolites, bicarbonatemia, serum and urine immunofixation, fraction 3 and 4 of complement, crioglobulinemia, HbA1c, arterial gas analysis, evaluation of urine rate every 6 hours, daily urine collection, urine sediment. We also collected anamnesis on eventual nephrotoxic concomitant therapies like ASA, FANS, clinical parameters and objectives signs of RI (edema, symptomatic disionia). On the second day of hospitalization we requested protein electrophoresis on serum and urine, chest X-ray, ultrasonography of abdomen, ecocardiography and electrocardiography. On the day three we evaluated results of previous exams and we decided, if necessary, eventual biopsieson (bone marrow in suspected unknown MM pts, renal in suspected CN pts, umbilical fat for amyloidosis). All analyses were daily and collegialy discussed between Internists and Nephrologists.

Results: From March to December 2016 we admitted 57 pts with RI and monoclonal component (29 F, 28 M, 41-83 yrs range), 20 are known MM pts and 37 de novo pts. We diagnosed 11 de novo MM, 13 knowed MM with a de novo RI, 12 diabetes related RI, 3 amyloidosis, 16 other causes.
Summary/Conclusions: The implementation of the International Myeloma Working Group Reccomendations in a routine clinical practice confirmed its feasibility and utility in the optimal workout of MM pts. We obtained diagnosis of RI within 4 days, both in known and in de novo MM pts, with a positive impact on reduced hospitalization, uncessary dyalisis and steroids overtreatment.

PB2013

NOCARDIOSIS PROVOKED BY NOVEL AGENTS AT RELAPSED MULTIPLE MYELOMA:CASE SERİES

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Background: The proteosome inhibitors and immunmodulatory drugs which are used in MM treatment enhance the risk of infection by several mechanisms.Nocardial infections are rare in Turkey.
Aims: Here,we present three relapsed myeloma cases which developed nocardia pneumonia.
Methods: Case-1 :66 year old man, who has a history of autologous SCT 4 years ago and lenalidomide usage because of IgG kappa type myeloma, has been prescribed bortezomibe for the relapse of the disease.He was immunocompromised not only because of the myeloma, and also because of the diabetes and renal failure without dialysis. He was admitted to the hospital because of the productive cough.His lymphocyte count was 1290/mm3 and flow-cytometric analysis showed CD5:%68 and CD20:%2. Thorax CT showed 39x39x45 mm mass like lesion.Broncoscopic lavage examination showed branched bacillus via modified acid-fast and Gram stain.This typical morphological appearence was defined as Nocardia spp. Imipenem/cilastatin treatment started and control CT was performed after ten days and it showed regression of the infiltration.He was discharged with oral TMP/SMX antibiotherapy. Case-2:71 year old woman,who has a history of two auotologous SCT 12 and 5 years ago because of IgG kappa type myeloma;admitted to the hospital with productive cough during pomalidomide treatment.Her lymphocyte count was 2300/mm3 and flow-cytometric analysis showed CD5:%88 and CD 20:%1.HRCT showed a 7x6x6 cm sized mass like lesion with a cavity.Branched Gram positive bacillus (Nocardia sp.) was detected from broncoscopic specimen analysis,so imipenem-cilastatin therapy has been started.She responded well to therapy and was discharged with TMP/SMX antibiotherapy. Case-3:72 year old man,who has a diagnosis of IgG kappa type myeloma and a history of autologous SCT 4 years ago following bortezomibe treatment,relapsed 5 months ago. He has been admitted to the hospital with nonproductive cough complaint under the treatment of lenalidomide and dexamethasone.His lymphocyte count was 520/mm3.Flow-cytometric analysis couldn't be performed.Thorax CT showed 4 cm sized cavity and sputum microscopy showed acid rezistant branched bacillus thought to be consistent with nocardiosis.The imipenem/cilastatin and TMP/SMX treatment have begun and 12 days later, a control CT was performed and showed regression.He was discharged with oral TMP/SMX antibiotherapy.
Results: See Table 1 and Figure 1.

Table 1.

Genereal features of the cases	Case -1	Case-2	Case-3
Age	66	71	72
Gender	Male	Female	Male
Lymphocyte Count(/mm³)	1290	2300	520
Myeloma Type	IgG kappa	IgG kappa	IgG kappa
Previous Treatment	Autologous SCT 4 years ago Lenalidomide-Dexamethasone	Autologous SCT 5 and 12 years ago Bortezomibe-Thalidomide-Dexamethasone	Autologous SCT 4 years ago Bortezomibe-Thalidomide-Dexamethasone
Recent Treatment Before Nocardiosis	Bortezomibe	Pomalidomide	Bortezomibe-Thalidomide-Dexamethasone

Case-1: A mass like infiltration at right lung superior lobe posterior segment suggesting Nocadia

Figure 1.

Summary/Conclusions: The proteosome inhibitors and immunmodulatory drugs which are used for the treatment of MM;make T cell disfunction and considering B cell disfunction is also present because of the nature of the disease;this situation tends to provoke rare opportunistic infections such as nocardiosis.Thus,in these patients;it is significant to follow the lymphocyte count closely and to keep in mind that kind of rare microorganisms.

PB2014

LENALIDOMIDE IN PATIENTS WITH DIALYSIS-DEPENDENT END STAGE RENAL FAILURE (ESRF) AND MULTIPLE MYELOMA

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Background: Lenalidomide is an oral immunomodulatory medication with clinical efficacy in relapsed/refractory and treatment naïve multiple myeloma (MM), 5q- myelodysplasia and lymphoma. Lenalidomide is eliminated predominantly unchanged by urinary excretion. Renal impairment is common in MM (15-40%) and approximately 10% of MM requires dialysis. However, there is a paucity of clinical safety data of Lenalidomide in ESRF. There is evidence that Lenalidomide can be safely used in patients with moderate and severe renal dysfunction with dose adjustment. However, published data in hemodialysis-dependent patients is limited to a handful of patients across small retrospective analyses and case reports. Patients with ESRF have generally been excluded from clinical trials investigating Lenalidomide. Phase III trials in the relapsed setting (MM-009, MM-010) excluded patients with a serum creatinine >221µmol/L. The FIRST trial (MM-020), investigating upfront use, excluded patients dependent on dialysis. There is no accepted clinical standard on the most appropriate dosing of Lenalidomide in dialysis. The manufacturer has provided guidelines, being 5mg daily, day 1-21, every 28 days (equivalent to 105mg per cycle). There is alternate well-cited pharmacological data that the more appropriate starting dose is likely 15mg, three times per week, given post-dialysis (equivalent to 135mg per cycle). **Aims:** To provide real-world evidence of an institutional experience of the use of Lenalidomide in dialysis-dependent MM.
Methods: We performed a retrospective audit of our in-centre experience with treating dialysis-dependent MM with Lenalidomide and included patients who completed at least one cycle of therapy. Patients were assessed for haematological toxicity, significant infective complications, thrombosis, disease response and progression-free survival. Best response was stratified by IMWG criteria. Patients' baseline characteristics, prior therapies, cytogenetics and FISH data were collected.
Results: We identified 5 patients treated between 2010 and 2017, aged between 54 to 73 years old. All patients had relapsed/refractory MM and dialysis dependent ESRF. The median number of prior therapies was two. One patient had t(11,14) on FISH and died from progressive disease. Dose schedules are shown in the Table 1. Almost all patients experienced grade III-IV haematological toxicity and 60% had grade III-IV infection. There was a positive correlation between dose and toxicity, and furthermore there appeared to be an inverse relationship between age and tolerated dose. Haematological toxicities and infection were ameliorated by dose adjustment in most instances. There was no drug related mortality, however one patient died of progressive disease. Four of the five patients were prescribed aspirin thromboprophylaxis, with no proven thrombotic complications seen. Where possible to assess, the ORR was 75% (3/4), with 2 patients achieving a very good partial response (VGPR), 1 partial response and 1 progressive disease. The lowest starting dose in this cohort was 10mg twice/week and the maximum dose was 25 mg three times/week.

Table 1.

	Age	Dose Schedule	Cumulative Dose/cycle	Number of Cycles	Grade III/IV Neutropenia	Grade III/IV Anaemia	Grade III/IV Thrombocytopenia	Clinically Significant Infection	PFS (months)	Response
Patient 1	63	15mg three times weekly	135mg	12	Yes	No	No	No	9	VGPR
Patient 2	54	i) 25mg three times weekly ii) 25mg twice weekly iii) 25mg three times weekly	225mg 150mg 225mg	26 36 8	No No Yes	No No No	No No Yes	Yes No Yes	85	VGPR
Patient 3	67	i) 25mg twice weekly ii) 10 mg twice weekly	150mg 60mg	13 21	Yes No	Yes No	Yes No	Yes Yes	48	Partial Response
Patient 4	73	10mg twice weekly	60mg	2	Yes	No	No	Yes	2	Disease Progression
Patient 5	46	5mg daily	105mg	1	§	§	§	No	¶	¶

§ Disease-related pancytopenia ¶ Too early to assess

Summary/Conclusions: Our experience builds on the emerging evidence that reduced dose of Lenalidomide can be safely prescribed for dialysis-dependent MM, with clinical efficacy. In our cohort most patients took Lenalidomide on days of dialysis only. There was significant variation of dose-related tolerability between patients. However, toxicity was manageable with diligent monitoring and dose adjustment.

PB2015

STUDY USE OF 18-F FDG PET / CT SCANNING INTO THE FIRST FOLLOW UP OF PATIENTS WITH MULTIPLE MYELOMA AND ASSOCIATION WITH BIOCHEMICAL RESPONSE

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Background: Positron computed tomography (PET / CT) with ^{18}F fluorodeoxyglucose-labeled glucose (FDG) is a reliable technique with high sensitivity and specificity for assessing skeletal involvement and recent studies propose it as a method for predicting treatment response in multiple myeloma. Conventionally, the response is measurable by the monoclonal component in both serum and urine and Minimal residual disease (MRD) by flow cytometry has been established as a mandatory tool. The studies are aimed at combining the measurement of paraprotein with imaging tests that help to promptly define response or failure to the treatment.
Aims: The primary endpoint was the correlation of the biochemical response with the FDG PET/CT in a second evaluation after first line treatment. The secondary endpoint was the correlation between MRD and with second FDF PET/CT.
Methods: We included in this retrospective and observational study at Universitary Hospital of Vall d' Hebron, all patients with newly MM and PET/CT before to start a first line treatment and a second PET/CT when completing treatment. PET/CT were analyzed by the department of Nuclear Medicine with experience to grade the lesions in MM, were evaluated and categorized into positive or negative according to the criteria proposed by Zamagni, *et al.* The biochemical response was defined according to the standard IMWG response criteria.
Results: Eighteen patients (8 males and 10 females) with untreated MM entered, seven patients were classified with ISS III, fifteen had a good performance status, none presented renal lesion, only 16% had hypercalcemia and 66% showed immunoparesis. Ten patients were IgG isotype, six were classified as light chains myeloma and two patients were oligosecretors. Seventeen patients had bone marrow infiltration with a median of 42% plasmatic cells. Two patients had a extramedullary plasmocytoma and nine had an anormal ratio of light chains. Seventeen patients were treated with bortezomib-based regimens, (median 5.5 cycles) included VTD, MPV, VLD and VD. After treatment fourteen patients achieved completed response, two partial response and two had progressive disease. PET/CT was positive in all patients pretreatment, 15 focal lesions, 2 diffuse bone marrow involvement plus focal lesions and 1 involvement of bone marrow alone. Twelve patients had more than 3 focal lesions and two had extramedullary disease. At the end of first line treatment, PET/CT was negative in eight patients (44%) and fourteen had complete biochemical response (78%). 62% of the patients with negative PET/CT showed negative flow minimal residual disease (MRD) and biochemical complete response . Two patients had PET/CT with progression disease and corresponded to a biochemical progression.
Summary/Conclusions: The correlation between PET/CT and biochemical response obtained after treatment was positive in patients with complete response. We found discordant data in two patients with oligosecretory myeloma. No correlation was shown between PET/CT and flow MRD.
Are necessary more long term studies that include greater number of patients to confirm that the PET/CT negative is an image technique that could be a tool to follow up patients after the first line treatment added to the evaluation of the biochemical response.

PB2016

MULTIPLE MYELOMA IN BORNEO SARAWAK: A DEVELOPING WORLD'S EXPERIENCE

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Background: Sarawak, is the largest state of Malaysia situated on the island of Borneo. Sarawak General Hospital is the tertiary referral center of Sarawak (serving a population about 1 million people). It is 980 km away from its main hematology/transplant referral center in Kuala Lumpur, Malaysia, which is accessible only by airplane. Hence, treatment of patients with multiple myeloma in this part of the state is a big challenge due to its geographical constraint.
Aims: To identify demographics and clinical characteristics of patients with multiple myeloma; To establish treatment and outcome of patients with multiple myeloma.
Methods: This is a retrospective study examining basic characteristics and clinical outcomes of patients diagnosed with multiple myeloma between 2010 and 2016 in Sarawak General Hospital. Patients' case notes were traced and the relevant information was entered into a pre-designed data collection form. Data was analysed and interpreted via IBM SPSS Statistics version 24.0.
Results: There were a total of 63 patients with the male to female ratio of 3:2. The median age for patient was 61 years old (range 31 to 86 years old). Majority of them are local natives of Iban or Bidayuh descendants (n=32, 50.8%) followed by Chinese (n=20, 31.7%) and Malays (n=11, 17.5%). Most common type of multiple myeloma is of IgG variant (n=27, 42.9%). The most common myeloma related organ or tissue impairment (ROTI) are anaemia (n=54, 85.7%) followed by bone lesion (n=48, 77.8%), renal impairment (n=27, 42.9%) and hypercalcaemia (n= 18, 28.6%). More than half presented late with Durie Salmon stage III disease (n=34, 54%). Majority of patients were treated with dexamethasone/thalidomide (n=25, 39.7%). Sixteen patients (25%) received bortezomib based treatment. Three patients (n=3, 4.8%) undergone bone marrow transplant. Thirty five patients died (n=35, 55.6%). Median survival time was 21 months (95% CI: 16,26). One year, two years and five years survival rate was 67.4%, 43.6%, 31.6%. Patients who were 60 years old and above have lower median overall survival (20 months) compare to patients who were 60 years and below (36 months) even though they are not statistically significant (p=0.565).
Summary/Conclusions: Baseline characteristics of patients with multiple myeloma in Borneo Sarawak are similar to the rest of Asia. However, our median overall survival was comparatively lower to our counterparts. Limitation wise, due to logistic and economic reasons, we do not have good access to cytogenetic and genetic profiling that enables us to prognosticate patients accordingly.

PB2017

A RETROSPECTIVE AND PROSPECTIVE AUDIT OF RADIOLOGICAL INVESTIGATIONS FOR SUSPECTED CASES OF PLASMA CELL DYSCRASIAS/MYELOMA IN THE ALTNAGELVIN AREA HOSPITAL

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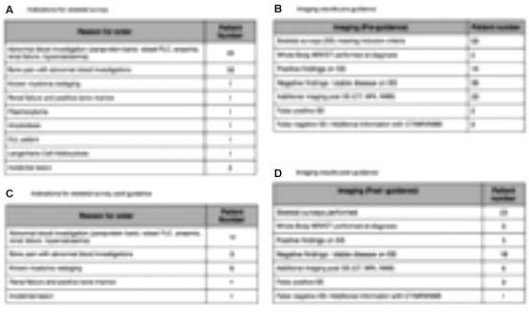
Background: The updated NICE guidelines for diagnosis and management of myeloma (2016) suggests whole-body MRI as first-line imaging for people with suspected myeloma and consideration of MRI/CT/PET in newly diagnosed myeloma to assess for bone disease or EM plasmacytoma.
Aims: Our aims were to ascertian; 1) Our current practice regarding radiological investigation for myeloma (2) Whether additional diagnostic information was gained using CT/MRI imaging (3) Since its release, is the trust compliant with the NICE guidance (4) The estimated cost of meeting the current NICE guidance
Methods: This retrospective and prospective audit included all patients having a skeletal survey performed for suspected multiple myeloma within the Altnagelvin Area Hospital (AAH). Retrospectively from 10/2/15 until 9/2/16 data was collected using the advanced search feature of the Sectra IDS7 PACS system. The 'Reason for examination' for each study was then analysed and those ordered for reasons other than suspected myeloma were excluded. Each case was analysed individually and any follow up MRI/CT/NMB imaging performed in the 6 month period following the skeletal survey were included in the data collection. The same information was gathered prospectively from 10/2/16-30/5/16 following the NICE guidance. 54 skeletal surveys where performed for suspected/restaging myeloma pre guidance.
Results: The indications for requesting imaging is shown in Table 1A. No WB MRI/CT was performed in this period. 26% patients had new lytic lesions on skeletal survey. 23 patients had further imaging in the form of MRI or CT following skeletal surveys. All the positive MRI findings offered additional diagnostic information - including examples of missed multiple spinal deposits. The results of imaging are summarised in Table 1B. The false negative rate for skeletal surveys was 39% and the false positive rate was 22%. Following NICE guidance publication 23 patients had skeletal surveys performed for suspicion of myeloma between 10/2/16 and 30/5/16. The indications are summarised in Table 1C. No WB imaging was performed. 5 patients had positive skeletal surveys. 6 patients had subsequent CT/ MRI imaging. A skeletal survey was reported normal with a subsequent MRI showing multiple spinal deposits. The imaging results are summarised in Table 1D.

Table 1.



Summary/Conclusions: The expected cost of implementing WB imaging for 60 patients per year in the AAH is £18,240. In comparison the cost of performing skeletal surveys would be £4200 per annum. NICE guidance 2016 offers an

economic model for imaging with WB MRI. In addition it reviews evidence which links time to diagnosis to survival and myeloma related complications. The NICE guidance offers clear evidence that WB-MRI should be the investigation modality of choice for suspected myelomatous disease. It offers a diagnostic and cost-effective strategy that will ensure health improvements for myeloma patients. This audit offers further evidence of the diagnostic accuracy of MRI imaging. At present failure to comply with NICE guidance will lead to delayed diagnosis of myeloma in certain patients and potential patient harm. Therefore I offer a business and health improvement case for the Western Trust to instigate WB-MRI imaging for all suspected myelomatous bony disease.

PB2018

TONI DEBRE FANCONI SYNDROME DURING MYELOMA, ABOUT 8 CASES

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Background: The cast nephropathy with cylinders is the most frequent renal complication of the myeloma, which results from a catabolism of the light chains by the tubular cells and can lead a tubular chronic suffering showing itself by a syndrome of acquired Toni-Debré-Fanconi marked by a glycosuria, a phosphaturia, an aminoaciduria, a sometimes severe and sometimes revealing hypokalemia.
Aims: We reporting some observations informed by Multiple Myeloma complicated with a Fanconi syndrome.
Methods: From January 2000 till December 2010: 78 cases of Multiple Myeloma were brought together, whose circumstance of discovery 22 cases with renal failure, it's was a evolutes complications in 12 cases; and in 10 cases it's discovered at diagnosis. The renal achievement is dominated by Tubule disease in 11 cases, Randall syndrome 8 cases, and Nephrotic syndrome in 3 cases. The tubule disease of Fanconi is suspected at only 8 patients: in front of the presence of a renal glycosuria (without associated diabetes) and a frank proteinuria in the majority of the cases, with a hypophosphatemia and a fickle hypokalemia.
Results: The clinico-epidemiological and immuno-biological characters of these 8 patients are the following ones: - The median age is of 64 years (39-76), sex ratio=3. The osseous pains and the muscular cramps dominate the clinical presentation with constant diffuse demineralization in the radiology. - The patients were classified (according to the Salmon-Durie classification): IIIB (3 cases) and IIB (5 cases). ISS 3 in majority of the cases. - The monoclonal immunoglobulin observed: IgG kappa: 4cases, IgA kappa: 2cases, light chain kappa: 2cases. With a Bence Jones proteinuria isotype kappa and a glycosuria in the majority of the cases. - The gravity of the renal faillure, based on the clearance of the creatinine: with an average clearance of 16,19 ml/min (4-37): several in 5cases, terminal in 3cases. - We note more of hypocalcaemia while the hypercalcaemia is noted in a single case, the hypophosphatemia is found in half of the cases. The therapeutics is double: - Symptomatic: alkaline hydration, correction of the metabolic disorders and sometimes the renal extra purge (indicated in 3cases). - Specific: chemotherapies VAD 7cases, a patient died by cardio-vascular complication. Under treatment the recovery of the renal function is obtained in 3 cases, to the rests of the patients persists a stable renal failure.
Summary/Conclusions: The Syndrome of Fanconi is a frequent and often formidable complication during Myeloma, observed to 30-40% of the patients in an autopsique series. It is necessary to think to it in front of any renal achievement in myeloma of kappa light chain with renal glycosuria, a generalized amino-aciduria and a hypophosphatemia resulting respectively from a defect of the transport of the glucose, from amino acids and from phosphates by the renal proximal tubule. To improve the osseous and renal appearances, it is necessary to realize a calcic supplementation, phosphorous and by the vitamin D active, as well as the correction of the acidose and a specific treatment reducing the excretion renal of the light chains.

PB2019

DEPP RESPONCES WITH CARLFIZOMIB-LENALIDOMIDE-DEXAMETHASONE IN RELAPSED/REFRATORY MULTIPLE MYELOMA PATIENTS: A REAL LIFE EXPERIENCE

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Background: Carfilzomib is a new proteasome inibitor with in contrast to the reversible binding of bortezomib, binds irreversibly and selectively to its target: the chymotrypsin-like activity of the 20S proteasome. The phase IB/II PX-171-006 study was the first study in which carfilzomib was combined with lenalidomide and dexamethasone. In the phase I dose-escalation part the maximum planned dose was established as well tolerated and in the phase II part the study focused the efficacy and toxicity in the subgroup treated with maximum planned dose. The ASPIRE trial showed superior response rates and progression free survival for carfilzomib-lenalidomide-dexamethasone compared with lenalidomide-dexamethasone in relapsed/refractory Multiple Myeloma patients.
Aims: The aims is explorer the efficacy and tolerability of carfilzomib-lenalidomide-dexamethasone in relapsed/refractory Multiple Myeloma patients in real life.
Methods: All patients received carlfilzomib 20/27 mg/m2 days 1,2,8,9,15 and 16; lenalidomide 25 mg days 1-21 and daxamethasone 20 mg days 1,2,8,9,15,16, 22 and 23, according to post approval access protocol. After 2, 4, 6 and 8 cycles the responses, disease progression and toxicity were assessed using the International Myeloma Working Group Uniform Response Criteria and WHO score respectively.
Results: From January 2016 to February 2017 in hematology "Cardinale G.Panico Hospital" and "Bari Policlinico", treated 15 relapsed/refractory Multiple Myeloma patients with carfilzomib-lenalidomiode-dexamethasone. Six patients male (40%), 9 female (90%), mean of age 62 years (range 38-79); 10 (66%) and 5 (34%) relapsed/refractory multiple myeloma respectively. Median time from diagnosis to carfilzomib-lenalidomiode-dexamethasone was 46 months (range 12-92); median of prior therapy was 3 (range 1-6); 9 (60%) received autologous transplantation while 1 (6%) allogeneic; 11 (73%) prior therapy with lenalidomide; 15 (100%) prior therapy with borthezomib; 2 (14%) prior therapy with pomalidomide (Table 1). Eleven (73%) patients achieved after 2 cycles a response rate ≥PR, of these 3 VGPR. After 4 cycles, 5 (33%) and 1 (7%) have obtained at least a VGPR and CR respectively (Figure 1). Three patients were not evaluated for treatment discontinuation because of rapid progression disease and died during first cycle with a median of 5 prior lines therapy. Most grade 3-4 adverse events were haematological and well manageable, 10 (80%) trombocitopenia and 5 (35%) neutropenia grado 3-4. Dyspnea, fatigue and pyrexia were higher but were mostly grades 1 and 2. Only 2 patients developed respiratory failure and pneumonia while cardiac failure, ischemic heart disease and hypertension not were detected.

Table.1; Baseline patient characteristics.

MEAN OF AGE, years (range)	62 (38-79)
MULTIPLE MYELOMA, n (%)	
RELAPSED	10 (66)
-	5 (34)
REFRACTORY	
MULTIPLE MYELOMA SUBGROUP, n (%)	
-	
IgG	6 (40)
-	2 (14)
IgA	7 (46)
-	
MICROMOLECULAR	
STAGING, n (%)	
DURIE-SALMON	
-	
I-II	
-	
III	3 (20)
ISS	12 (80)
-	
I-II	
-	7 (47)
III	8 (53)
MEDIAN TIME FROM DIAGNOSIS TO KRD, months (range)	46 (12-92)
MEDIAN OF PRIOR THERAPY, lines (range)	3 (1-6)
PRIOR TRASPLANT, n (%)	
-	
AUTOLOGOUS	9 (60)
-	1 (6)
ALLOGENEIC	
PRIOR THERAPY, n (%)	
-	
LENALIDOMIDE	11 (73)
-	15 (100)
BORTHEZOMIB	2 (14)
-	
POMALIDOMIDE	

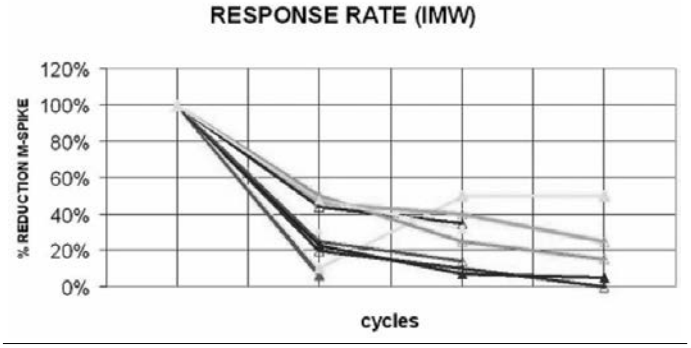

Figure 1.

Summary/Conclusions: Carfilzomib-lenalidomide-dexamethasone is a powerful and efficacy association in relapsed/refractory Multiple Myeloma patients, which allows the achievement of deep responses from the first cycle of therapy. Non haematological adverse events of grade 3 or higher were reported in only 2 patients.

PB2020

CARFILZOMIB-LENALIDOMIDE-DEXAMETHASONE IN THE MANAGEMENT OF RELAPSED AND REFRACTORY MULTIPLE MYELOMA: A REAL-LIFE EXPERIENCE

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Background: Carfilzomib is an epoxyketone proteasome inhibitor of second generation, proved to be effective in relapsed and refractory Multiple Myeloma (rrMM).
Aims: In this retrospective observational trial, it has been evaluated efficacy and tolerance of Carfilzomib, in combination with lenalidomide-dexamethasone (KRD) as salvage regimen in patients with relapsed and refractory MM (rrMM), whose prognosis is particularly severe.
Methods: 21 patients (12 M/9 F, Table 1), with rrMM, median age at diagnosis 62 years (r. 47-75), median age at start of treatment 65 years (r. 53-81) treated with several lines of treatments (median 3, r. 2-10), included 2 patients refractory to Bortezomib, underwent to KRD regimen (ASPIRE trial schedule: Carfilzomib starting dose 20 mg/sqm on days 1,2 of cycle 1, target dose 27 mg/sqm thereafter; Lenalidomide 25 mg on days 1 through 21; Dexamethasone 40 mg on days 1,8,15 and 22, every 28 days) for a median treatment cycles of 2 (r 1-8). ISS was equally distributed, and cytogenetic was evaluable in 8 patients, and in particular one del13q14 1qgain, one del 13q14 and one t(11;14). 86% of patients had previously been treated with schedule containing bortezomib and IMIDs, and 33% had also received radiotherapy. 57% of them had undergone at least to a single auSCT.
Results: Carfilzomib was well tolerated, with grade 2 anemia in 28% of patients, without necessity of blood transfusions; 5% grade 1 and 9.5% grade 3 neutropenia (no ospedalization was required, no septic shocks were observed); 33% grade 2, 19% grade 3 and 5% grade 4 thrombocytopenia, without hemorrhagic events and necessity of transufsions. Concerning severe extrahematologic toxicity, it was observed grade 1 pneumonia in 47% of patients, treated by common antibiotic drugs; grade 2 Hypertension in 24% of patients; grade 3 arrhythmias in 5% of patients; grade 2 dyspnea in 5% of patients; grade 1 fatigue in 9.5% of patients. According to IMWG criteria, after a median follow-up of 3 months (r.1-13), ORR was 66,7% (14/21 : 8 VGPR, 6 PR) with 3 progressive diseases and 2 patients in stable disease, which can be considered as an impressive result in this subset of rrMM patients. In particular, for 1 patient, KRD was, after having achieved at least a PR, a bridge to second auSCT. Median time to response was 2 months (r.1-4), median OS from diagnosis was 47 months (9-170 range), median OS from start of Carfilzomib was 3 months (range 1-13).

Table 1.

Baseline characteristics of patients	
Age	
Median (range) - yr	median male: 66 years/female: 55,5
> 65 yr - no. (%)	
Male:sex - no. (%)/female	14 M (73,7%)/ 4F (27,3%)
White race - no (%)	
ECOG - no./total no. (%)	22 pz
0-1	5 pz (22,8%) ECOG 0/ 13 pz (59%) ECOG 1
2	4 pz (18,2%) ECOG 2
ISS disease stage at study entry - no. (%)	22 pz
I	8 (36,3%)
II	11 (50%)
III	3 (13,6%)
Type of measureable disease - no. (%)	
IgG K/L	8 (36,4%)/ 6 (27,3%)
IgA	3 (13,6%)
Other	
Detected in urine only	3 (13,6%)
Detected in serum free light-chains only	
Not evaluated	2 (9,1%)
Creatinine clearance - ml/min	
Mean - ml/min	
Distribution - no. of patients (%)	22 pz
30 to <50 ml/min	5 (23%)
≥50 ml/min	17(77%)
Unknown or other value	
Serum β2-microglobulin - no. Of patients (%)	
< 2.5 mg/liter	19 (83,4%)
≥ 2.5 mg/liter	3 (13,6%)
Median time since initial diagnosis of multiple myeloma (range) - months	46 months (13-214)
Cytogenetic features - no. of patients (%)	22 pz
Standard risk	19 (85,3%)
High risk	3 (13,6%) del 13
No. Of prior therapies - no. Of patients (%)	
Median no. (range)	2.5 (1-5)
Distribution - no. of patients (%)	
1	1 (4,5%)
2	4 (18,2%)
3	16 (72,8%)
4	0
5	1(4,5%)
Prior stem-cell transplantation - no. of patients (%)	
Prior first stem-cell transplantation	11 pz first stem-cell transplantation
Prior first AND second SCT	6 pz second stem cell transplantation

Summary/Conclusions: KRD has shown significant efficacy in a particularly severe setting of patients, relapsed and refractory to all available therapeutic resources, and, in particular cases, it could be considered as a bridge to a second autologous or allogenic SCT.

PB2021

IMWG ´14 DIAGNOSTIC CRITERIA TO INITIATE TREATMENT IN NEW DIAGNOSED MULTIPLE MYELOMA: REAL-WORLD STADISTICS

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Background: Diagnostic criteria for Symptomatic Multiple Myeloma (MM) Published in 2003 by the International Myeloma Working Group(IMWG'03) established for the presence of a bone Marrow infiltration by plasma cells (BMPC) in any percentage And / or the presence of a monoclonal component of any amount Along with the presence of signs or symptoms of organ damage (CRAB) attributable to the proliferation of plasma cells. These criteria have not changed in the last decade until the Recent revision of diagnostic criteria and treatment that IMWG Published by the end of 2014, which proposes an initial Pathologic condition (>10% BMPC or demonstration of a Plasmacytoma) as a preliminary condition before starting treatment. Due to "CRAB redefined" and / or the presence of markers of Rapid progression to "classical-symptomatic" MM criteria.
Aims: There are few information about real-life statistics in NDMM according to new criteria to initiate treatment.
This 2year analysis shows a percentage of patients (22%) who have initiated new treatments superior to those described in the literature
Methods: We have performed a retrospective analysis with all new MM cases diagnosed from Dec-2014 (after new criteria were published) to Feb-2017 (28 months). 55 patients were diagnosed of MM. 26 were male and 29 female. The median age at diagnosis was 74 years (52-87), 11 were under 65 (U65) and 44 were over 65 (O65).
Results: 3 were diagnosed after biopsy of plasmacytomas. None of them have Bone Marrow (BM) infiltration but with criteria of MM after PET-CT multitopic involvement. 7 of these NDMM were smoldering MM (sMM). All of then completed initial staging with more sensitive imaging tests than conventional radiology (MRI and / or PET-CT) 2 of these sMM were under 65 years old and were included in a clinical trial. The other 5 were older than 65 and after a median of 16 months of follow-up did not meet criteria in initiate treatment. Of the 41 patients who started treatment, 10 of them were new criteria, the rest met criteria for classic organic disease (CRAB) Figure 1. 6 patients were diagnosed after performance of PET-CT (3 of them after plasmacytoma biopsy; initial diagnosis: solitary plasmacytoma), 1 after PET-CT negative but MRI positive, 2 with FLC ratio criterium and the last one with BM Plasmatic Cell (BMPC) >60%, MRI image and FLC criteria. Although these data are quite different from those reported previously, accurate diagnosis in initial stages may increment the proportion of real-active MM. We don´t observe increments in incidence rate in these period *vs* pre´2014 (reported to 22nd EHA abstract). We observe that the early mortality is decreasing in the last 5 years (from 2013). The effect of early diagnostic may contirbute to get these improvement of survival.

	1	2	3	4	5	6	7	8	9	10
CRAB	-	-	-	-	-	-	-	-	-	-
FLC	-	-	-	-	-	-	+	+	+	-
BMPC	-	-	-	-	-	-	-	-	+	-
PET-CT/MRI	+	+	+	+	+	+	-	-	+	+

Characteristics of the 10 patients who got treatment due to new criteria

Figure 1.

Summary/Conclusions: One of the hypotheses for introducing new criteria for initiating treatment was that the initiation of adequate and early treatment may improve the prognosis of patients with symptomatic NDMM. In an aging population such as the one we present, we believe that these new criteria to initiate treatment can improve the medium- and long-term prognosis of this group of people with few chance to start intensive or a lot of lines of treatment because of increasingly comorbidities by age. Further follow-up and evaluation of survival comparing the "classical" group *vs* new-criteria group are guaranteed to assess if these early treatment will improve survival.

PB2022

POMALIDOMIDE PLUS LOW-DOSE DEXAMETHASONE: A CHANCE FOR RELAPSED AND/OR REFRACTORY MULTIPLE MYELOMA. A REVIEW OF A CASE SERIES DIAGNOSED IN A SINGLE CENTER

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Background: The treatment of patients with multiple myeloma (MM) has dramatically changed over the past decade due in part to the development of new agents and myeloma-specific targets. Nowadays, new effective treatments exist for patients with RRMM not responding to bortezomib and lenalidomide. Pomalidomide alone has shown limited efficacy in patients with RRMM, but synergistic effects have been noted when combined with dexamethasone.
Aims: To show our experience with the use of 28-day cycles of pomalidomide (4 mg/day on days 1–21, orally) plus low-dose dexamethasone (40 mg/day weekly, orally) (Pom/dex) in RRMM.
Methods: This is a retrospective study performed between May 2014 and January 2017 in the Hospital of Guadalajara (Spain). Eight patients (3M, 5F), with a median age of 67 years (range, 40-81), diagnosed with MM were included. Four were classified as high-risk myeloma (Patients 1-4). Patient 1 (P1) had plasma cell leukemia and received Pom/dex plus bortezomib; Patient 2 (P2) presented complex karyotype and received Pom/dex after three previous regimens and an autologous transplantation; Patient 3 and Patient 4 (P3 and P4) had extramedullary plasmacytoma and received Pom/dex±local radiotherapy. The eight patients of this study had failed to bortezomib and lenalidomide-based therapy, and received Pom/dex until disease progression or unacceptable toxicity. Pom/dex was associated with ciclophosphamide in two patients, and with bortezomib in another two patients. The primary endpoint was progression-free survival (PFS).
Results: The median number of prior regimens was 2 (range, 1-4) and five of eight patients (62.5%) had previously received autologous transplantation. Median time from diagnosis to Pom/dex was 51.5 months (range, 28-155). Patients received a median of 6 cycles of Pom/dex (range, 2-16). In the whole series, the median follow-up was 60.5 months (IQR: 56.0-80.25), and median PFS was 11 months; 75% of patients had not progressed after 5 months, and 50% of patients after 11 months. The overall response rate was 87.5% (only one patient discontinued therapy for non-response). In standard-risk MM patients, median follow-up was 61 months (IQR: 46.25-140.25), and median PFS was 13 months; 75% of patients had not progressed after 2 months, and 50% of patients after 13 months. Regarding the high-risk group of patients, P1 achieved complete response after 6 cycles of Pom/dex+bortezomib; P2 achieved PFS of 11 months; P3 achieved plasmacytoma resolution after 6 cycles of Pom/dex plus local radiotherapy; P4 abandoned Pom/dex after 3 cycles because of severe neutropenia and sepsis. In this group median follow-up was 60.5 months (IQR: 56.3-79.8), and median PFS was 6 months; 75% of patients had not progressed after 5 months, 50% of patients after 6 months, and 25% of patients after 11 months. Regarding adverse events, they were present in two patients: one had neutropenia, and the second one pneumonia plus pulmonary venous thromboembolism. Both of them died (Figure 1).

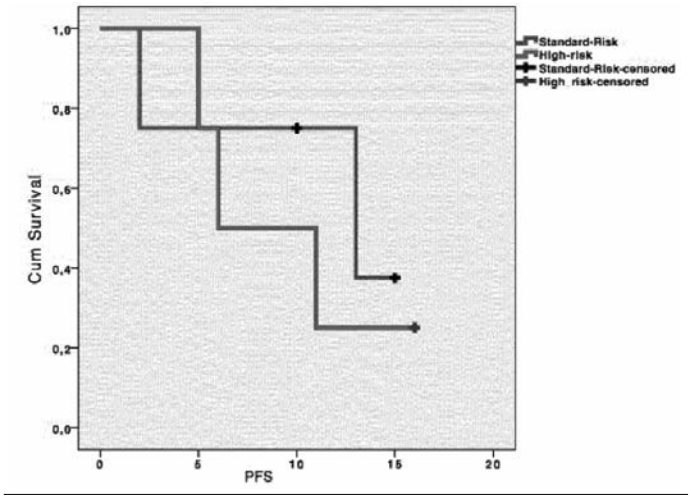

Figure 1.

Summary/Conclusions: In our experience, Pom/dex regimen has prolonged PFS of patients with RRMM, with an improvement of health-related quality of life. This regimen has been even valuable in high-risk patients who received Pom/dex after ≥2 treatment regimens. Pomalidomide plus low-dose dexamethasone, an oral regimen, could be considered a new treatment option as a standard of care for patients with RRMM who have poor prognosis and a high need for effective treatments.

Myeloproliferative neoplasms - Biology

PB2023

ROUTINE SCREENING FOR KIT M541L IS NOT WARRANTED IN THE DIAGNOSTIC WORK UP OF PATIENTS WITH HYPEREOSINOPHILIA

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Background: The role of the KIT M541L variant in patients with hypereosinophilia (HE) is controversial. On the one hand, this variant is a recognised inherited single nucleotide polymorphism (c.1621 A>C; rs3822214) with a minor allele frequency of 0.08 in the ExAC database and classified as benign/likely benign on ClinVar. On the other hand, it has been suggested that KIT M541L increases the sensitivity of the KIT receptor to stem cell factor (Foster R *et al.*, Br J Dermatol. 2008;159:1160-9) and may be somatically acquired in imatinib responsive CEL-NOS patients negative for PDGFRα/β abnormalities (Iurlo A *et al.*, Oncotarget. 2014;5:4665-70). Consequently it has been suggested that HES patients should be screened for KIT M541L, as positive cases may benefit from imatinib treatment.
Aims: We aimed to (i) compare the KIT M541L allele frequency between patients referred for investigation of HE and normal healthy controls (ii) investigate the variant allele frequency (vaf) to determine if KIT M541L mutations may be acquired somatically and (iii) investigate the KIT M451L status in cases negative for PDGFRα/β abnormalities who responded to imatinib.
Methods: We screened healthy controls (n=214) and patients referred for investigation of *FIP1L1-PDGFRA* negative HE (n=220) for KIT M541L using an amplification refractory mutation system (ARMS) PCR designed to amplify allele specific products of different sizes, and able to detect KIT M541L down to 5% vaf. Fishers exact two tailed test was used to compare the allele frequency between the control and HE groups. Digital droplet PCR (ddPCR) was used for patients heterozygous for KIT M541L by the ARMS assay to determine whether the KIT M541L mutation burden was close to 50% (consistent with a constitutional polymorphism) or <50% (suggestive of a somatic mutation). We also studied pre-treatment DNA from 3 patients with hypereosinophilic syndrome who were treated with imatinib (400 mg/day) and showed normalization of eosinophil counts at a median of 0.8 months (0.4-5.0) after treatment for a duration of 13.6 months (range, 3.7-44.8).
Results: Forty two (19%) of HE cases tested positive for KIT M541 compared to 38 (18%) of healthy controls. The KIT M541L allele frequency was no different between cases and controls (0.095 *versus* 0.098; P=0.91). Of the 42 KIT M541L heterozygous HE cases, 40 had sufficient DNA for analysis by ddPCR. The mean allele burden was 50.4% (range 48.3%>56.0%), consistent with all instances being constitutional. None of the three imatinib responders tested positive for KIT M541L prior to treatment.
Summary/Conclusions: Whilst we cannot exclude the possibility that KIT M541L may be acquired somatically in very rare cases, we conclude that there is no clinical value in screening for this variant on a routine basis for patients with HE or HES.

PB2024

MUTATIONS OF THE JAK2 GENE AND CYTOGENETIC ABNORMALITIES ARE PREDICTIVE OF PROGRESSION TO HEMATOLOGICAL NEOPLASMS IN PATIENTS WITH IDIOPATHIC LEUKOCYTOSIS

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Background: Idiopathic leukocytosis and erythrocytosis are hematological disorders without specific causes. Frequent V617F mutations on the *JAK2* gene have been reported in patients with polycythemia vera (PV), essential thrombocythemia, and primary myelofibrosis. We also found *JAK2* V617F mutations in one of 11 patients with idiopathic erythrocytosis. Mutations of the *CSF3R, SETBP1,* and *ETNK1* genes have been found in chronic neutrophilic leukemia and atypical chronic myeloid leukemia (CML). Furthermore an autosomal mutation was found in the *CSF3R* gene in a family with chronic neutrophilia. However, little is known about mutations associated with idiopathic leukocytosis.
Aims: We previously analyzed the *JAK2, CSF3R, CALR*, *SETBP1,* and *ETNK1* genes in 10 patients with idiopathic leukocytosis (EHA20). To elucidate the relevance of genetic alterations, we extended the analysis with 17 genes known to be involved in hematological neoplasms in 16 patients with idiopathic leukocytosis.
Methods: Leukocytosis is defined as a total white blood cell count more than two standard deviations above the mean, or a value greater than 11,000/μL. Those patients who satisfied the following criteria were included in the study: leukocytosis (predominantly neutrophils); the absence of apparent causes of leukocytosis; and documentation of the leukocytosis over a prolonged period

of time. The period of observation was 1 year or longer in most patients. Sixteen patients with idiopathic leukocytosis were analyzed in the study. Neutrophils or mononuclear cells were collected after obtaining written informed consent from the 16 patients. Neutrophils from peripheral blood were purified by dextran sedimentation followed by hypotonic lysis and centrifugation with Ficoll-Conray. Mononuclear cells were isolated from bone marrow by Ficoll-Conray gradient centrifugation. Genomic DNA was extracted using the QIAamp DNA blood mini kit (Qiagen, Valencia, CA, USA). Mutations within hot spots of the *CSF3R, JAK2, CALR, SETBP1, ETNK1, CBL, TET2, ASXL1, EZH2, IDH1/IDH2, DNMT3A, U2AF1,* and *CEBPA* genes were analyzed by direct sequencing in both directions using a 3730xL DNA Analyzer (Life technologies, Carlsbad, CA, USA) and/or allele specific polymerase chain reaction analysis. Total RNA extraction and reverse transcriptase polymerase chain reaction (RT-PCR) were performed between the *ETV6* and *ABL1* genes in 10 patients. *BCR/ABL1* gene was analyzed by RT-PCR or fluorescence *in situ* hybridization in 8 patients. The current study was conducted within the guidelines and with the approval of ethical committee.
Results: *JAK2* V617F mutations were found in one of the 16 patients with idiopathic leukocytosis. No mutations were found in the other genes in the 16 idiopathic leukocytosis patients. *ETV6-ABL1* fusion gene was detected in one of the 10 patients. No *BCR/ABL1* fusion gene was detected in the 8 patients. One idiopathic leukocytosis patient with *JAK2* V617F mutation has developed PV. Another patient with sustained leukocytosis for 20 years showed cytogenetic abnormalities during observation and has developed Philadelphia chromosome negative CML (Ph-CML). *ETV6-ABL1* fusion gene was detected in this patient. Another patient with normal karyotype progressed to blast crisis of Ph-CML accompanied by cytogenetic abnormalities. Of the remaining 13 patients with idiopathic leukocytosis, one resolved the disease and twelve had a stable disease.
Summary/Conclusions: Idiopathic leukocytosis comprises heterogeneous conditions. *JAK2* mutations and cytogenetic abnormalities are predictive of progression to hematological neoplasms.

PB2025

EVALUATION OF EXPRESSION OF MIRNAS ISOLATED MICROVESICLES OF PATIENTS WITH MYELOFIBROSIS ASSOCIATED WITH DISEASE
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Background: Myelofibrosis is a hematological disease inserted in the group of myeloproliferative neoplasias. It has as main characteristic fibrosis of the bone marrow, consequence of a variety of histological changes presented in the medullary microenvironment. The development of the disease is related to the clonal expansion of myeloid stem cells, abnormal expression of cytokines, hypercellularity of myeloid lines and also extramedullary hematopoiesis. The pathophysiology of myelofibrosis involves the activation of signal transduction pathways, which may occur due to genetic rearrangements and mutations that alter the structure of protein tyrosine kinases, making hematopoietic progenitor cells independent or hypersensitive to cytokines, generating anomalous cellular behavior. Myelofibrosis is a hematological disease inserted in the group of myeloproliferative neoplasias. It has as main characteristic fibrosis of the bone marrow, consequence of a variety of histological changes presented in the medullary microenvironment. The development of the disease is related to the clonal expansion of myeloid stem cells, abnormal expression of cytokines, hypercellularity of myeloid lines and also extramedullary hematopoiesis. The pathophysiology of myelofibrosis involves the activation of signal transduction pathways, which may occur due to genetic rearrangements and mutations that alter the structure of protein tyrosine kinases, making hematopoietic progenitor cells independent or hypersensitive to cytokines, generating anomalous cellular behavior.
Aims: Recent studies have shown that microvesicles produced by cells of the organism may be associated with the cellular communication process due to their intravesicular content and that miRNAs also found in this content are able to regulate diverse cellular processes. The expression of some microRNAs is associated with hematopoietic processes such as the transformation of myeloid, erythroid and megakaryocytic progenitors. These can regulate the hematopoiesis of normal stem cells and also of compromised progenitors, having an important role in the pathogenesis of some acquired hematological malignancies. The objective of this work is to investigate the presence of specific miRNAs in microvesicles excreted in peripheral blood plasma of patients with myelofibrosis, which may be related to cellular communication.
Methods: Microvesicles were isolated from the plasma by ultracentrifugation method and through molecular biology techniques it was possible to validate their presence. Assays by qPCR were performed to evaluate the presence of specific miRNAs.
Results: We used the miRNAs described in the literature as influential in the process of hematological disorders. They are: mir146b, mir 150, mir 29a and 155. After analysis of miRNA differential expression, miR-29a and miR-155 were less expressed in MF patients compared to healthy donors (P <0.02 and P <0.03), and miR-223 did not Presented a statistically significant difference. Data on miR-29a corroborate in part with the literature, since the data presented here relate to miRNA carried by VEs rather than serum / plasma. However, low levels of miR-29a expression are related to aberrant auto-renewal of hematopoietic progenitor cells, thus indicating that VEs may contribute to this mechanism. As for miR-155, the data obtained do not corroborate with the literature and, possibly, the VEs do not participate in the mechanism of regulation of Megacariopoiese by miR-155. We used the miRNAs described in the literature as influential in the process of hematological disorders. They are: mir146b, mir 150, mir 29a and 155.After analysis of miRNA differential expression, miR-29a and miR-155 were less expressed in MF patients compared to healthy donors (P <0.02 and P <0.03), and miR-223 did not Presented a statistically significant difference. Data on miR-29a corroborate in part with the literature, since the data presented here relate to miRNA carried by VEs rather than serum / plasma. However, low levels of miR-29a expression are related to aberrant auto-renewal of hematopoietic progenitor cells, thus indicating that VEs may contribute to this mechanism. As for miR-155, the data obtained do not corroborate with the literature and, possibly, the VEs do not participate in the mechanism of regulation of Megacariopoiese by miR-155.
Summary/Conclusions: MiRNAs present in the microvesic content may collaborate in the cellular communication process in myeloproliferative diseases and induce hematopoietic disorders.

PB2026

COMPREHENSIVE STUDY OF BCR/ABL GENE EXPRESSION IN PROGRESSION OF "CLASSIC" MYELOPROLIFERATIVE DISORDERS
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Background: Classic myeloproliferative disorders (MPDs) also known as chronical MPDs, include several clonal hematologic diseases (such as polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF)), which are negative for t(9;22) translocation, result to BCR/ABL chimeric gene expression . Characterization of JAK2V617F mutation, deletion in 12 exon of JAK2 gene, MPL W515L/K, mutation in calreticulin gene (CALR) contributed a lot to understanding of molecular pathogenesis of MPDs. However, detailed molecular mechanism underlying the progression of MPD remains unclear. Several cases of MPDs with detected BCR/ABL expression were described repeatedly in previons publications. The phenomen of simultaneous coincidence of mentioned molecular markers in each clinical case requires comprehensive study.
Aims: The aim our work was to investigate BCR/ABL expression in therapy resistant MPD patients with disease progression.
Methods: Peripheral blood samples 175 patients with progressive MPD and 67 patients with primary MPD was used as a biological material for experiments. Qualitative and quantitative analysis of BCR/ABL gene (p190, p210, p230) was performed by two-step PCR and real-time PCR. Jak2, Jak2-e12, MPL, CALR mutations were determined by direct sequencing and allele-specific PCR. RAG1 and RAG2 expression was analyzed by real-time PCR.
Results: 175 patients with progressive MPD were analysed: 35(20%)- PV, 38(22%)- ET, 102(58%)-PMF. BCR/ABL gene expression was identified in 47 cases (32,83%). We found 44 cases with BCR/Abl /p210, 3 cases with BCR/Abl/p190, and no cases of BCR/Abl/p230. We have observed hepatomegaly (17/43- 40%) and elevated WBC in BCR/ABL positive cases. JAK2V617F mutation was identified in 139 patients, deletion in 12 exon of JAK2 gene was found in 2 patients. One case with MPL W515L mutation and 11 cases with CALR mutation were identified. Group of primary MPD patients (N= 67)- 26(47%)-PV, 21(38%)-ET, 8(15%)-PMF, contained 55/67(82%) patients with JAK2V617F mutation. Expression of BCR/ABL/p210 was detected only in 2/55(3,6%). We also found that expression levels of key components of V(D)J recombinase RAG1 and RAG2 in granulocytes is higher in Jak 2V617F-positive MPDs patients(46/49- 94% cases) compared to heathy donors (3/42- 7%).
Summary/Conclusions: The normal rearrangement of immunoglobulin receptors in maturing B-lymphocytes depends on JAK-kinases activation. Therefore we suppose that activation of key components of V(D)J recombinase (RAG1/RAG2) could lead to appearance of additional clone with chimeric BCR/ABL due to increased tension of Jak-STAT pathway.. The expression of BCR/ABL gene could be the possible reason for MPD progression and should be considered as an indication for complementary therapy.

PB2027

IMMUNOHISTOCHEMICAL ANALYSIS OF CALRETICULIN MUTATIONS IN PRIMARY MYELOFIBROSIS (PRMF) PATIENTS: RESULTS OF OUR INSTITUTION IN THE JAK2V617F MUTATED AND JAK2V617F WILD TYPE PRMF PATIENTS
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Background: Myeloproliferative neoplasms (MPNs) are a group of chronic myeloid cancer characterized by overproduction of mature hematopoetic cells. Mutations in one of three genes; Janus kinase 2 (JAK 2), myeloproliferative leukemia protein (MPL) and calreticulin (CALR), have been found in most patients with BCR-Abl negative MPNs. JAK2 mutations are present virtually all cases of Polycythaemia Vera and 50-60% of prMF and Essential Thrombocythemia (ET). Recently, mutations in CALR gene were found in 50-80% of JAK2 and MPL mutation negative ET and prMF patients.
Aims: To evaluate immunohistochemical results of CALR gene mutation in the bone marrow samples of the JAK2V617F mutated and JAK2V617F wild type Primary Myelofibrosis (prMF) patients.
Methods: Material: Bone marrow biopsy samples from 32 patients previously diagnosed as primary myelofibrosis with known JAK V617F mutation status were obtained from archives of Marmara University Pathology Laboratory. Bone marrow samples of two patients were already known as CALR mutated by PCR analysis. Bone marrow samples of three JAK2 wild type and CALR mutated ET, two JAK2 wild type, CALR mutated prMF patients and two CALR wild type ET patients were used as positive and negative control tissues for CALR immunohistochemistry. Immunohistochemistry: 4-µm unstained sections of each bone marrow biopsy specimens were cut onto electrostatically charged glass slides. Immunohistochemistry was performed on an automated immunostainer (Ventana BenchMark Ultra; Ventana Medical Systems, Inc). CALR antibody (clone CAL2, Dianova, Germany) staining used a 1:100 dilution.Any cytoplasmic staining of the cells with CAL2 antibody was considered positive immunostaining.
Results: We studied 32 bone marrow specimens of primary myelofibrosis with 15 (47%) of them having JAK2 V617F mutation and 17(53%) of them lacking JAK2 V617Fmutation. CALR immunoreactivity was seen in 8 (25%) of all pr MF patients. CALR immunoreactivity was seen in 8 (47%) of patients with PMF myelofibrosis who are negative for JAK2V617F mutation. CALR immunoreactivity was not seen in patients with PMF myelofibrosis who are positive for JAK2V617F mutation. CALR immunoreactivity was seen in 3 (100%) of patients with ET and 2 (100%) of patients with known CALR mutation. CALR immunoreactivity was not seen in patients with CALR wild type ET patients. We observed that CAL2 immunostaining was seen mainly in the cytoplasm of the small and large megakaryocytes, and atypical megakaryocytes as found in fibrotic PrMF. Pale immunostaining was seen in myeloid and erytoid cell precursors. This immunostain also stained some small cells appearing as micromegakaryocytes.
Summary/Conclusions: An immunohistochemical stain easily detects the CALR mutation by staining of megakaryocytes in formalin-fixed bone marrow biopsy specimens . This method would be a easy, rapid, and cost effective way to detect CALR mutations in daily routine hematopathology biopsy evaluation of the myeloproliferative patients.

PB2028

THE HIF1A/2A MRNA INDEX HAS A SIMILAR TREND AS THE CHANGES OF EXPRESSION MRNA CALR AND MDR1 GENES IN WHOLE BLOOD SAMPLES OF PATIENTS WITH JAK2 V617F POSITIVE MPN

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Background: Various groups have reported that isoforms of hypoxia-inducible transcription factor 1a (HIF-1a) and 2a (HIF-2a) can regulate both overlapping and distinct target genes. HIF-1a and HIF-2a have been shown to play opposite roles in the regulation of macrophage function [Takeda N. e.a., 2010]. HIF-index incorporated as a strong prognostic biomarker of renal cell cancer [Szendrői A. e.a., 2016]. Only HIF1a was known as regulator expression of multidrug resistance gene (MDR1) and response to chemotherapy [Comerford K.M. e.a., 2002]. New data have shown exclusive role of HIF-2α in regulates the proliferation and glucose metabolism in erythroleukemia cells [Xu Q.Q. e.a., 2016]. No any information about relations between index of HIF and mRNA gene expression level MDR1 or CALR in patients with myeloproliferative neoplasms (MPN).
Aims: Investigate the mRNA expression levels of HIF-1a and HIF-2a, MDR1 and CALR genes in whole blood samples from patients with JAK2 V617F positive MPN.
Methods: Real-time PCR was performed to detect of HIF1a, HIF2a, MDR1 and CALR mRNA transcripts levels in white blood cells 14 healthy volunteers (median age 22 years, range 21-58 years, 57% males) and 11 (median age 44 years, range 20-77 years, 45% males) patients with JAK2 V617F-positive MPN, median of allelic burden is 36%, range 9-87%. Venous blood were collected in tube with RNAse inhibitor. Total RNA was isolated using "RIBO-zol-D" (Aplisens) and were transcribed using "Reverta-L" (Aplisens). PCR was optimized for the thermocycler CFX96 (Bio-Rad). The results were calculated utilizing the delta Ct method in the software package of "R". The threshold cycles (Ct) genes and housekeeping genes (TBP, GUS, ABL) determined using Cy0 method. The results was normalization with this reference genes. Mann-Whitney U test was used to evaluate significant difference between the groups, the degree of correlation (r) was assessed using Spearman test.
Results: We observed a lower mRNA expression MDR1 and CALR in whole blood samples of patients with MPN compared with a group of healthy volunteers (Figure 1). The expression level of mRNA HIF2a not changed and for HIF1a it should be noted a tendency for statistical significance. It found no correlation between allelic burden and mRNA expression level. Index HIF 1a/2a more clearly showed a correlation with the fall of MDR1 and CALR mRNAs (r= -0.64 in control and r= -0.7 in MPN group, p<0.05). CALR gene unlike MDR1 gene is not known among the target HIF regulation, but their unidirectional change indicates the possible metabolic links.

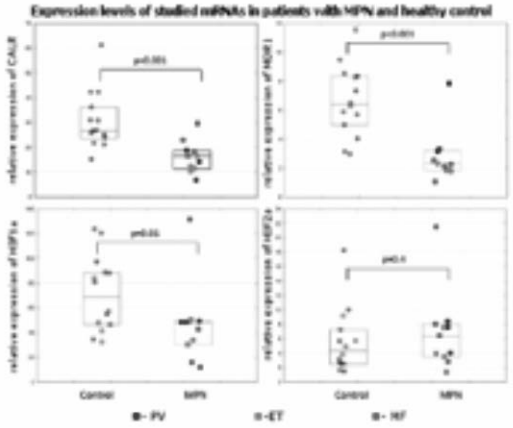

Figure 1.

Summary/Conclusions: We assume that the studied gene expression changes reflect the metabolic processes in the bone marrow progenitor cells. Probably JAK2 V617F mutation leads to more favorable microenvironment and reduced willingness to autophagy, causing the index shift HIF1a/2a. We found reduced of mRNA CALR expression in blood cells at MPN and this fact require further investigation.

PB2029

CD177 EXPRESSION IN PERIPHERAL BLOOD NEUTROPHILS IN HEALTH AND DISEASE STATES

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Background: Objective and specific assays are required in the identification of both chronic myeloproliferative disorders and myelodysplastic syndromes.
Aims: Exploration of the possibility of using the CD177 expression in the peripheral blood neutrophils for the diagnosis of either entity.
Methods: The 213 subjects were organized into 4 main groups; benign neutrophil leukocytosis group, secondary erythrocytosis group and clonal myeloid neoplasms group together with a haematologically normal group as controls. All cases were subjected to clinical assessment as well as the flow cytometry determination of the percentage (%) and mean fluorescent intensity (MFI) of peripheral blood neutrophils expressing CD177.
Results: Skewed high peripheral blood neutrophil CD177 MFI was significantly associated with Philadelphia-negative cMPDs patients (2.9-37.4; median 14.1) compared to controls (0.8-20.5; median 8.8). The MDS patients did not show a significant difference in either CD177% or MFI compared to the controls. Polycythemia Vera (PV) patients had similar results of CD177 expression (% and MFI) compared to Essential Thrombocytosis (ET) patients. However, they had higher CD177 MFI levels compared to the secondary erythrocytosis patients and controls (4.8-37.4; median 16.5, 1.58-25.7; median 5.81, 0.85-20.5; median 8.8 respectively). CD177 MFI showed statistically significant higher values in ET patients compared to the haematologically normal control group (2.9-34.5; median 13.4 *versus* 0.85-20.5; median 8.8 respectively). No correlation between CD177 expression and JAK2 V617F allele burden could be detected in either PV or ET patients. With a 20 p.d.u cutoff, the specificity of neutrophil CD177 MFI in Philadelphia-negative cMPDs patients' diagnosis and differentiation of PV from secondary erythrocytosis was 93% and 85% respectively. The CD177% had a low accuracy of in the diagnosis of MDS patients. The CD177 patterns observed were one positive peak and bimodal pattern (Figure1).
Summary/Conclusions: The CD177 expression is highly associated with Philadelphia-negative cMPDs. It could reliably represent a useful potential marker in detecting those disorders and differentiating them from reactive cases.

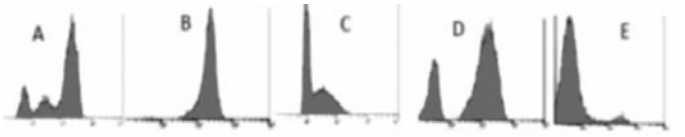

Figure 1. Patterns of peripheral blood neutrophil CD177+ve cells expression observed in flow cytometry. A) Bimodal pattern. B, C, D, E) CD177 single positive peak varieties.

PB2030

DETECTION OF THE MUTATIONS IN GENES JAK2 AND MPL IN THE DIAGNOSIS OF CHRONIC MYELOPROLIFERATIVE DISORDERS

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Background: Chronic myeloproliferative diseases is a group of clonal Ph-negative hematological diseases, which include erythremia (polycythemia Vera, PI), chronic megakaryocytic leukemia (essential thrombocythemia, ET) and subleukemicmyelosis (primary myelofibrosis, PMF, chronic idiopathic myelofibrosis). The origin of these diseases is linked to transformation of hematopoietic stem cells, the result is the excessive production of mature cells of erythroid, granulocytic and megakaryocyte shoots with relatively long course of the disease. The frequency of occurrence of mutation V617F of gene JAK2 exon 12 and MPL gene varies in different literature.
Aims: Determination of the frequency of occurrence of mutations in genes JAK2 and MPL and identifying the importance of the verification of these diseases.
Methods: The study included 350 patients with chronic myeloproliferative diseases — with polycythemia Vera 150 patients, with essential thrombocythemia 78, with chronic idiopathic myelofibrosis 55 and 67 patients were examined with the purpose of differential diagnosis with Ph(-) Chronic myeloproliferative diseases. The age of patients ranged from 20 to 70 years, median age was 54 years. Isolation DNA of patients was carried out using a set of reagents "AmpliPrep RIBO-prep" (OOO Interlabservice, Russia). The concentration and purity of isolated DNA was determined by Nano Drop 2000 instrument (USA). Detection of gene mutation JAK2V617F and MPL gene was carried out by standard polymerase chain reaction on a thermal cycler 2720 "Applied Biosystems" (USA), using a set of "Litech" (Moscow).
Results: The result of the research showed that the incidence of the V617F mutation in JAK2 was varying in patients depending on the type of disease. In polycythemia Vera the mutation V617F in the JAK2 gene was identified in 147 patients of 150 (98,3%), with essential thrombocythemia in 42 patients of the 78 (54,2%), with chronic idiopathic myelofibrosis in 27 patients of 55 (49.1%). In 67 patients with no hematological profile, wich examined with the purpose of differential diagnosis with Ph(-) chronic myeloproliferative diseases, V617F in JAK2 was detected in 6 (8,6%), which allowed to confirm Ph(-) Chronic myeloproliferative diseases. A mutation in exon 12 of the JAK2 gene was detected in 2 of 33 (2,9%) of those surveyed V617FJAK2-negative patients exclusively diagnosed with polycythemia Vera. The MPL W515L mutation gene was detected in polycythemia Vera and chronic idiopathic myelofibrosis 2.2% (1 of 41) and 2% (1 of 52) of patients.
Summary/Conclusions: Thus established, our data confirm that mutations in the genes JAK2 and MPL are highly specific diagnostic markers in patients with Ph-negative chronic myeloproliferative diseases.

PB2031

ASSOCIATION OF MYELOPROLIFERATIVE NEOPLASM AND LYMPHOPROLIFERATIVE DISORDER IN 3 PATIENTS

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Background: Lymphoproliferative disorders (LPD) and myeloproliferative neoplasms (MPN) are two very different sets of hematological pathologies. However, several studies have shown that the risk for LPD onset in patients with MPN is higher than in the general population (1)(2). No single LPD seems to be more at cause and all MPN are likely to present the onset of an associated LPD.
Aims: We present 3 cases diagnosed in the Department of Hematology, « Groupement Hospitalier Est », Lyon, France, of patients bearing an association of MPN and LPD: an essential thrombocythemia (ET) with myeloma, ET with marginal zone lymphoma and a chronic myeloid leukemia with chronic lymphoid leukemia.
Methods: Diagnosis have been made thanks to cytology of peripherical blood, bone marrow aspirate and biopsy and confirmed by cytogenetic and molecular biology techniques.
Results: *Case number 1.* A 68 year old woman known to have essential thrombocythemia as a MPN, with V617F mutation of the JAK2 protein kinase. After 19 years of treatment by Hydrea, she developed a splenomegaly , anemia and slight lymphocytosis of 4.77 G/L. The blood smear, the bone marrow aspirate and biopsy examination revealed myeofibrosis evolution and an infiltration by 30% of a small sized clonal lymphoid population CD20+, CD5-.Medullar karyotype was normal: 46, XX[10].In conclusion the ET has evolved into myelofibrosis and is associated with a lymphoproliferative syndrome, possibly marginal zone lymphoma.No additional treatment has been implemented. *Case number 2.* A 64 year old woman know to have ET with V617F mutation of the JAK2 protein kinase treated by acetic salicylic acid. 5 years after, she presented with IgG kappa type monoclonal gammopathy up to 28 g/L, without any associated clinical manifestation nor cytopenia. Medullar blood was diluted but showed slightly atypical plasmocytes remaining under 10%.Myeloma was diagnosed anyway and the patient received 5 cures of Velcade-Melphalan-Prednisone which resulted in complete remission. The MPN remains stable to this day. *Case number 3.* A 62 year old man with chronic lymphoid leukemia, treated by six cycles of R-FC. While in remission since 2 years, hemogram shows hyperleucocytosis (WBC: 18.3 G/L) with thrombocythemia (platelets: 1886 G/L) without anemia (Hb: 13.7 g/dL).Blood smear examination reveals 3% of myelemia and basophilia (3,66 G/L).BCR-ABL transcript is positive in 43% and karyotype points out a 9;22 translocation. (46, XY, t (9 ;22) (q34 ;q11)[1] nuc ish (BLX3, BCRx3,ABL.con BCRX2)[48/100].) Before starting Nilotinib, cytoreductive treatment by Hydrea was decided. Treatment is under way.
Summary/Conclusions: The three cases described highlight the diverse situations observed in cases of combined MPN/LPD pathologies. MPN with secondary onset of LPD are most frequently encountered, as was the case with patients 1 and 2. Cases of preexisting LPD and late onset MPN are rare (1), and cases of simultaneous discovery of both pathologies even more so (3). Several hypotheses have been formulated to explain the frequency of onset of these pathological associations: genomic instability due to JAK2 protein kinase activation, or due to BCR-ABL mutation, or exposure to cytotoxic chemotherapy or radiations (3).

Myeloproliferative neoplasms - Clinical

PB2032

CLINICAL AND ANALYTICAL DIFFERENCES BETWEEN CALR TYPE-1 AND CALR TYPE-2 MUTATION IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA AND PRIMARY MYELOFIBROSIS: A SINGLE CENTER STUDY

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Background: The JAK2V617F is a main molecular marker in myeloproliferative neoplasms (MPN) and is harbored in about 50-60% of essential thrombocythemia (ET) and primary myelofibrosis (PMF). Recently, CALR mutation was described in ET and PMF JAK2V617F negative patients. There are two main variants of CALR mutation: type 1 (a 52-bp deletion) and type 2 (a 5-bp insertion).
Aims: To compare clinical and analytical data of ET and PMF patients with CALR type-1 vs CALR type-2 mutation.
Methods: We performed a single center study on 471 patients: 87 PMF and 384 ET. The JAK2V617F mutation was analyzed in DNA from peripheral blood leukocytes by PCR ARMS method. In all JAK2V617F negative patients detection of CALR mutation was performed by fragment length analysis and the results were confirmed by sequencing. Statistical data analysis was performed using Statistica 12.5 software for Windows.
Results: From 384 ET patients 254 were JAK2V617F positive (66%), 80 were CALR positive (21%) and 51 were JAK2V617F and CALR negative (13%). From CALR positive patients: 36 (51%) had type-1, 34 (45%) type-2 mutation, and 10 (12%) type-3 mutation. From 87 PMF patients 56 were JAK2V617F positive (64%), 18 were CALR positive (21%) and 13 (15%) were JAK2V617F and CALR negative. From CALR positive groups: 13 (72%) had type-1 and 5 (28%) had type-2 mutation. Compared with ET carrying JAK2V617F mutation, patients ET CALR positive (type-1 plus type-2) had lower hemoglobin (13.3 *vs* 14.5 g/dl, p<0.001) and leukocyte (8.2 *vs* 9.7 G/L, p<0.001), higher platelet counts (1067 *vs* 800 G/L , p<0.001) but with no significant differences in frequency of thrombosis. In ET, CALR mutation was associated with increased odds of myelofibrotic transformation (odds ratio [OR]=2.61; 95% CI: 1.28 - 5.34; p=0.009) comparing with JAK2V617F positive patients. Patients ET CALR type-1 had higher leukocyte counts than ET CALR type-2 mutation (9.6 *vs* 7.3 G/L, p= 0.008), but we did not find significant differences in hemoglobin, platelet counts, frequency of thrombosis or myelofibrotic transformation. Within PMF, no significant differences were observed. Moreover in PMF, there was no significant differences between the JAK2V617F, CALR type-1 and type-2 mutation status respect to the International Prognostic Score System (IPSS).
Summary/Conclusions: 1. In our population, the frequency of JAK2 and CALR mutation was similar to previously described. 2. Compared patients with ET JAK2V617F positive, ET CALR positive (type-1 plus type-2) had higher platelet count but no higher frequency of thrombosis was observed. 3. Myelofibrotic transformation was more frequent in ET CALR positive *versus* JAK2V617F positive patients. 4. ET patients CALR type-1 *versus* type-2 had higher leukocyte count but there were no more significant differences between these two groups. 5. There were no significant differences within PMF group (to small number of patients). 6. In PMF patients, there was no relations between IPSS and mutational status (JAK2V617F, CALR type-1 and type-2).

PB2033

ESSENTIAL TRHOMBOCYTHEMIA: STUDY OF TREATMENT LINES REQUIRED. EXPERIENCE OF A SINGLE CENTER

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Background: Essential thrombocythemia (ET) is a chronic myeloproliferative neoplasm that shows similar survival prognosis as general population, with a very low rate of transformation to myelofibrosis and acute leukemia.
There are different treatments for these patients with optimal responses at first. For the first line, it is usually threated with hydroxyurea, although in young patients it is usually replaced by anagrelide / interferon.
There are publications of hydroxyurea side effects, especially cutaneous, but there are not many studies about how many lines of treatment are needed to control the disease.
Aims: Study type and lines of treatment needed in patients with ET in a cohort of patients from January 1997 to January 2017.
Methods: We studied patients diagnosed of essential thrombocythemia in one area of the region of Murcia from January, 1997 to January, 2017. Those who started treatment and those who needed change were analyzed, either by resistance or by intolerance.
Results: In our area we have registered a total of 152 patients diagnosed with ET. Of these, 71% (108 patients) have required at least one treatment line. Table 1 shows the number of treatment lines required for the control of the disease. As it is shown in the Table, more than 20% of treated patients needed a second line and 6,5% required more tan 2 lines. At last, Table 3 shows current treatment of ET patients.

Table 1. Number of line treatmentes required for disease control.

Treatment lines	N (%)
1	76 (70,3)
2	23 (21,2)
3	7 (6,48)
4	1 (0,92)
5	1 (0,92)

Table 2. Drugs used in patients with ET.

Treatment	N
Hydroxiurea	99
Anagrelide	31
Interferon	10
Busulfan	4
Melphalan	1
Danazol	1

Table 3. Current treatment of ET patients.

Current treatment	N
No treatment	34 (29 never treated, 5 no currently)
in treatment	108
Hydroxyurea	76
Anagrelide	22
INF	6
Busulfan	1
Danazol	1
Hydroxyurea + Anagrelide	2

Summary/Conclusions: This study highlights that, although ET has a very good prognosis, there is a significant percentage of patients that will need a change of treatment, either because of resistence or intolerance.

PB2034

THROMBOTIC AND BLEEDING RISK FACTORS IN ESSENTIAL THROMBOCYTHEMIA

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Background: Thrombosis and hemorrhage are the main category of complications, that affects the overall survival (OS), quality of life and therapy option choice in essential thrombocythemia (ET). Molecular marker presence (*JAK2*V617F, *MPL, CALR*) or its absence (triple-negative status (TN)) in ET supposed to impact on the clinical course, thrombosis rate and ET prognosis.
Aims: The aim of this study was to investigate interactions between the presence of molecular marker, thrombosis/bleeding rates and the OS in ET.
Methods: Outpatient's charts of 240 ET patients, who had been diagnosed with ET at our institution according to WHO 2008 criteria. The following data were assessed: complete blood count, bone marrow biopsy results, bone marrow cytogenetic, the restriction fragment length polymorphism (RFLP) results used for *JAK2*V617F detection, in case of *JAK2*V617F-negative status the PCR-RFLP (*MPL* detection) and the direct sequencing (*CALR* detection) results. Different thrombotic/bleeding complications rates were analyzed. The OS in ET patients was compared according to molecular markers revealed.
Results: According to their mutational status 182/240 (75.9%) patients (pts) were *JAK2*V617F-positive (*JAK2*+); 30/240 (12.5%) – *CALR*-positive (*CALR*+): type 1 (*CALR1*+) – 13/30 pts (43.3%), type 2 (*CALR2*+) – 17/30 pts (56.7%). Only two pts were *MPL*-positive (*MPL*+) (0.8%), TN were 26/240 pts (10.8%). Among 240 pts 183 (76.3%) hadn't any thrombotic complication or bleeding event (no complications/NC), 57/240 (23.7%) had complications: 49/57 (85.9%) reported arterial or/and venous thrombosis, stroke or heart failure (thrombosis+) and 11/57 (19.3%) had bleeding events (hemorrhage+). Thrombotic complications in *JAK2*+ had 27.4% (50/182) pts, in TN – 30.7% (8/26) pts, in *CALR1*+ – 18.2% (2/11) pts and no cases of thrombosis were detected in *CALR2*+ and *MPL*+ subgroups (p<0,001). There were significant statistical differences in

median platelet count as follows: 742x10^9/l (thrombosis+) and 937x10^9/l (hemorrhage+) (p=0.003). No significant statistical differences in median hemoglobin and leukocyte count (p=0.75 and p=0.47) were detected. There were more than a half pts older than 60 years in groups NC (51%) and thrombosis+ (59%) and in group hemorrhage+ only 36% (p<0,001). Cardiovascular risk factors were reported in 24% pts (NC), 69% pts (thrombosis+) and 36% pts (hemorrhage+) (p<0,001). There were no significant statistical differences in follows risk factors as thrombosis >1000x10^9/l and leukocytosis >11x10^9/l (p=0.85 and p=0.72). No significant differences in OS among groups NC, thrombosis+ and hemorrhage+ (p=0.12) were found (Figure 1).

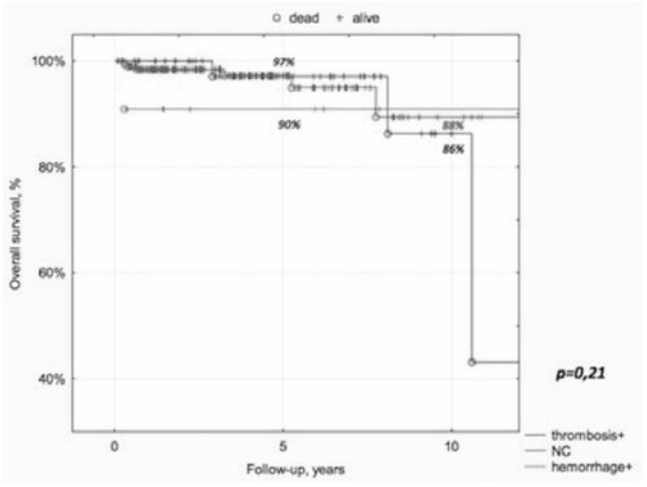

Figure 1.

Summary/Conclusions: Leukocytosis >11x10^9/l and thrombocytosis >1000x10^9/l cannot be assessed as independent thrombosis risk factors in ET. *JAK2*V617F mutation was associated with increased risk of thrombotic complications in ET. *CALR* mutations were correlated with lower thrombosis risk and better OS rate, comparing to *JAK2*+ and TN status despite the fact of *CALR*+ patients had higher platelets level. Along with common thrombosis risk factors (age >60 and cardiovascular risk factors) mutational status may help to identify ET course and to optimize individual therapy option choice.

PB2035

DETECTION OF JAK2 EXON 12 MUTATIONS BY HETERODUPLEX ANALYSIS AND PYROSEQUENCING

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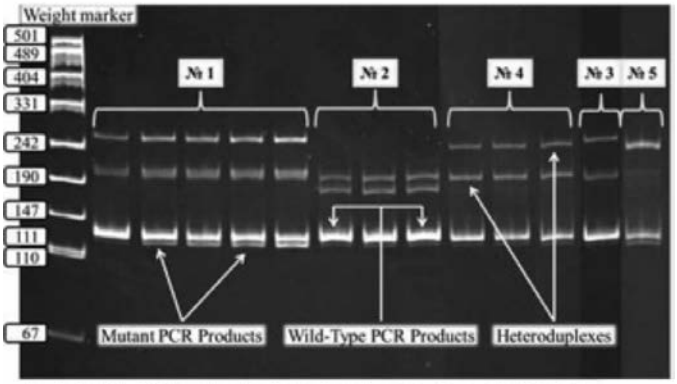
Background: Somatic mutations in codons 533-547 of JAK2 exon 12 are highly specific to confirm the diagnosis of polycythemia vera (PV). We have previously proposed techniques for the detection and quantification of JAK2 exon 12 allele burden using a pyrosequencing method (Subbotina T et al, Haematologica 2014). However, due to the high cost of sequencing, developing a two-stage algorithm for detect mutations in JAK2 exon 12 using inexpensive screening is of immediate practically necessity.

Aims: The aim of this study was to demonstrate the feasibility of using heteroduplex analysis with separation of the PCR product by electrophoresis on non-denaturing PAGE as the preliminary screening test for detection of JAK2 exon 12 mutations.

Methods: 274 patients with PV or unclear erythrocytosis and with a low JAK2V617F allele burden or unmutated JAK2V617 (51 women, mean age 52.2±15.7 years and 223 men, mean age 43.6±15.6 years) were included in this study. The informed consents from these patients were obtained. The PCR with the additional stage of formation heteroduplexes was performed using the Real-time PCR kit (Syntol, Russia) and CFX 96 Real Time System (Biorad, USA). PCR products were analyzed by electrophoresis in 8% PAGE. The presence of the mutations was identified and confirmed by pyrosequencing method with PyroMark Q24 (Qiagen, Germany). To verify the presence of mutations, the DNA sequences extracted from the clinical samples were cloned into pGem-T vector using standard protocol («Promega», USA), and obtained clones were sequenced using reagents and equipment of the «Applied Biosystems» (USA). JAK2 exon 12 variance^{MUT} was calculated as a measure of relative changes in allele burden between the baseline and follow-up sample (Theocharides A et al, Haematologica, 2008).

Results: We detected JAK2 exon 12 mutation in five out 274 patients. The results of electrophoresis on non-denaturing PAGE are reported in Figure 1. The type of №1-5 patient mutations was determined by pyrosequencing: N542-E543del (c.1624_1629delAATGAA); I540-E543delinsKK (c.1619_1627 TCAgAAATgK (c.1622_1627delGAAATG) and p.H538_K539>L (c.1612_1616CACAA>TT). These mutations have been already described. Main characteristics of 5 patients with JAK2 exon 12-mutated PV are reported in Table 1. The PV diagnosis of №1, 2, 3 and 5 patients was confirmed by bone marrow trephine biopsies histological examination. All five patients with JAK2 exon 12-mutated PV have an increased number of red blood cells, along with an accompanying increase in the concentration of hemoglobin and hematocrit level in the peripheral blood. Some of them had increase number of leukocytes and platelets in the disease dynamics. №1-4 patients was treated phlebotomy only and did not received any cytoreductive treatment to date. Patient №5 receives hydroxyurea (HU). Importantly, two out five patients with JAK2 exon 12-mutated PV also have a mutation JAK2V617 (<1%). JAK2 exon 12 allele burden in sample from №1 patient is significantly increased in the disease dynamics.



Analysis of the PCR product by electrophoresis on non-denaturing PAGE (in the disease dynamics for the №1, №2 and №4 patients).

Figure 1.

Table 1.

Characteristics of 5 patients with JAK2 exon 12-mutated PV.

№	Age of manifestation (years)	Sex	Disease	HU	JAK2 V617F mutation	JAK2 12 exon mutation	% JAK2 12 exon mutation baseline	% JAK2 12 exon mutation last sample	JAK2 12 exon variance MUT	Time between two assessments (mo)
1	61	M	PV*	No	Neg	N542_E543 del	15	50	+233	41
2	48	M	PV*	No	<1%	I540_E543 delinsKK	11	13	n.s.	15
3	27	M	PV	No	Neg	N542_E543 del	15	11	n.s.	43
4	~72	F	PV	No	Neg	R541_E543 >K	21	26	n.s.	36
5	29	M	PV	Yes	<1%	H538_K539 >L	30	-	-	-

* – The allele burden was determined at the primary address to the doctor with MPN symptoms

Summary/Conclusions: The proposed variant of the heteroduplex analysis with separation of the PCR product by electrophoresis on non-denaturing PAGE can be recommended for use as the preliminary screening test which is carried out before the confirming pyrosequencing. The two-stage approach allows to optimize the algorithm of the JAK2 exon 12 mutation detection and to improve the efficiency of testing for patients suspected of having PV in whom a JAK2V617F mutation is not detected or detected in a low allele burden. In five out 274 patients we detected JAK2 exon 12 mutation and confirmed the diagnosis of PV.

PB2036

INTRODUCTION OF AN NGS GENE PANEL INTO CLINICAL SERVICE FOR MYELOPROLIFERATIVE NEOPLASMS

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Background: In the West Midlands region of the UK, all patients with a suspected myeloproliferative neoplasm (MPN) have access to quantitative analysis

of *JAK2* V617F by droplet digital PCR as standard of care. The British Committee for Standards in Haematology recommends that suspected MPN cases have investigation of *JAK2* exon 12, *CALR* and *MPL* genes if *JAK2* V617F is negative.
Aims: The aim of the project was to improve the MPN service by substituting sequential analysis of individual target regions within the *JAK2*, *CALR* and *MPL* genes with a single assay, and to increase the number of genes available for analysis.
Methods: A commercial next generation sequencing (NGS) gene panel (Oxford Gene Technology, SureSeq Myeloid Panel), coupled with the Illumina MiSeq platform was validated and implemented. The gene panel utilises hybridization based enrichment technology and consists of 25 MPN–related genes. During the validation stage the following were enriched and analysed: 29 positive control samples with 30 known pathogenic variants, 30 negative control samples without known pathogenic variants in the *JAK2, CALR and MPL* genes, and 24 MPN samples of unknown mutational status. Thus so far over 200 clinical samples have been analysed and reported since the service was introduced in October 2016.
Results: The panel has successfully identified: a large range of known pathogenic variants at high sensitivity (*JAK2* V617F variant allele frequency 1%, *CALR* Type I frameshift variant allele frequency 3%), a potential alternative driver mutation in a known low level *JAK2* V617F positive patient, a rare *MPL* exon 4 pathogenic variant and also the detection of low level *CALR* pathogenic variants, which would not have been detected by Sanger sequencing analysis. In one patient the panel identified the presence of two different *JAK2* exon 14 pathogenic variants in cis (*JAK2* V617F and *JAK2* C618R). The *JAK2* C618R prevented the hybridization of the probe binding site of the *JAK2* V617F ddPCR assay which had led to a false negative result by ddPCR. The validation procedure also explored coverage and limits of sensitivity, potential chemistry specific artefacts and identified common polymorphisms for all 25 genes.
Summary/Conclusions: The panel has replaced the current sequential analysis of *CALR, MPL* and *JAK2* exon 12 in *JAK2* V617F negative patients and reduced turn-around-times with increased accuracy and sensitivity compared to Sanger sequencing and fragment analysis. Our current clinical service operates on a two tier system whereby clinicians can request analysis of the full 25 gene panel or a 4 gene subset (*JAK2, CALR, MPL, CBL* as an *in silico* analysis).

PB2037

IN JAK2V617F POSITIVE MYELOPROLIFERATIVE NEOPLASMS, BLEEDING RISK CORRELATES WITH ALLELE BURDEN
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Background: Myeloproliferative neoplasms (MPN) are characterized by the presence of JAK2V617F mutation that is almost invariably associated with polycythemia vera (PV), but also occurs in the majority of patients with essential thrombocythemia (ET) or primary myelofibrosis (PMF). JAK2V617F-positive patients display different laboratory and clinical features from JAK2-wild type, but no clear correlation was found between the JAK2V617F allele burden and natural history of the disease. The most common causes of morbidity and mortality in MPN are thrombotic and hemorrhagic complications, albeit bleedings are less frequent than thrombosis and mostly represented by minor hemorrhages (ecchymosis, epistaxis, menorrhagia and gingival hemorrhage). The impact of different allele burden on bleeding risk is uncertain.
Aims: Aim of our study is to explore whether there is an association between JAK2V617F allele burden and hemorrhagic complications in a large cohort of MPN diagnosed and followed in a single center.
Methods: We selected 253 MPN (121 ET= 47.8%, 124 PV=49% and 8 PMF=3.2%) carrying JAK2V617F mutation. The median follow-up of patients was 8.8 years (0.1 – 37.3 y). Complete medical history and anti-thrombotic drugs use were recorded. Hemorrhagic complications were classified as "major" or "minor" in agreement with ISTH criteria. The patients were categorized into four groups according to the amount of JAK2 mutant allele, (1st quartile 1-25%, 2nd quartile 26-50%, 3rd quartile 51-75% and 4th quartile 76-100%). Nominal variables were compared with X^2 test or Fisher's exact where indicated. Survival has been evaluated only for groups with different prevalence of events during follow-up and were calculated with the Kaplan Meier method and compared with the Log Rank test.
Results: Three patients (1.2%) bleed at diagnosis (1 major and 2 minor hemorrhages) while 27 (11.8%) suffered for hemorrhages during follow-up (10 major and 17 minor). Prevalence of hemorrhages results higher in 4th quartile compared both to 2nd (p=0.003) and to 1st (p<0.001) quartiles. Hemorrhages-free survival is confirmed lower in 4th quartile compared both to 2nd (p= 0.004) and to 1st (p<0.001). The incidence rate of hemorrhages are respectively 0.7/100 pats /y for 1st quartile, 0.65/100 pats /y for 2nd quartile, 1.26/100 pats /y for 3rd quartile and 3.23/100 pats /y for 4th quartile with a IRR of 5 and of 4.6 for the 4th quartile respectively *versus* 2nd and 1st one. No statistically significant difference has been demonstrated in the use of anti-thrombotic drugs among patients of the different quartiles.
Summary/Conclusions: Risk factors for hemorrhage in MPN are not well defined, and there is no risk estimation model for this outcome. Acquired von Willebrand disease, entity of platelet increased count and aspirin use have been implicated in bleeding occurrence. Previous reports fail to demonstrate a correlation between JAK2 mutation and bleeding risk. In contrast, in our cohort we found a significantly higher incidence of bleeding manifestations during follow-up in patients with higher allele burden. Interestingly no differences were seen in administration of anti-thrombotic drugs among quartiles, suggesting an independent role of JAK2 allele burden in the different distribution of hemorrhagic events.

PB2038

JAK2 ALLELE BURDEN IN PATIENTS WITH PHILADELPHIA NEGATIVE MYELOPROLIFERATIVE NEOPLASMS
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Background: The JAK2V617F allele burden (JAK-AB) plays a central role in chronic myeloproliferative neoplasms (cMPNs); its presence has also been advocated in the differential diagnosis of cMPNs and as independent risk factor for venous thromboembolic complications. New treatment with Ruxolitinib may decrease JAK-AB but at the present, it is not clear the clinical advantage of such reduction
Aims: Primary aim of the current study was to evaluate at diagnosis the JAK-AB in patients with Philadelphia negative cMPNs, in order to evaluate any association with standard demographic, clinical and laboratory parameters with particular reference to thrombotic risk.
Methods: Peripheral blood samples from patients with Ph-negative cMPNs were collected, DNA from leucocytes was analysed for Jak-2 (V617F) gene mutation with amplification-refractory mutation system (ARMS) PCR, subsequently a real-time quantitative polymerase chain reaction (qRT-PCR) for JAK2V617F allele burden measurement was applied. A multivariate analysis was than performed to evaluate any association of AB with demographic and clinical data.
Results: One hundred and twelve patients with Philadelphia negative cMPNs were investigated: 52 females with a median age at diagnosis of 69 years (age range: 18-95 years), 60 males with a median age of 68 years (age range: 18-82 years). Thirty-four patients had Essential Thrombocythemia (ET), fifty-two had Polycythaemia Vera (PV) and twenty-six had primary myelofibrosis (PMF). JAK2-AB of patients with an age of <69 years and ≥69 years, was respectively evaluated. Patients older than 69 years showed a significantly higher JAK2-AB . JAK-AB was significantly reduced in ET, when compared to PV and PMF. No correlation was found between median values of allele burden and IPSS and DIPSS scores. In patients with PV (n=52), a significant correlation was observed between allele burden and WHO2008 scoring system. No significant correlation was found between allele burden and thrombotic risk according to IPSET-t and IPSET-ET for PV and ET, respectively. Patients with a previous history of thrombosis had the highest JAK2-AB. In PMF, a positive correlation between JAK-AB and grading of fibrosis was found only for the highest grades (PMFIII and IV). JAK-AB had a positive correlation with splenomegaly in PMF.
Summary/Conclusions: Our report cannot confirm any correlation between allele burden and thrombotic risk, according to currently adopted scoring systems. A previous history of thrombosis is however associated with the highest AB in all cases.

PB2039

COMPARISON OF CLINICAL AND LABORATORY DATA, INCLUDING JAK-2 46/1 HAPLOTYPE, BETWEEN PATIENTS WITH IDIOPATHIC ERYTHROCYTOSIS AND POLYCYTHEMIA VERA.
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Background: Idiopathic erythrocytosis (IE) is a relatively rare finding characterized by an increased red blood cell mass without an identifiable cause. Diagnosis of IE is based on the exclusion of primary and secondary erythrocytosis including JAK2-wild-type polycythemia Vera (PV).
Aims: In the current study, we report clinical features and laboratory data able to discriminate IE from PV, at diagnosis
Methods: We have here analyzed clinical and laboratory parameters, including Jak-2 46/1 haplotype, from patients with a confirmed diagnosis of IE and PV, followed from January 2010 to December 2016. Data were statistically analyzed, nominal variables were compared with X^2 test and continuous variables with the Mann-Whitney test.
Results: Overall, 40 patients with IE and 93 patients with PV were included in the current analysis (Table 1). Splenomegaly and itch were reported only in one patient with IE. History of thrombosis and cardiovascular events was positive in one case with IE. Jak-2 (V617F) and exon 12 mutations were negative in all patients with IE, while Jak-2 46/1 haplotype was found at heterozygous state in 18 patients and at homozygous state in 2 patients with IE.

Table 1.

	PV	IE	p
Patients N.	93	40	
MALE N. (%)	59 (63,44%	38 (95%)	0.0001
FEMALE N (%)	34 (36.56%)	2 (5 %)	0.0001
MEDIAN AGE AT DIAGNOSIS, YEARS	66 (23 - 95)	58 (17 - 83)	0.007
SPLENOMEGALY N° (%)	38 (40,86%)	1 (2,5%)	0.000001
ITCH	36 (38,70%)	1 (2,5 %)	0.00000 1
MEDIAN WBC COUNT X10⁹	9,3 (4,535,2)	8.1 (4,2-14,3)	0.03
MEDIAN HB g/dl	17,5 (15,1-21,0)	17,4 (16,1-19.1)	0.9
MEDIAN HT %	53,3 (48.4 - 64.3)	52,4 (48.2 - 55.3)	0.1
MEDIAN PLTS COUNT X 10⁹	435.0 (270-1013)	216 (178.339)	0.001
V617 F OF JAK2 POSITIVE N. (%)	86 (92,47)	0	
JAK2 EXON 12 MUTATION N. (%)	2 (2,15%)	0	
HAPLOTYPE 46/1 OF JAK2, ETHEROZIGOUS	42 (45,16%)	18 (45,0%)	0,98
HAPLOTYPE 46/1 OF JAK 2, HOMOZIGOUS	25 (26,88%)	2 (5 %)	0.008
PATIENTS WITH CARDIOVASCULAR EVENTS OR THROMBOSIS N %	32 (34,4%)	1 (2,5%)	0.000008

Summary/Conclusions: In the current study, we highlight peculiar clinical and laboratory findings of IE, in comparison with Polycythemia Vera. As shown by available studies, Hb and HCT level do not easily discriminate between the two categories of patients while gene panels may be useful to improve diagnostic accuracy of IE. We have here first observed the presence of Jak-2 46/1 haplotype in approximately half patients with IE, even in absence of JAk-2 mutations; the homozygous status was statistically different among PV and IE patients. The role of such association deserves further specific studies.

PB2040

LABORATORY RESPONSIVENESS OF LOW-DOSE ASPIRIN IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

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Background: The essential thrombocythemia (ET) is a myeloid neoplasm characterized by platelet hyperreactivity and thrombosis. The daily low-dose aspirin (ASA) is a cornerstone in the prevention of the thrombotic events. In the ET an accelerated platelet turnover translates in a renewal of the drug target shortening the duration of cyclooxygenase (COX-1) inhibition and may dictate new dosing strategies particularly in ASA "low-responders" patients.
Aims: Therefore, we evaluated platelet count, β-thromboglobulin (β-TG) and platelet factor 4 (PF4), as markers of platelet activation, the platelet function activity (PFA), as indicator of ASA platelet sensitivity.
Methods: We studied 60 patients (20 men, 40 women; mean age 51 years, range 32-70) with ET according to WHO criteria. The mean duration of disease was 11 years. All patients were on ASA 100 mg once daily. Of the 60 patients, 45 were on anagrelide hydrochloride (daily dose 1.5 mg) (10 men, 35 women), 15 were on hydroxyurea (daily dose 2 mg) (10 men 5 women). None had inherited or acquired thrombotic risk factors. Sixty subjects served as controls. Platelets were measured by automated analyzer. β-TG and PF4 were determined by ELISA. ASA platelet sensitivity was measured by Platelet Function Analyzer (PFA-100).
Results: The mean platelet count was 455±200x109/L. All patients had normal β-TG and PF4 ((12±5 IU/ml and 4±1 IU/ml) and prolonged C/EPI closure time (T, unit: s, n.v. 84-160 s) (249±40 s).
Summary/Conclusions: These findings suggest that in ET patients the daily low-dose ASA represents an optimal dosing strategy and that PFA test may be an useful tool to distinguish between the ASA "normal-responder" and "low-responder" ET patient.

PB2041

CLINICAL AND EXPERIMENTAL CHARACTERISTICS OF MYELOID/ LYMPHOID NEOPLASMS DISPLAYING PDGFRA OR PDGFRB REARRANGEMENT

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Background: According to the 2016 revision to the WHO classification of myeloid neoplasms and acute leukemia, the cases with rearrangement of tyrosine kinase (TK) genes PDGFRA, PDGFRB are classified in Myeloid/lymphoid neoplasms with eosinophilia and rearrangement of PDGFRA, PDGFRB, or FGFR1, or with PCM1-JAK2. It is a rare event that patients presented rearrangements with these genes. And in the past decade, the dose of TKI to cases with PDGFRA and B abnormal was inconclusive.
Aims: The goal of the study was to assess the clinical and experimental characteristics and observe the response of Imatinib(IM) therapy of Myeloid/lymphoid neoplasms with PDGFRA or B abnormal.
Methods: Cytogenetic examination of bone marrow cells obtained from patients was performed by 24h culture method. R banding technical was used for karyotype analysis. PDGFRA and B gene rearrangement were detected by FISH using triple-color of 4q12 and dual color break-apart PDGFRB probes. The fusion genes of rearrangements of PDGFRA and B genes were detected by RT-PCR. Immunophenotype analysis was carried out by flow cytometry. Most of all cases were treated with IM and followed up.
Results: The diagnoses included 27 cases of MPN, 1 case of AML-M2 and 1 case of non-hodgkin lymphoma. 21 cases were PDGFRA rearrangement, the other 8 were PDGFRB abnormal, 7 of 8 were EP fused gene, one of which concurrent with DEK-CAN fused gene, and the eighth had MYO18A-PDGFRB. 7 cases of the 8 PDGFRB rearrangement had a primary abnormality with t(5;12)(q33;p13) and the other one had a secondary abnormality of AML-M2. PDGFRA and B genes rearrangement detected by FISH and multiple-RT-PCR were positive. The immunophenotypical analysis showed myeloid or lymphoid. These cases achieve rapid and durable remissions on IM.
Summary/Conclusions: In summary, patients with significantly anemia and eosinophilia should be screened for the presence of PDGFRA and B rearrangements. The dual-colour FISH is a simple approach and should be added into the diagnostic work-up because these patients respond to imatinib therapy, and sustained responses have been observed. The OS of PDGFRA and B abnormal was similar with a previous report in a western population and another Chinese hematology center.

PB2042

PLATELET AGGREGATION STUDY OF ESSENTIAL THROMBOCYTHEMIA TREATED WITH ANAGRELIDE

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Background: Essential thrombocythemia (ET) is a myeloproliferative neoplasm characterized by thrombocytosis and abnormal megakaryocyte proliferation. Patients with elevated platelet count are considered to be a high-risk group for thromboembolic and/or hemorrhagic complications. In Japan, anagrelide treatment was recently approved for the 1st line as a cell reduction therapy on ET. Even now, there are few study whether the risk of thrombosis has decreased after anagrelide treatment. Moreover, the platelet count problem uncertainty remains what is the best practice to follow when the platelet count in platelet-rich plasma (PRP) exceeds about 600 x10⁹/L, in the recent recommendations for the standardization of light transmission aggregometry by the platelet physiology subcommittee of Scientific and Standardization Committee /International Society of Thrombosis and Hemostasis.
Aims: The aim of this study was to characterize the platelet aggregation (PA) in patients with ET. We would also clarify whether there were any changes of hemostatic side effect and platelet aggregability before and after treatment with anagrelide.
Methods: This study has been conducted with blood sample obtained from six healthy subjects, compared to 18 consecutive patients with ET. None of the patients was taking anticoagulants or cytoreductive agents. We also studied six anagrelide-treated patients with ET. Whole blood aggregometry (WBA) and LTA using PRP were performed. ADP-induced PA or collagen-induced PA used natural count PRP and platelet count adjusted PRP with platelet-poor plasma. Data were compared in the groups using the Tukey-Kramer test. This study was approved by the Ethical committee of our hospital. All study procedures were performed in accordance with the Declaration of Helsinki.
Results: The result of WBA was not obtained, because the filter was obstructed by giant platelets. In the natural PRP, even over 900x10⁹/L, the platelet aggregability was markedly increased compared with the control (ADP-induced PA: p=0.023, collagen-induced PA: p=0.001), but, was not significantly different (ADP-induced PA: p=0.703, collagen-induced PA: p=0.986) in the count adjusted PRP. These results were not confirmed in cases with platelet counts of less than 600x10⁹/L. There was no decrease in platelet aggregation before and after treatment with anagrelide (ADP-induced PA: p=0.3403, collagen-induced PA: p=0.514).
Summary/Conclusions: In the ET patients with platelet counts more than 900x10⁹/L, the platelet aggregation by LTA with natural count PRP was remarkably accelerated and this data seemed to reflect the disease state. Although treatment with anagrelide showed cyto-reductive effect without any hemorrhagic complication in patients with ET, it did not fully reduce platelet aggregability.

PB2043

A SINGLE CENTRE EXPERIENCE OF MASTOCYTOSIS

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Background: Mastocytosis considered as a subcategory of myeloid neoplasms based on World Health Organization (WHO) 2016 classification, is characterized by expansion and accumulation of abnormal clonal mast cells in

one or more organs. KITD816V mutation and other KIT mutations play as driver mutations in the pathogenesis of disease. KITD816V mutation is positive in%80 of systemic mastocytosis patients. Recent studies show that high allele burden of KITD816V and high serum tryptase levels correlate with aggressive disease. Recently the importance of CD30 expression on neoplastic mast cells has been confirmed. CD30 is expressed aberrantly on neoplastic mast cells in patients with advanced systemic mastocytosis.

Aims: In this study we aimed to present demographic data, clinical follow-up and treatment of patients with mastocytosis and identify the impact of KIT D816V allele burden and expression of CD30 by mast cells in systemic mastocytosis.

Methods: We performed a retrospective study on 54 adult patients with mastocytosis (24 female, 30 male; mean age:44±13) who fullfilled WHO criteria between 2006 and 2016. These patients comprise cutaneous mastocytosis (CM) (n=10), ındolent systemic mastocytosis (ISM) (n=30), smoldering systemic mastocytosis (SSM) (n=2), aggressive systemic mastocytosis (ASM) (n=4) , systemic mastocytosis associated hematologic neoplasm (SM-AHN) (n=3), mast cell leukemia (MCL) (n=4) and mast cell activation syndrome (MCAS)(n=1).

Results: At diagnosis, age of patients with advanced disease was higher than ISM and SSM group (p=0.001). Most frequent symptom of disease was skin lesion (urticaria pigmentosa) (%64). Skin lesions were significantly higher in patients with ISM and SSM than with advanced disease (p=0.009). But B symptoms were significantly higher in advanced disease variant (p=0.013). Anemia, trombocytopenia, elevation of ALP and GGT, hypoalbuminemia were significantly higher in advanced disease than in ISM and in SSM. Osteopenia was higher in patients with ISM and SSM than with advanced disease,%56 and%18 respectively. KITD816V mutation was detectable in peripheral blood in 33 of 40 mastocytosis patients (%82) with a median Ct value 36±4. Median Ct value was significantly lower in advanced SM (Ct: 32±5) than in SM and SSM (Ct: 36±4)(p=0.028) showing a significantly higher allele burden. Expression of CD30 on mast cells in bone marrow biopsies with immunohistochemistry investigation was detectable in 20 of 32 systemic mastocytosis patients (%62). There was no significant difference expression of C30 on mast cell between patients with ISM (%65) (13/20) and advanced SM (%87) (7/8) (p=0.371). There was no significant correlation between elevated serum tryptase level and CD30 expression (p=0.114).

Summary/Conclusions: The definition of disease subcategories in systemic mastocytosis is important for choosing the treatment modality (cytoreduction or allogeneic stem cell transplantation *vs* treatment of the mediator symptoms) for the individual patient. CD30 is a diagnostic marker and also a possible therapeutic target.

PB2044

JAK2 PSEUDO-KINASE AND KINASE MUTATIONS IN THE ETIOLOGY OF THROMBOCYTOSIS

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Background: Thrombocytosis is defined as an abnormally increased number of platelets (>450x10^9/L) in the blood counts, whose cause can be primary or secondary, hereditary or acquired. Hereditary thrombocytosis is a rare congenital disease due to germ line mutations affecting thrombopoietin signaling genes such as THPO, MPL and, more recently, JAK2.

Aims: To describe five cases of persistent thrombocytosis in young patients with *JAK2* mutations.

Methods: Four children (2F: 2M), median age of 8,8 years and 1 young adult (F) 21 years-old, with sustained elevation of platelet counts. None had previous history of thrombo-hemorragic events. Main causes of secondary thrombocytosis were excluded, and all patients tested negative for *BCR-ABL1*, *JAK2*V617F, *CALR* and *MPL* mutations. Sanger sequencing of exons 12 to 20 of JAK2 was performed in all patients. Family studies were possible in 3 families.

Results: Median CBC values: platelets- 630±90x10^9/L; hemoglobin - 13,3±1,2 g/dl and leukocytes- 9,3±2,3x10^9/L. Four different *JAK2* mutations were identified in the 5 patients (Table 1): *JAK2* S591L/R867Q/T875N/T875I. The patient with the *JAK2* T875N mutations had a discrete splenomegaly Familial studies allowed the identification of *JAK2* T875I mutation in 3 adults previously characterized as essential thrombocythemia (ET) triple negative

Summary/Conclusions: *In vitro* studies performed by other authors have demonstrated that *JAK2* R867Q and *JAK2* S591L, described in familial thrombocytosis, promote JAK-STAT activation. The germline nature of *JAK2* T875N mutation, previously described in an acute megakaryoblastic leukemia primary cell line, was confirmed in DNA obtained from hair follicle. Two patients presented a non-described *JAK2* T875I mutation. Familial studies clarified the etiology of thrombocytosis in 3 adults previously diagnosed as ET triple negative. The identification of different *JAK2* germline pseudo-kinase and kinase domains mutations has settled the etiology of persistent thrombocytosis in 4 children and 1 young adult. Therefore, particularly in children, after excluding the main causes of secondary and acquired thrombocytosis, *JAK2* gene sequencing should be incorporated in the differential diagnosis of this condition. The characterization of these rare forms of thrombocytosis and the follow up of these patients across generations, will improve the understanding of this entity.

Table 1.

Patient characterization and familial studies

	JAK2 GENE						CLINIC AND LABORATORIAL DATA		
	Mutation	Exon	Domain	Age diag (years)	Hb g/dL	WBC x10⁹/L	Plat x10⁹/L	Bone Marrow Biopsy	Family studies	Clinical findings
P1	T875N (c.2624C>A)	20	Kinase	5	13,7	8.61	757	Mild Megakaryocytic hyperplasia	Absent in family, positive in follicle DNA sample	Mild splenomegaly (on ultrasound)
P2	T875I (c.2624 C>T)	20	Kinase	10	13,8	8,79	560	Mild Megakaryocytic hyperplasia		Asymptomatic
P2	T875I (c.2624 C>T)	20	Kinase	24	15,9	10,4	814	Mildly hypocellular	Father	17 years follow-up
P2	T875I (c.2624 C>T)	20	Kinase	56	12,5	5,3	427	Mild Megakaryocytic hyperplasia	Grandmother	Thrombofebitis 9 years follow-up
P2	T875I (c.2624 OT)	20	Kinase	28	14,2	11,2	622	n.a.	Uncle	8 years follow-up
P3	T875I (c.2624 C>T)	20	Kinase	21	13,8	8,3	660	n.a	n.a Positive in follicle DNA sample	Asymptomatic
P4	R867Q (c.2600 G>A)	20	Kinase	4	14,2	7,77	528	n.a	n.a	Fetal hydrops (anemia and thrombocytopenia at birth)
P5	S591L (C.1772 OT)	13	Pseudo-Kinase	16	13	11	512	Normal		Asymptomatic
P5	S591L (c.1772 C>T)	13	Pseudo-Kinase	41	14,4	6,96	417	Normal/Mildy hypocellular	Mother	Asymptomatic

n.a. = not available

PB2045

COMPARISONS OF PATIENT MANAGEMENT IN MYELOPROLIFERATIVE NEOPLASM PATIENTS IN THE UK *VS* REST OF WORLD: ANALYSIS FROM THE INTERNATIONAL LANDMARK SURVEY

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Background: Patient (Pts) with myeloproliferative neoplasms (MPNs), including myelofibrosis (MF), polycythemia vera (PV), and essential thrombocythemia (ET), experience a substantial disease burden. The international MPN LANDMARK survey evaluated the patient-reported impact of MPNs in pts across 6 countries and identified current treatment strategies in these pts.

Aims: To analyze differences in treatment strategies used by physicians and pts to manage their MPN between the UK and the Rest of Surveyed World (ROSW).

Methods: A cross-sectional survey was conducted in Australia, Canada, Germany, Italy, Japan, and the UK. The Internet-based survey was administered separately to MPN patients and treating physicians (the two samples were not linked). Observed differences between the UK and ROSW are described in terms of treatment patterns and patient physician communication.

Results: A total of 699 pts (UK, n=286; ROSW, n=413) and 219 physicians (UK, n=31; ROSW, n=188) completed the survey. UK physicians were more likely to start treatment rather than delay at diagnosis of PV or ET. A greater proportion of ROSW physicians reported they would observe >25% of patients at diagnosis (UK - 54% MF, 30% PV, 37% ET; ROSW – 51% MF, 48% PV, 50% ET). No difference was observed in the most commonly received treatments for each disease, but a greater proportion of UK patients reported receiving treatments than ROSW. This difference was greater in PV and ET than MF. For MF the most commonly received treatments were ruxolitinib (UK 55%, ROSW 50%), aspirin (UK 53%, ROSW 37%), hydroxyurea (HU) (UK 31%, ROSW 28%) and transfusion (UK 27%, ROSW 23%), for PV they were aspirin (UK 83%, ROSW 58%), phlebotomy (UK 76%, ROSW 67%) and HU (UK 63%, ROSW 36%) and for ET they were aspirin (UK 94%, ROSW 52%), HU (UK 62%, 30% ROSW) and anagrelide (UK 14%, ROSW 18%). Physician reported data on treatments prescribed demonstrated a similar pattern as a greater proportion of UK physicians reported using treatments than ROSW. UK physicians reported that their patients were more likely to 'often' disagree with their primary treatment recommendation than ROSW (16% *vs* 7%) but despite this UK patients were more likely to be 'completely' satisfied with their physicians understanding and support of their treatment goals (UK, 51%; ROSW 35%). Patients rated who they thought should be the main decision maker on a scale of 1 (the patient) to 10 (physician). UK patients were slightly more inclined to want to be involved in treatment decisions (mean: UK, 6.25; ROSW, 7.01). UK physicians supported this as more 'agreed strongly' with the statement '*I involve my MPN patients in treatment decisions"* (UK, 39%; ROSW 28%).

Summary/Conclusions: In comparison with ROSW: UK physicians were more likely to prescribe drug treatments for ET/PV. Interestingly, UK patients desired to be more involved in treatment decisions, and this was reflected in the physician's perspective to involve their patient in treatment decisions more. UK patients were also more likely to disagree with their physician on primary treat-

ment recommendations. However, this had no impact on satisfaction suggesting that UK patients welcome an open discussion on treatment options with their physician. These data highlight the importance of maximizing patient physician communication in order to improve patient satisfaction with treatment in the UK.

PB2046

ANALYSIS OF EMERGING MOLECULAR SIGNATURES AND ASSOCIATED CLINICAL FEATURES IN MPN

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Background: Myeloproliferative neoplasms (MPNs) are a group of clonal hematological disorders that arise from transformation of a multipotent hematopoietic stem cell which includes polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF).Driver mutation's confer growth advantage on the cancer cell and most likely is selected in the tissue microenvironment within which the neoplastic cells arise. Three-quarters of these patients carry the unique JAK2V617F mutation, JAK2 exon 12 mutations are found in 5% of patients with PV, MPL exon 10 mutations are present in about 5% ET/PMF and CALR mutations are found in 50-70% patients with ET/PMF.
Aims: In this study we investigated the prevalence of these so called driver mutations in patients with MPN's from January 2007 – January 2017 reported in our center.
Methods: We analyzed 3000 samples with suspected MPN for JAK2V617F mutation by ARMS-PCR and their allele burdens were reported by RQ-PCR. We have screened a cohort of 500 patients for JAK2/MPL/CALR mutations by a sequential molecular analysis which includes PCR, RT-PCR and fragment analysis.
Results: JAK2V617F mutation is present in 50% of patients with MPN. Among 600 cases submitted for sequential molecular analysis identified 372 cases with JAK2V617F mutation, 70 cases with CALR mutation, and 6 cases with MPL mutations. Allele burden study on JAK2V617F positive patients revealed that patients with ET has the lowest allele burden, those with PV an intermediate one and those with PMF showed the highest burden. Measurement of JAK2V617F allele burden by RQ-PCR for a PMF case after allogeneic transplant (ASCT) reported that allele burden of 2.9% after 20 days of transplant and a negative result after 60 days of transplant *vs* 13% before ASCT.CALR mutation is found in ET and PMF cases that are mutually exclusive with JAK2V617F and MPL exon 10 mutations in ET whereas 2 cases with PMF found to be positive for JAK2V617F and CALR mutations. We found 40 cases with a 52-bp deletion, 4 cases with a 14bp deletion and 26 cases with a 5bp insertion. CALR variants reported in our cohort were 54% type 1 and 46% type 2 mutations. We found a tendency towards older age among type 2 carriers compared to type 1 carriers (median age at diagnosis: 57 years *versus* 52 years) or compared to non-type 2 carriers (median age at diagnosis: 57 years *versus* 53 years). Similarly, platelet count at diagnosis tended to be higher in the subgroup of type 2 mutation carriers than in patients with the type 1 mutation while hemoglobin levels and white blood cell count were lower compared to those with non-type 2 mutation. The mutual allele burden of JAK2V617F /CALR exon indel mutations of two PMF patients found as 10%/65% and 15%/55% respectively. In our cohort, 10% of the patents with CALR mutation had anemia, 21% had splenomegaly, and 43% had megakaryocytes at time of diagnosis. Compared with JAK2 V617F-positive ET and PMF, CALR-mutant ET and PMF are clinically correlated with lower WBC, leukocyte and hemoglobin counts, higher platelet counts, and a reduced risk of thrombosis.
Summary/Conclusions: Analysis of JAK2/MPL/CALR genes as molecular marker's for MPN's, allows the diagnosis of 95% of patients with MPN.As a novel mutation, CALR testing also has a prognostic significance and it was not mutually exclusive with JAK2V617F mutation. Measurement of *JAK2* V617F allele burden early after transplantation is an important predictive parameter in monitoring patients following this treatment.The knowledge of driver mutations can provide valuable information for diagnosis and prognosis, which ultimately can be highly useful for clinical decision making for the management of patients with MPN.

PB2047

IMPACT OF THE TYPE OF CALR MUTATIONS ON THE CLINICAL AND LABORATORY FEATURES OF ESSENTIAL THROMBOCYTHEMIA AND PRIMARY MYELOFIBROSIS

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Background: In 2013, in the majority of JAK2V617F negative patients with essential thrombocytosis (ET) and primary myelofibrosis (PMF) have been identified mutations in the 9 exon of CALR gene. Described more than 30 different mutations, subdivided into two subtypes: deletions (type I) and insertions (type II). There are data on the phenotypic effects, depending on the version of CALR mutations. However, the prognostic significance of mutations CALR is still insufficiently clear.
Aims: To assess the impact of the type I and type II mutations of CALR on the clinical and laboratory features of ET and PM.
Methods: A multicenter retrospective study was carried out. Samples of peripheral venous blood was obtained from 149 patients with ET (n=76) and PMF (n=73). Patients that were negative for JAK2V617F and MPL515L/K mutations were studied for CALR mutations presence as described in original paper (T.Klampf, 2013). CALR Mutations were detected in 34 patients with ET (10 - men, 24 - women) and 25 patients with PMF (13 - men, 12 - women). Statistical data processing was carried out in the program STATISTICA for Windows 6.0.
Results: The frequency of mutations CALR was comparable in patients with ET and PMF (44.7% and 35.6%). Mutations of type II is 2 times more common in ET than with the TFM: 17.1% *vs* 9.6% (p=0.178). Mutations of type I detected in 21 cases in ET, in 18 cases - in PMF, type II in 13 cases - in ET and 7 - in PMF. The median of follow-up period of patients with ET with type I mutation was 36 months (3-87), with type II - 22 months (2-90). In PMF, the median of follow-up in the group with type I mutation was 46 months (3- 133), type II - 77 months (4-115). Hematological parameters in patients with ET showed higher levels of WBC in patients with type I mutation (p=0.043), the level of Hb in this variant was lower (p=0.009). In PMF levels of Hb were similar in the studied groups. Type of mutations had no significant effect on the number of WBC in patients with PMF. However, PLT was higher in PMF patients with type II mutations of CALR (p=0.014). Spleen size in ET patients on the time of the diagnosis date was slightly different: in type I - 106,5mm, type II - 119,6mm (p=0.076). The type of mutation in our study had no effect on the stratification according to the IPSET. Also there were no significant differences in assessing of the effect of therapy. Spleen size on the time of the diagnosis date in PMF patients with type I mutation were slightly larger (180,9mm *vs* 169,9mm). Revealed more pronounced fibrotic changes of the bone marrow (BM) in patients with type I CALR mutations (p <0,005). CALR mutation type had no influence on the distribution of patients with PMF, depending on the risk groups on the scale of IPSS and DIPSS.
Summary/Conclusions: The effect of the type of CALR mutation on the clinical and laboratory features of the ET and PMF has found. Type of CALR mutations in our study had no effect on the number of PLT in ET, but have a value for this index in PMF. Type I mutations in ET accompanied higher WBC level and a lower level of Hb. The published studies have not shown the influence of the type of mutation in the Hb level and the number of WBC in ET. An important observation was the detection of the effect of type I mutations on development fibrotic changes of BM in PMF. Our data are consistent with previously published studies that showed no effect on the stratification of patients according to the scale on the IPSS.

PB2048

Abstract withdrawn.

PB2049

THE UNIQUE CASE OF GERMLINE CEBPA MUTATION IN PATIENT WITH FIP1L1/PDGFRA ASSOCIATED MYELOID/LYMPHOID NEOPLASM WITH EOSINOPHILIA

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Background: Myeloid/lymphoid neoplasms with eosinophilia (MLNe) associated with PDGFRA rearrangement are rare disorders. The most frequent PDGFRA abnormalities is FIP1L1/PDGFRA (F/P) fusion gene results from a cryptic interstitial deletion at 4q12 with constitutive activation of tyrosine kinase (TK) activity. Although known since 2003, many questions remain in understanding the biology, disease course and response to therapy. The F/P fusion gene may clinically present as chronic eosinophilic leukemia (CEL), T-cell lymphoblastic lymphoma (T-LBL) or both concurrently. Acute myeloid leukaemia (AML) may also occur at presentation or during the course of the disease. While F/P is the driver mutation, to date there are few data about genetic variants of the disease that may contribute to clinical outcome. CCAAT/enhancer binding protein alpha (CEBPA) gene functions as key regulator of granulocytic differentiation. CEBPA mutations contribute to leukemogenesis by promoting proliferation and blocking differentiation of myeloid lineage in AML. Germline CEBPA mutations is a very rare and account about 1% in AML only.
Aims: We present the first case of detection of familial germinal CEBPA muta-

tion in a patient with F/P MLNe who received related allogeneic transplantation from brother.
Methods: A 26 year-old male patient was presented with a 4-week history of fever, fatigue, difficulty in swallowing. Physical examination revealed generalized lymphadenopathy, splenomegaly, tonsils enlargement, leukocytosis ($20x10^9$/L), with marked eosinophilia ($4,0x10^9$/L). A bone marrow aspirate showed 2% blasts, 21% eosinophils. Histological examination of an cubital lymph node biopsy showed diffuse proliferation of medium-sized lymphoblasts. Immunohistochemistry and flow cytometry showed that the lymphoblastic population expressed CD2, CD5, CD7, CD4, CD99, TdT and CD1a. Polymerase chain reaction (PCR) analysis from samples of the lymph node and bone marrow failed to detect clonal T-cell receptor rearrangement. A diagnosis of T-cell lymphoblastic lymphoma (T-LBL) associated with reactive eosinophilia was rendered. The patient began standard multiagent chemotherapy in accordance with ALL-2009 protocol (ClinicalTrials.gov Identifier: NCTO1193933) and achieved complete clinical remission. As he was planned to conduct autologous hematopoietic stem cell transplantation (HSCT), blood hematopoietic stem cells have been successfully harvested after stimulation of hematopoiesis. However, within 10 days after the discontinuation of G-CSF he developed leukocytosis ($130x10^9$/L) with 21% of eosinophils (absolute number $27,3x10^9$/L) and cubital lymphadenopathy. Histological examination of lymph node showed T-LBL relapse. Bone marrow biopsy revealed the expansion of predominantly eosinophilic cells. The study was carried out to exclude second myeloproliferative disease. Molecular and cytogenetic examinations of bone marrow failed to reveal BCR-ABL, FLT3 and NPM1, but showed CEBPA (TAD2) mutation. FISH probe revealed deletion 4q12 (F/P rearrangement), confirmed by RT-PCR. The same changes were also found in the lymph node cells. So, in accordance with 2008 WHO classification, he was diagnosed as «PDGFRA-associated MLNe». The patient was subsequently treated with imatinib mesylate at the dose 100mg daily and showed a good clinical response. After 4 months minimal residual disease still persisted in bone marrow (RT-PCR positive for F/P and PCR for CEBPA mutation) and he received an allogeneic HSCT from his brother. Routine testing of chimerism at 2 months after HSCT revealed the recipient DNA less than 5% and positive probe for F/P and CEBPA. We hypothesized the germinal origin of CEBPA mutation.
Results: The same N-terminal (TAD2) CEBPA mutation was found in the patient`s skin, lymph node and bone marrow, and in the patient's brother bone marrow samples. Unfortunately, no materials from parents was available for analysis at that time.
Summary/Conclusions: Germline CEBPA mutations is very rare event and have been identified as causative gene mutations in familial AML. For the first time to our knowledge this mutation was detected in patient with PDGFRA-associated MLNe. This observation is of particular interest because it will provide novel insight about the genetic basis and the additional events responsible for the course of the disease.

PB2050

DEVELOPMENT AND DESIGN OF A RANDOMIZED CONTROLLED TRIAL USING ONLINE YOGA FOR SYMPTOM MANAGEMENT IN MYELOPROLIFERATIVE NEOPLASM PATIENTS

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Background: Patients with myeloproliferative neoplasms (MPNs) suffer from symptom burdens (*i.e.* fatigue, weight loss, night sweats, insomnia, sexual dysfunction, and pruritus) often not alleviated by JAK inhibition. We previously demonstrated feasibility of an online, home-based yoga 12 week intervention, in MPN patients on stable medical therapy. Specifically, online yoga was feasible in MPN patients (N=38) (*i.e.*, 68% satisfied with study, 75% felt it was helpful for symptom management and weekly yoga participation averaged ~51 min/week) and effective to alleviate residual MPN symptom burden (effect size [ES]=-0.36, p=0.004), anxiety (ES=-0.67, p=0.002), depression (ES=-0.41, p=0.049), sleep (ES=-0.58, p<0.001), and fatigue (ES=-0.33, p=0.04).
Aims: To conduct a randomized controlled pilot study further investigating the preliminary effects of online streamed yoga to improve MPN patient symptom burden compared to a wait-list control and to determine the feasibility (*i.e.*, implementation, practicality) of collecting biomarkers that are potentially related to MPN symptoms and disease (*i.e.* inflammatory cytokines and cortisol) in a national study. The study is underway with early feasibility data reported herein; efficacy results to be reported at conference upon study completion.
Methods: MPN patients (on stable medications including JAK inhibition) were recruited (via online MPN organizations) and randomized to a yoga group (intervention - 12 weeks, 60min/week at Udaya.com) or wait-list control (usual care). The yoga "intervention" was developed with MPN-specific concerns in mind (*i.e.*, enlarged spleen/liver, older adult population) and based on feedback received from our feasibility study. Weeks 1-2 were introductory (5-6, 5-10 min videos), with weeks 3-12 progressing in video duration and intensity (2-3, 20-30 min videos). All participants completed weekly activity logs, symptom burden questionnaires (MPN-SAF TSS and NIH PROMIS) at weeks 0,7,12, and 16 (follow-up), and wore a Fitbit Flex (objective activity and sleep monitor) through week 12. The yoga group only was also asked to receive a blood draw (*i.e.*, CBC, TNF-a, IL-6) and to provide saliva samples (*i.e.*, salivary cortisol) at baseline and will be expected to do so at post-intervention. Control group participants are asked to maintain usual levels of activity and will have access to the same videos as the yoga group after follow-up (week 16).
Results: A total of 260 MPN patients completed the eligibility questionnaire, of which 96 were eligible. Of those, the first 62 eligible were enrolled into the study (completed an informed consent). Thirty-four participants were randomized to the intervention group and 28 to the control group. Of those enrolled (n=62), 31% were diagnosed with PV (n=19), 37% with ET (n=23), and 32% with MF. The mean age of participants is 57.8 years (±10.3), 87% female (n=54), 95% Caucasian (n=59), and the mean BMI is 26.8 kg/m^2. A total of 85% (n=29/34) of yoga group participants completed the baseline blood draw and 83% (n=15/18 eligible for saliva samples) completed the baseline saliva sample. Of those that completed their blood draw (distance travelled to receive blood draw ranged from 1.2 miles – 91.6 miles, avg. 16.3 miles). To date, seven participants have dropped out of the study due to: 1) study too time consuming/laborious (n=1), 2) Fitbit device being too difficult to use/wear (n=1), 3) non-study personal issue (n=2), or 4) never responding after completing the informed consent (n=2). Outcomes will be complete by May 8, 2017 with results to be presented at EHA.
Summary/Conclusions: Data presented here will inform next steps for a RCT investigating the effectiveness of online yoga for symptom management in MPN patients.

PB2051

COMPARISONS OF SYMPTOM BURDEN IN MYELOPROLIFERATIVE NEOPLASM PATIENTS IN THE UK *VS* REST OF WORLD: ANALYSIS FROM THE INTERNATIONAL LANDMARK SURVEY

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Background: Patients with myeloproliferative neoplasms (MPNs), including myelofibrosis (MF), polycythemia vera (PV), and essential thrombocythemia (ET), experience a substantial disease burden. The international MPN LANDMARK survey evaluated patient-reported impact of MPNs across 6 countries.
Aims: To analyze differences in disease and symptom burden of MPN patients between the UK and the Rest of Surveyed World (ROSW).
Methods: A cross-sectional survey was conducted in Australia, Canada, Germany, Italy, Japan, and the UK. The internet-based survey was administered separately to MPN patients and treating physicians (the two samples were not linked). Observed differences between the UK and ROSW are described in terms of symptom burden.
Results: A total of 699 pts (UK, n=286; ROSW, n=413) and 219 physicians (UK, n=31; ROSW, n=188) completed the survey. UK patients reported more symptoms than those in ROSW (9.02 *vs* 5.95 respectively). A higher proportion of UK patients reported experiencing symptoms compared with ROSW (*e.g.* fatigue and tiredness UK - 87% MF and PV, 86% ET; ROSW – 64% MF, 39% PV, 45% ET). This pattern was observed for 28 of the 31 symptoms recorded. A similar difference was seen when physicians were asked about frequency of patient-reported symptoms (*e.g.* fatigue and tiredness UK – 90% MF, 67% PV, 70%; ROSW – 71% MF, 55% PV, 48% ET). Patients rated symptom severity from 1 (not severe at all) to 10 (worst possible). The UK was higher than ROSW for the three most common symptoms; fatigue and tiredness (mean: UK 6.73, ROSW 6.18), difficulty sleeping (mean: UK 6.09, ROSW 5.38) and loss of concentration (mean: UK 6.01, ROSW 5.67).This difference was not observed when physicians were asked to rate symptom severity. An overall symptom burden was calculated as a function of all patient-reported symptoms. UK patients were disproportionately represented in the high symptom burden group (UK 35% *vs* ROSW 16%) and had an average overall symptom burden score of 40.1 compared with 24.1 among ROSW patients. UK patients were also more likely to have been classified with a high risk score at diagnosis (UK 22% *vs* ROSW 9%). Despite the consistently greater symptom burden experienced by UK patients, little difference was observed in patient satisfaction with their physician's communication (UK 81% satisfied *vs* ROSW 90%) and disease management (UK 87%, ROSW 90%). However, UK patients were more likely to disagree with the statement '*My doctor understands how much my condition impacts my life*' (UK 39% *vs* 22% ROSW). UK physicians had more MPN patients under their care than ROSW (mean patients under care in last 12 months; UK - 25 MF, 46 PV, 47 ET; ROSW - 15 MF, 31 PV, 20 ET) and were also more likely to agree with the statement '*There is not enough time during the appointment to discuss all of the symptoms a patient is experiencing'* (UK 74% *vs* ROSW 54%).

Summary/Conclusions: UK patients perceive a higher symptom burden than ROSW in terms of frequency and severity. While UK physicians agree with regards to frequency, they didn't perceive a greater symptom severity in their patients compared to ROSW physicians. Patient/physician disconnect was unlikely to be the cause as satisfaction was high and similar to that in ROSW. However, UK physicians not only have more patients under their care than their ROSW counterparts, they are also more likely to feel they don't have enough time to discuss all symptoms. This is likely to be impacting on the ability of patients and physicians to communicate fully on symptoms and to agree on the best disease management plan.

PB2052

MPN10 SCORE AND SURVIVAL OF MOLECULARLY ANNOTATED MYELOPROLIFERATIVE NEOPLASMS PATIENTS; A FIRST REPORT ON AN EGYPTIAN COHORT

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Background: The vast majority of myeloproliferative neoplasms (MPNs) patients are characterized by a molecular genetic background and by variable symptoms reflecting disease burden that may correlate with prognosis.
Aims: To study the impact of driver gene mutations: Janus kinase 2 (JAK2), calreticulin (CALR) and myeloproliferative leukemia virus oncogene (MPL) on disease burden and correlating mutational status with symptom severity calculated by MPN10 score, degree of bone marrow (BM) fibrosis, clinical characteristics and survival in MPNs patients.
Methods: MPN Symptom Assessment Form Total Symptom Score (MPN-SAF TSS) was assessed as mean/median of 10 items: fatigue, concentration, early satiety, inactivity, night sweats, itching, bone pains, abdominal discomfort, weight loss and fever. $JAK2^{V617F}$and exon12 mutations were performed by allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) while $CALR^{exon9}$ insertion/deletion and MPL $W515^{exon10}$ mutations were assessed by high-resolution melting (HRM).
Results: 93 MPNs patients (48 males and 45 females): 18 polycythemia vera (PV), 41 essential thrombocythemia (ET), 24 primary myelofibrosis (PMF), 10 Post-ET/PV-myelofibrosis (post-ET/PV-MF) were included. Median age at diagnosis was 55 years (17-75) and was lower in ET than PV and PMF patients; 44 (19-75) years *vs* 56 (34-70) years and 56 (20-75) years, respectively (p<0.001). JAK2 mutation was positive 53/93 (57%); 16 (90%) PV patients, 14 (34%) ET patients, 15 (62%) PMF patients, 8(80%) post-ET/PV-MF patients (p<0.001). CALR mutation was positive in 14/93 (15%); 10 (24%) ET patients, 4 (17%) PMF patients, zero (0%) Post-ET/PV-MF patients (p=0.050). MPL mutation was positive in 3/93 (3%); 2 (5%) ET patients, 1(4%) PMF patients, zero (0%) Post ET/PV-MF patients. 23/93 (25%) patients were triple negative; 15 ET, 2 PV, 4 PMF and 2 post ET-MF. Median MPN10 score was 21 (4-45) in ET *versus* 37.5 (25-56) in PV, 54 (15-80) in PMF and 59 (45-75) in Post-ET/PV-MF (p<0.001). From 39 patients with BM fibrosis, 6 (15%) were triple negative *vs* 33 (85%) mutant patients (p=0.007). Among 52 patients with splenomegaly; 7 (13.5%) patients were triple negative *vs* 45 (87%) patients with a positive mutational status (p<0.001). Median MPN10 score was 48 (5-76) in JAK2 positive patients *vs* 25 (4-80) in JAK2 negative (p<0.001) and was 22.5 (4-65) in CALR mutants *vs* 35 (5-80) in CALR negative (p<0.050). Median MPN10 score was 21 (10-48) in triple negative patients *vs* 40 (4-80) in MPNs JAK2/CALR/MPL mutants (p<0.001).After a median follow-up period of 36 months (6.6-102), progression free survival (PFS) and overall survival (OS) of the whole cohort was 85% and 95%, respectively. PFS of JAK2 positive *vs* negative patients was 62% *vs* 100% (p<0.001). PFS of CALR positive *vs* negative patients was 100% *vs* 78% (p=0.067). PFS of triple negative *vs* mutant patients was 100% *vs* 75% (p=0.004). OS of JAK2 positive *vs* negative patients was 85% *vs* 100% (p=0.011). OS of CALR positive *vs* negative patients was 100% *vs* 92% (p=0.197). OS of triple negative *vs* mutant patients was 100% *vs* 90% (p=0.015) (Figure 1).

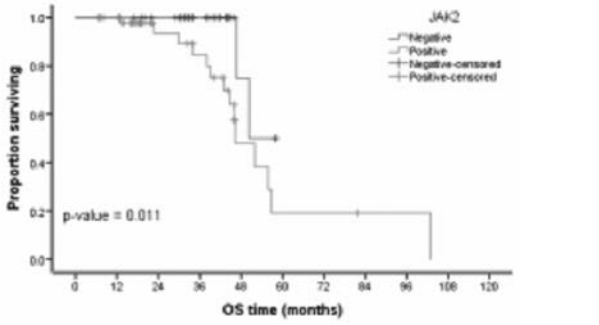

Figure 1.

Summary/Conclusions: MPN10 score is directly affected by JAK2 and CALR positivity and can be used as a major predictor of survival in MPNs patients. Triple negative ET patients in our cohort have significantly lower MPN10 score, show lower incidence of BM fibrosis and splenomegaly which may indicate a more indolent disease course.

PB2053

FINAL RESULTS FROM PEN-PV STUDY, A SINGLE-ARM PHASE 3 TRIAL ASSESSING THE EASE OF SELF-ADMINISTRATING ROPEGINTERFERON ALFA-2B USING A PRE-FILLED PEN IN POLYCYTHEMIA VERA PATIENTS

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Background: Interferon-alpha (IFNa) based therapies have been successfully used in myeloproliferative neoplasms for over thirty years. A known burden for long-term therapy applying IFNa in otherwise fit outpatients is the necessity of frequent hospital visits for product administration. Ropeginterferon alfa-2b (AOP2014) is a novel long-acting monopegylated IFNa allowing initially bi-weekly and, in long-term maintenance, monthly administration. To further improve on convenience and compliance, a pre-filled, dose-adjustable pen was developed for patient self-administration at home.
Aims: Open-label, single arm, multicenter phase III trial assessing the self-administration of AOP2014 using a pre-filled, dose-adjustable pen (NCT: 2014-001356-31).
Methods: The study was performed in 18 sites in 8 European countries. Patients were eligible who completed the AOP2014-arm in the PROUD-PV study (12 months of treatment). A total of 7 visits was scheduled within 3 months (two supervised self-administrations at site, followed by four self-administrations in the home-setting, and a final assessment visit at study site).
Results: A total of 36 patients were enrolled and received the AOP2014 pen for self-administration. The mean age was 58.5 years (range 37 to 77 years), 23/36 (63.9%) were male patients and a large proportion of patients (15/36 [41.7%]) entering the study received a baseline dose of 500 µg AOP2014. 72.2% (26/36) used 2 pens (dose >250 µg) and 27.8% of patients (10/36) used one pen (dose up to 250 µg) to administer the appropriate dose. At the first supervised visit, 80.6% (29/36) of patients had achieved full success, defined as no technical problems with the pen experienced by the patient during the injection, and no early withdrawal of the pen (before injection was complete), both observed by the investigator. At the second supervised visit the full success rate was 91.7% (33/36). The majority of observations resolved after the second supervised visit. Only 5 patients (13.9%) needed one additional supervised visit prior using the pen correctly in a home-setting. All patients had achieved full success at Visit P7 (supervised assessment visit at study end).
The patients responded favourably to the use of the pre-filled pen for the administration of AOP2014 and the accompanying instructions. Based on the Investigator's assessment, no patients exhibited any visible pain or physical discomfort, appeared to be dissatisfied when using the pen or exhibited any frustration using the pen, nor did the patient report any pain arising from the use of the pen. The majority of patients (32/36 patients) rated the instructions for the AOP2014 pen (*i.e.* scope and structure of the leaflet, clarity and comprehensibility of the text, clarity of the images and design of the leaflet), and the AOP2014 pen itself (*i.e.* setting the dose, user-friendliness, injection procedure) as "very good" or "good". The haematological parameters and spleen size remained stable throughout the study, and the rate of responders (haematological response with and without spleen size) was maintained during the entire study, suggesting that the use of the pen device did not affect drug activity. Of the 47 adverse events (AE) reported during the study, 19 were related. Most AEs were mild to moderate in intensity. One serious AE (mild atrial fibrillation, unrelated), one pen-related AE (mild nervousness reported prior first administration in the home setting), and one Grade 3 TEAE (pain in extremity, related) were recorded, but none led to a dose reduction.

Summary/Conclusions: The AOP2014 pen was well accepted and no major difficulties were reported. The study drug performed as expected and there were no safety concerns arising from the administration of AOP2014 using the pen device. The AOP2014 pen allows for individual dosing and a patient-convenient mode of self-administration of ropeginterferon alfa-2b at home and is expected to support adherence and compliance in the long-term treatment of PV patients.

PB2054

JAK2, CALR AND MPL MUTATIONS: CORRELATION WITH PHENOTIPE DISEASE AND HISTOPATHOLOGICAL FEATURES OF BONE BIOPSY

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Background: Drivers mutations JAK2, CALR and MPL are mutually exclusive in Essentials thrombocytemia (ET) and these are included in the diagnostic criteria of mieloproliferative neoplasms (MPNs). Consistent with know literature, the molecular characterisation have implications in the phenotipe disease and it might be interesting to study if these are associated with the histopathological characteristics of bone marrow biopsy
Aims: The purpose of this work is analyse the correlations between clinical-biological and histological characteristics of bone marrow biopsy and the mutational status (JAK2, CALR, MPL).
Methods: The study included 76 patients with ET diagnosed according to WHO criteria at the Haemathology Departament from Hospital de Jerez from January 2005 to December 2015. We examined the prevalence, and clinical and laboratory correlations of JAK2/CALR/MPL mutations. To evaluated the histology, one pathologist with expertise in haematopathology review the bone marrow biopsies corresponding to 44 patients with ET. We incluyed only bone marrow biopsies of at least 10 mm in length and/or minimun 8 inter-trabecular areas. The pathologist only had access to age and gender data. Mutations JAK, CALR and MPL were analysed by PCR real time and sanger sequencing.
Results: There where 55 (72%) patients JAK2, 12 (15.5%) patients CALR, one patient MPL and 9 (11.8%) patients triple-negative (TN). The main clinical and laboratory features of the patients are show in Table 1A. As can be seen, a 75% of patients belonged a high risk group, 18 (23%) patients presented thrombotic events before diagnosis and only 4 (5.3%) during the evolution. Clinical and molecular characteristics of patients as age, sex, hemoglobin level and stratification of risk were statistically significant. (Table 1A). Thromboembolics events seemed to be more frequent in patients with JAK2 mutation, although statistical significance was not achieved. The correlation between histopathological characteristics and mutational status are shown in Table 1B. We observed differences between the presence of laxes groups of megacaryocytes according with the mutational status and there were more frecuently in patients with CALR mutation (p= 0.01). With a median of follow up of 4 years (ranger 0.3-11 años) a total of 6 patients had died. Two patients evolved to overt, one of them to acute leukaemia and the other one to myelofibrosis at 66 and 44 months from ET diagnosis respectively.

Table 1.

A

CLINICAL FEATURES	PATIENTS (n=76)
MEDIAN AGE (IQR)	72 (54.25 - 79)
AGE > 60 años n (%) FEMALE n (%)	47 (62%)
HEMOGLOBIN g/dl median (IQR)	14.2 (13.1 - 15.3)
LEUKOCYTES x10⁹/µL median (IQR)	8.1 (7.6 - 11.7)
PLATELETS x10⁹/µL median (IQR)	891 (787 - 999)
LEUKOCYTES x10⁹/µL *ESSG n (%)	8 (2.25 - 14.25)
PLATELETS > 1000x10⁹/µL *ESSG n (%)	2 (2.25%)
RISK GROUP THROMBOSIS LOW / INTERMEDIATE / HIGH	4 (5.3%) / 14 (18.4%) / 58 (76.3%)
THROMBOSIS BEFORE DIAGNOSIS *ESSG	9 (11.8%)
THROMBOSIS AFTER DIAGNOSIS *ESSG	4 (5.3%)
TRANSFORMATION HEMATOLOGY NEOPLASM *ESSG	1 (1.3%)

B

HISTOLOGYCAL PARAMETERS	JAK2 (n= 51)
PERCENTAGE OF CELLULARITY NORMAL INCREASED n (%)	19/51 (37.3%)
GRANULOCYTE/ERYTHROID RATIO NORMAL/DECREASE/INCREASE n (%)	15/51 (29.4%)
MEGACARYOCYTES SERIES NORMAL/INCREASED +VERY INCREASED n (%)	13 (25.5%)
DISPOSITION MEGACARYOCYTES GROUPS	
LAXES GROUPS *ESSG n (%)	31 (60.8%)
DENSE CLUSTERS *ESSG n (%)	8 (15.7%)
BOTH GROUPS *ESSG n (%)	8 (15.7%)
PARATRABECULAR LOCATION *ESSG n (%)	8 (15.7%)
NUCLEAR ABNORMALITIES	
HYPERLOBULATED *ESSG n (%)	21 (39%)
BULBOUS *ESSG n (%)	11 (21.6%)
HYPERCHROMASIA *ESSG n (%)	8 (15.7%)
DISPLASTIC *ESSG n (%)	23 (45.1%)
FIBROSIS GRADE 0/1/2 n (%)	8 (15.7%) / 31 (60.8%) / 12 (23.5%)

TRIPLE NEGATIVE n= 9 (11.8%)	0.01
0.48
0.27
0.07
0.14
0.24
0.31
0.43
0.17
0.25
NA
1
0.52
0.37
0.83

Summary/Conclusions: In our study we can confirm that there are differences between clinical and laboratory finding according with mutational status, as shown in previus studies. The most consistent finding of this study was the presence of laxes groups of megacaryocytes significantly higher in those with CALR mutations. The major limitations of this study include a small number of patients and biopsies, available to analysed, this might be the mayor causes for the lack of the data demostrating clinical and histologycal relevance. But our results should not be underestimated because, to our knowledge, this is the second study thas has investigated this relation.

PB2055

CLINICAL IMPLICATION OF QUANTITATIVE JAK2 V617F ANALYSIS WITH DROPLET DIGITAL PCR IN MYELOPROLIFERATIVE NEOPLASMS

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Background: JAK2 V617F is the most common genetic mutation in myeloproliferative neoplasms (MPN) and included in the major diagnostic criteria. Beyond the description of existence, quantification of mutational load is proposed as a useful information to classify subgroups of MPN and to predict prognosis. Droplet digital PCR (ddPCR) is a novel assay which has an advantage in accurate and reproducible quantitative analysis.
Aims: This study was planned to verify the correlation of ddPCR with pyrosequencing in diagnosis of MPN and to investigate clinical implication of the mutation burden in disease course.
Methods: Between 2012 and 2016, peripheral blood or bone marrow samples were obtained from 56 patients at diagnosis and every 3 months after enrollment. Inclusion criteria were 1) older than 20 years, 2) who were newly diagnosed with MPN and 3) diagnosed with MPN before, not met the indication of JAK2 inhibitor treatment yet. JAK2 V617F mutation was detected by pyrosequencing as diagnostic work-up. The ddPCR was performed using the same samples with pyrosequencing to prove correlations between assays and to establish a detection sensitivity cutoff. Clinical aspects and hematologic profiles of enrolled patients were reviewed.
Results: The lowest value of measured JAK2 V617F allele by ddPCR except negative samples in our study was 0.01%, which was approximately 0.07 copies/uL of mutant allele. Some discrepancies were observed from 0.0001% to 0.01% concentration between the expected and measured values in ddPCR detection sensitivity assay, 0.1% was determined as the cutoff. Forty-two patients (75%) were positive for JAK2 V617F by pyrosequencing and 46 (82.1%) were positive by ddPCR. The mean mutated allele at diagnosis was 37.5%±30.08%. With ddPCR, the mean was 40.7%±31.2%. Pyrosequencing and ddPCR were highly correlated (r=0.9712, P<0.001). JAK2 V617F burden measured with ddPCR was significantly different by subgroups (P<0.001). In comparison of one disorder with another, polycythemia vera (PV) had more amount of mutant allele than essential thrombocytosis (ET) (P=0.001), however, differences between PV-myelofibrosis (MF) and ET-MF were not statistically significant. Follow-up samples were available in 12 patients and 8 were JAK2 V617F positive. Among them, reduction of mutant burden after treatment was observed in 6 patients (75%). JAK2 V617F burden showed initial reduction in a MF patient treated with JAK2 inhibitor, however, after dose reduction for toxicities, the JAK2 V617F mutation increment with hematologic aggravation was discovered. Mutation burden decrease showed a tendency consistent with hematologic improvement.Hematologic characteristics and JAK2 V617F load at the initial diagnosis and follow-up after treatment (Table 1, Figure 1).

Table 1.

Pt. No	Sex/Age	Subgroup	Initial JAK2 V617F allele (%)	Follow-up JAK2 V617F allele (%)	Difference	Initial CBC (WBC-Hb-Plt)	Follow-up CBC (WBC-Hb-Plt)	Treatment
1	F/56	PV	15.67	53.64	+37.97	15700-16.1-429K	4270-14.1-314K	Interferon alpha
2	F/63	PV	46.11	76.28	+30.17	10090-20.2-491K	6350-13.5-329K	Hydroxyurea, phlebotomy
3	M/72	PV	61.56	43.25	-18.31	15740-19.8-304K	7840-14.8-268K	Phlebotomy
4	F/52	PV	60.00	46.95	-13.05	18060-19.2-605K	6100-14.2-339K	Hydroxyurea
5	F/68	ET	11.35	4.94	-6.41	11620-11.5-780K	7270-12.3-540K	Hydroxyurea
6	F/49	ET	34.68	28.25	-6.33	6630-14.7-600K	5200-14.5-89K	Hydroxyurea
7	M/75	MF	21.23	16.55*	-4.68	8700-8.5-124K	7370-10.1-176K	Ruxolitinib
				25.71†	+9.16		11080-12.3-169K	
8	F/51	ET	0.17	0.00	-0.17	6920-10.8-396K	5940-9.7-329K	Hydroxyurea

* Data from the first follow-up sample.
† Data from the next follow-up sample in the same patient.

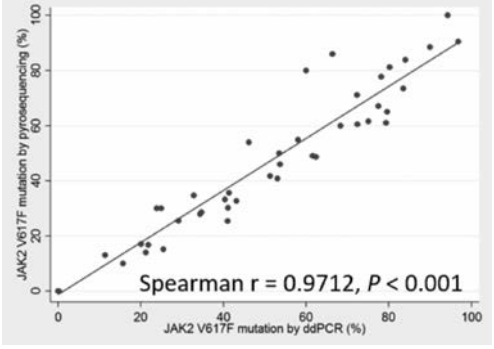

Figure 1.

Summary/Conclusions: Quantitative analysis of JAK2 mutation using ddPCR was highly correlated with pyrosequencing and might reflex clinical treatment response.

PB2056

CLINICAL IMPACT OF JAK2 AND CARLETICULIN GENE MUTATIONS ON PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

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Background: JAK2 (V617F) gene mutation is found in approximately 60% of patients with Essential Thrombocythemia (ET), while 5-10% of JAK2 (V617F) negative ET patients carry MPL gene mutations involving codon 515. Recently, mutations at the exon 9 of calreticulin (CALR) gene have been identified in approximately 50% of patients with ET, unmutated for Jak2 and MPL.
Aims: Primary aim of the current study was to analyze the prevalence of JAK2, MPL and CALR gene mutations in patients with ET; secondary aim was to evaluate the impact of gene mutations on clinical features of ET at diagnosis.
Methods: A cohort of consecutive patients with a diagnosis of ET followed between January 2013 and June 2016 were considered. JAK2 (V617F) gene mutation was detected by PCR testing; MPL and CALR mutations were analyzed by direct sequencing methods. Thrombotic risk score was calculated according to European Leukemia Net recommendations. Data were statistically analyzed.
Results: Overall**,** 148 patients were included: 107 (72, 30%) had JAK2 (V617F) gene mutation (JAK2+), 12 (8, 10%) carried a mutation at exon 9 of CALR gene (CALR+), 3 (2, 02%) carried a mutation at codon 515 of MPL gene, 26 (17.58%) patients were not mutated for JAK2, CALR and MPL genes (triple negative).CALR+ subjects, compared to JAK2+ patients, had a younger age at diagnosis: median 48 year (25-92) in CALR+ patients *vs* 72 years (18-93, respectively. Patients with MPL mutation had a median age of 82 years while triple negative subjects had a median age of 59 years (23-89). The mean score for thrombotic risk was 0 in CALR+ patients and 1 in JAK2+, MPL+ and triple negative patients. The distribution of International Prognostic Score for Essential Thrombocythemia (IPSET) categories was also statistically significantly different (p=0.003) for the three groups. The percentage of high-risk patients was 0 in CALR+ (0/12) group, 25, 60% (27/107) in JAK2+ group, and 18, 30% (5/26) in the triple negative group. The IPSETt model also stratified patients with statistically significant difference (p=0.001) among the three groups: the percentage of high-risk patients was 16, 66 (2/12) in the CALR+ group, 82, 35% (88/107) in the JAK2+ group, and 33, 33(9/29) in triple negative group. CALR+ patients belonged more frequently to the low /intermediate risk group than JAK2 +patients (80% *versus* 17, 5%, p=0.05). The incidence of thrombotic events at diagnosis of ET was 0 in the CALR+ group, 28,03% (30/107) in the JAK2+ group and 23,07% (6 /26) in the triple negative group. The median overall survival was not reached in any group.
Summary/Conclusions: CALR+ patients with ET are phenotypically distinct from JAK2+and triple negative patients. We can speculate a potential protective role of CALR mutation given the absence of thrombosis in IPSS and IPSETt high-risk patients.

PB2057

RUXOLITINIB IN MYELOFIBROSIS: A MULTICENTRE EXPERIENCE FROM THE EAST OF ENGLAND

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Background: Ruxolitinib, an oral Janus Kinase (JAK)1/JAK2 inhibitor, was approved in the EU in August 2012 for treating disease-related splenomegaly and constitutional symptoms in adults with primary myelofibrosis (PMF), post-polycythaemia vera myelofibrosis (PPV-MF), and post-essential thrombocythaemia myelofibrosis (PET-MF).
Aims: We present a retrospective multicentre analysis of MF patients treated with ruxolitinib from August 2012 to December 2016 at 3 centres in the East of England to assess its efficacy, safety, and tolerability in a 'real-world' clinical setting.
Methods: Retrospective data collection using electronic medical records and cancer registry data identified 49 MF patients treated with ruxolitinib at the James Paget, Norfolk and Norwich, and Ipswich hospitals (28, 14 and 7, respectively) over a 52-month period. Five had less than 3 months' follow-up and were excluded.
Results: The patient group was 61.4% male, with a median age of 71 years (41–91). There were 16 (36.4%) patients with PMF, 13 (29.5%) with PPV-MF, 9 (20.5%) with PET-MF, and 6 (13.6%) with post-myeloproliferative disorder (unclassified)-MF. The indication for treatment was painful splenomegaly in 20 (45.5%) patients, constitutional symptoms in 23 (52.3%), and portal hypertension in 1 (2.3%). Ruxolitinib was first-line therapy in 10 (22.7%) patients, second-line in 24 (54.5%), and third-line or greater in 10 (22.7%). Starting doses ranged from 5mg BD in 2 (4.6%), 10mg BD in 14 (31.8%), 15mg BD in 11 (25%) and 20mg BD in 17 (38.6%), with occasional dose reduction/interruption primarily due to thrombocytopaenia. Fifteen (34.1%) patients were IPSS 3+, 22 (50%) IPSS 2, 6 (13.6%) IPSS 1, and 1 (2.3%) IPSS 0. Mutation analysis was available for 32 (72.7%) patients, of which 29 (90.6%) were JAK2 V617F-mutated, 2 (6.3%) were JAK2 V617F/exon 12-unmutated, and 1 (3.1%) was CALR-mutated. The median duration of treatment was 16.4 months (3–45) and median time to progression was 15.5 months (7–32). Progression-free survival (PFS) was 65.9% and overall survival (OS) 68.2%. Seven patients died from AML, 5 from progressive MF, and 2 from pneumonia. Multivariate analysis showed that 'advancing age' and 'excess peripheral blasts (≥1%)' were predictive of poor outcomes (HR 1.08, 95% CI 1.01-1.16; p=0.024 and HR 4.38, 95% CI 1.12-17.09; p=0.033, respectively). Clinical assessment of spleen size was available for 29 (65.9%) patients and showed a reduction in splenomegaly in 16 (55.2%), an increase in 8 (27.6%) and no change in 5 (17.2%). Weight gain occurred in 32 (72.7%) and demonstrated a strong survival advantage (HR 0.21, 95% CI 0.07-0.65; p=0.006). The most common haematologic adverse events (AEs) were cytopaenias. Forty patients (90.9%) had anaemia and 22 (50%) were transfusion-dependent, compared with 29 (65.9%) and 10 (22.7%) pre-treatment, respectively. Thirteen (29.5%) patients also received an erythropoiesis-stimulating agent. Thirty-one (70.5%) patients had thrombocytopaenia (6.8% grade 4) compared with 13 (29.5%) pre-treatment. The most frequent non-haematologic AEs were minor infections, documented in 17 patients (38.6%), and included lower respiratory tract infections, candidiasis, and HSV/VZV reactivation. One patient died from Aspergillus pneumonia. Twenty-nine patients (65.9%) remain on treatment.
Summary/Conclusions: Ruxolitinib was well-tolerated and effective in improving constitutional symptoms in our 'real-world' study population. Therapeutic response and safety profile was similar to trial data although we observed a higher incidence of minor haematologic AEs that were readily managed with supportive care. Weight gain was associated with a strong survival advantage and could prove a useful clinical marker of response. The majority of patients remain on active treatment.

PB2058

MONITORING OF TRANSIENT MYELOPROLIFERATIVE DISORDER AND LEUKEMIA IN DOWN'S SYNDROME : A SINGLE UNIVERSITY HOSPITAL STUDY

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Background: Children with Down syndrome (DS) have a 10- to 20-fold increased risk of developing leukemia. But some patients don't suffer leukemia even they have significant numbers of blast cell in their peripheral blood. These condition called Transient myeloproliferative disoder(TMD), and it is a disease entity unique to DS newborns and is defined as the morphologic detection of blasts in DS less than three months of age.
Aims: This study gathered DS patients to find some difference between leukemia and TMD, to determine prognosis and risk factors.
Methods: We collect 317 patient's blood lab results in 433 DS patients. 102 patients has leukocytosis, and in 18 case found blast cells in their peripheral blood.
Results: 12 patients have found blast in three months of life, 11 of them finally diagnosed to TMD, and only 1 patient progress to Acute Myeloid Leukemia(AML) in 98 days of his life. Other 6 patients have blast in their blood after three months of life, and underwent chemotherapy due to hematologic malignancy. All patients with leukemia has anemia at diagnosis, which is not found in TMD patients*(p=0.018)*. In 7 Leukemia patients, 3 was acute Lymphoblastic Leukemia(ALL), 4 was AML. All AML patients has clonal change additional to trisomy 21 at their diagnostic point, which didn't found at TMD and ALL patients, even it didn't confirm former examination.
Summary/Conclusions: DS Patient who has blast in their peripheral blood before 3 months of life need closely follow up their Complete Blood Count and Chromosome analysis to find whether TMD progress to leukemia.

PB2059

INFECTIOUS EVENTS IN A COHORT OF PATIENTS WITH MYELOFIBROSIS UNDER TREATMENT COMPARING RUXOLITINIB WITH CONVENTIONAL THERAPY. A MONOCENTRIC EXPERIENCE OF 22 PATIENTS RETROSPECTIVELY ANALYSED

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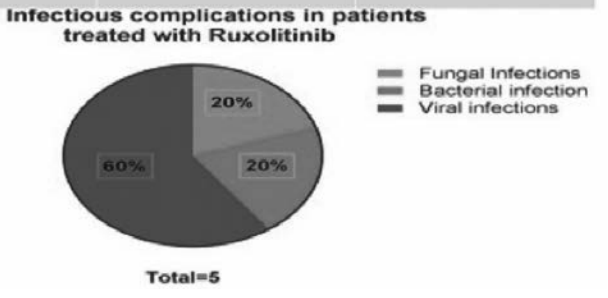
Background: Treatment with the Janus-activated kinase (JAK) 1 and 2 inhibitor ruxolitinib decreases constitutional symptoms and spleen size in myelofibrosis. However accumulating evidences suggest that the drug also exerts substantial immunosuppressive activity. The impressive clinical activity of ruxolitinib is predominatly mediated by its profound anti-inflammatory effects modulating dendritic cell (DC) function resulting in impaired CD4+ and CD8+ activity. Several studies have shown that Ruxolitinib affects different cytokines (IL1, IL6 and TNFalfa) and other immune processes and has been linked to increased incidence of opportunistic and no opportunistic infections. Herein we report our experience at our Centre.
Aims: In our retrospective study we analysed myelofibrosis patients treated with Ruxolitinib and cytoreductive treatment with Hydroxiurea and supportive therapy followed in our Department from 2012 to 2016 to evaluate rate of infections developed.
Methods: We reviewed 22 patients presenting myelofibrosis (median age 72, range 60-86)describing clinical and biological features (Table 1). Our aim was description of documented infections identified with conventional treatment and with Ruxolitinib. They were 11 treated with JAK inhibitors and 11 with Hydroxiurea taken orally, similar for age and clinical features.
Results: A total of 22 patients consecutively diagnosed were included in this analysis. There were 15 primary and 7 secondary myelofibrosis patients. According to the Dynamic International Prognostic Scoring System (DIPSS) 8 were low risk, 10 were intermediate risk and 4 were high. A total of 5 documented infections were identified throughtout the evaluation period, 4 were grade 1 and one grade 2. They are various including oral herpes simplex reactivation, pneumonia, recurrent viral flu syndromes, esophagitis fungal and urinary infections. All of them were present in the subgroup of patients undergoing therapy with Ruxolitinib (45%) after a medium time of 8 months from beginning of therapy (range 3-10). No patients received any anti-infective prophylaxis.
Median total daily dose of ruxolitinib was 10 mg (range 5-20). All of this infectious complications resolved after antimicrobial therapy and did not require hospitalization. None of patients were treated with concomitant immunosuppressive therapy. 3 of this patients presented renal impairment (median cratinine clearance of 46 ml/min).

Table 1.

CLINICAL AND BIOLOGICAL FEATURES OF PATIENTS REVIEWED

Characteristics	Group treated with Ruxolitinib	Group treated with conventional therapy
Gender M/F	8/34	29/13
Median age in years (range)	72 (60-86)	69 (51-88)
Number	11	11
Kind of disease	Primary Myelofibrosis (7), secondary Myelofibrosis (4)	Primary myelofibrosis (8), Secondary myelofibrosis (3)
JAK2V617F mutation	Positive (8), Negative (3)	Positive (6), Negative (5)
MPL mutation	None	1 (W515P)
DIPSS score	Intermediate -2 (7), High (4)	Low (8), Intermediate -1 (3)
Allelic Jak2V617F burden	45%, 65%, 99%, 38%, 88%, 79%, 82%, 65%	32%, 43%, 65%, 87%, 77%, 69%
Antiinfective prophylaxis	None	None
Cytogenetic evaluation	Deletion of chromosome 11 in one patient, rest are normal.	Deletion of chromosome 20 in one patient, rest are normal.
Renal impairment	5 patients	4 patients
Systemic symptoms	Present (11)	Present (7)
ECOG performance status 0-2	0-2 (8 patients), >2 (3)	0-2 (11 patients), >2 (0)



Summary/Conclusions: These data in our small series of patients suggest a higher incidence of ruxolitinib associated infections observed in clinical practice compared to traditional treatment. Immunosuppressive effect of Ruxolitinib is reported and the use of this drug in the transplant setting with beneficial effects on alloreactivity and on graft *versus* host disease is becoming more common. These patients might benefit from receiving prophylactic therapy with antiviral drugs or antibiotics or antifungal therapy or in alternative by careful monitoring. Finally nowadays physicians and patients should be aware of potential risks of using ruxolitinib including the risk of infections.
In summary, infections can occur in patients treated with ruxolitinib but are generally mild. Generally infections were non-life threatening and managed with appropriate supportive care.
Special care probably should be taken for patients older (more than 75 years old), treated with corticosteroid therapy and with renal impairment.
However larger studies are needed to confirm these observations.

PB2060

THE JAK2V617F MUTATION AND LEUKOCYTOSIS AS RISK FACTORS FOR INCIDENCE OF THROMBOTIC COMPLICATIONS IN PATIENTS WITH POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA

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Background: Polycythemia vera (PV) is a clonal, chronic, progressive myeloproliferative disease, caused by transformation of pluripotent hematopoietic stem cell. It is a malignant hematological disease that leads to excessive proliferation of erythroid, myeloid and megakaryocytic elements in the bone marrow. Essential thrombocythemia (ET) is a clonal disorder of unknown etiology that affects multipotent hematopoietic stem cell, and it is characterized by enhanced formation of megakaryocytes in the bone marrow and for no apparent cause, by markedly increased platelet counts in peripheral blood. PV and ET belong to a group of Philadelphia chromosome negative myeloproliferative neoplasms. Thrombotic and hemorrhagic complications are the most common cause of morbidity and mortality in patients with PV and ET. It is thought that the *mechanisms* that *lead* to *thrombosis* in *MPN* are the following: *increased blood cell mass, abnormal platelet function* and the *phenomenon* of *spontaneous aggregation*. The contribution to the incidence of thrombosis: increased level of products that are formed in the activation of platelets (thromboxane, p-selectin); increased production of microparticles that are parts of various cell membrane structures of platelet origin; JAK2V617F mutation. In patients with MPN there is increased activity of the coagulation system due to the resistance to the anticoagulant function of thrombomodulin.
Aims: The aim of this study is to monitor JAK2V617F mutations and *leukocytoses* as potential risk factors for the development of thrombotic complications in patients with polycythemia vera and essential thrombocythemia.
Methods: During the five-year period we monitored the occurrence of thrombotic complications in 56 patients (of both sexes, aged between 30 and 78 years), being diagnosed with PV and 22 patients (of both sexes, aged between 38 and 79 years) being diagnosed with ET. We used methods of clinical, laboratory, ultrasound and CT scans. With regard to the risk factors we followed the presence of JAK2V617F mutations and *leukocytoses.*
Results: Leucocyte count ranged from 5,2-27,1 x 10^9/L. The highest leucocyte count was recorded in the group of patients with PV ($p<0,01$). JAK2V617F mutation was also statistically more significantly present in patients with PV. The highest percentage of thrombotic complications (arterial and venous) was found in the group of patients with ET, which was statistically more significant relative to PV. Thrombotic complications in those groups were more frequent in percentage with patients diagnosed with leukocytosis, but statistical significance was present only in the group with PV. Thrombotic complications were in both groups more frequent in percentage with JAK2V617F positive patients, but without statistical significance. It is believed that activated neutrophils bind to platelets by influencing the increased expression of tissue factor activity, as well as the activation and damage of the endothelial cells, especially with JAK2V617F positive patients.
Summary/Conclusions: Leukocytosis and JAK2V617F may be considered as potential risk factors for the incidence of thrombosis in patients with PV and ET. Further follow-up of those patients, as well as a larger number of subjects are needed.

PB2061

RISK FACTORS FOR INCIDENCE OF HEMORRHAGIC COMPLICATIONS IN PATIENTS WITH PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

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Background: Myeloproliferative neoplasms (MPN) are the group of clonal, malignant hematopoietic stem cell disorders, characterized by the proliferation of one or more blood lines with normal or nearly normal maturing in the bone marrow and in extramedullar hematopoietic organs. Hemorrhagic syndrome is a complication that occurs in about a quarter of patients with PV and even 60% of patients with ET. Bleeding may complicate the clinical course of the IMF. It is manifested in the form of petechiae and ecchymoses, or may be life-threatening as uncontrolled esophageal bleeding. Bleeding occurs due to ineffective megakaryocytopoiesis, retention of platelets in the large spleen, qualitative

platelet disorders, acquired deficiency of factors V and vWF, disseminated intravascular coagulation.
Aims: The aim of this study is to monitor the count of erythrocytes, leukocytes and platelets, as well as hemoglobin and hematocrit values as potential risk factors for the incidence of hemorrhagic complications in patients with chronic myeloproliferative neoplasms.
Methods: During the three-year period we monitored the occurrence of hemorrhagic complications in 139 patients of both sexes, aged between 30 and 87 years, being diagnosed with Ph-myeloproliferative neoplasm. Patients were classified into the following groups: 1. Group with polycythemia vera (PV) (61); 2. Group with essential thrombocythemia (ET) (28); 3. Group with idiopathic myelofibrosis (IMF) (25); 4. Group with unclassified myeloproliferative neoplasm (MPNs) (25). The following possible risk factors were monitored: counts of erythrocytes, leukocytes and platelets, as well as hemoglobin and hematocrit values. We used methods of clinical, laboratory, endoscopy, ultrasound and CT scans.
Results: The highest percentage of hemorrhagic complications were in the group of patients with ET and IMF (p<0,01), followed by the group with MPNs (p<0,05). In all three groups, the incidence of hemorrhagic complications in patients older than 65 years of age was higher (p<0,001). The erythrocyte count ranged from 6,45-8,89 x 10^{12}/, leukocyte count 1,2-27,1 x 10^9/L and the platelet count ranged from 10,2 -1986,5 x 10^9/L. Hemoglobin values ranged from 176-210 g/L, and hematocrit from 0.58 to 0.83 L/L. The highest erythrocyte count, the highest hemoglobin and hematocrit values, as well as the highest leukocyte count was recorded in the group of patients with PV and MPNs (p <0,001) and the lowest in the group of patients with IMF (p <0,01). Among the group of patients with ET and MPNs there was no statistically significant difference in those parameters. In the group of patients with PV and MPNs hemorrhagic complications were more frequent in percentage in patients with leukocytosis and erythrocytosis, but without statistical significance. The highest platelet count was found in the group of patients with ET and MPNs (p<0,001), and the lowest in the group of patients with IMF (p<0,01). Among the group of patients with PV and MPNs there was no statistically significant difference with regard to platelet count. Hemorrhagic complications were more frequent both in patients with platelet count below 10x10^9/L (p<0,05) and in patients with platelet count over 1000x10^9/L (p<0,01). The increase in platelet count influences the adsorption of larger von Willebrand multimers on the platelet membrane, thus having an effect on their elimination from circulation and degradation.
Summary/Conclusions: The platelet count can be considered a significant parameter for monitoring the risk of hemorrhagic complications in patients with myeloproliferative neoplasms, particularly with ET and IMF. Deviation from the count of leukocytes, erythrocytes, hemoglobin and hematocrit values may be considered as a potential risk factor for bleeding in patients with myeloproliferative neoplasms, but further follow-up and a larger number of subjects are needed. The age of the patient can also be considered as a risk factor for the incidence of hemorrhagic syndrome in those patients. The follow-up of patients with unclassified myeloproliferative neoplasms has been particularly important, which showed a high prevalence of hemorrhagic complications, and with the purpose of their further differentiation.

PB2062

CLINICAL RELEVANCE OF JAK2V617F MUTATIONAL LOAD IN PATIENTS WITH PHILADELPHIA NEGATIVE MYELOPROLIFERATIVE NEOPLASMS FROM REPUBLIC OF MACEDONIA (SINGLE-CENTER EXPERIENCE)
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Background: Polycythemia vera (PV), essential thrombocytosis (ET, and primary myelofibrosis (PMF) are Philadelphia chromosome negative myeloproliferative neoplasms (MPN) characterized by the expression of an aquired activated JAK2V617F mutation. Up to date, it remains controversial how one mutation can lead to expression of three different clinical MPN phenotypes. However, several studies have shown that the JAK2V617F allele burden may correlate with specific MPN entity.
Aims: In order to further clarify these observations, we evaluated the JAK2 mutational status and its clinical implications in 233 JAK2 V617F+patients with different MPNs from the Republic of Macedonia.
Methods: We conducted a single center retrospective study which included 233 patients with JAK2V617F+MNP diagnosed according to WHO criteria, with median follow up period of 4 years. Quantification of the JAK2V617F mutation was analized with the Real Time PCR method using the Larsen protocol. Based on the mutational load patients were divided in three groups: first with <10% mutational load, second with 10-50% load and third with >50% mutational load. The correation of the allele burden with various clinical parameters was done by Mann-Whitney and student's tests using Statgraphics 4.3 software.
Results: Our study showe that median allele burden was lowest in patients with ET (22.8%), followe by PV patients (37.1%) and PMF pts (49.6%) (p<0.01). A higher mutation burden (>50% *vs* <10%) was associated with advanced age (67.5 *vs* 58,5 years and 65 *vs* 58 years in ET and PMF pts respectively), with higher leucocyte count (10^3 /µL) (9,87 *vs* 8,87, 13.8 *vs* 12.4, and 18.99 *vs* 14,8 in ET, PV and PMF pts respectively), with elevated erytrocyte count (5.76 *vs* 4.85 and 5.59 *vs* 4.52 in ET and PMF pts respectively), and with higher hemoglobin level (g/dL) and platelet count 10^3 /µL (15.45 *vs* 14.35 and 1071.5 *vs* 860.5 in ET patients respectively) (p<0.05 for all comaprisions)
Summary/Conclusions: Our study confirmed that higher allele burden is associated with advanced age, polycythemic features, and increased leucocytes and platelets counts regardless of the diagnosed MPN entity and suggests that implementation of JAK2V617F allele burden in the diagnostic workup of MPN pts could help for choosing the optimal treatment option in this group of patients.

PB2063

CSF3R T618-MUTATED CHRONIC NEUTROPHILIC LEUKEMIA: A RARE CASE SUCCESSFULLY TREATED WITH RUXOLITINIB
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Background: Chronic neutrophilic leukemia (CNL) is a rare BCR-ABL1–negative myeloproliferative neoplasm (MPN) with only 200 patients reported to date according to the WHO criteria. These cases are characterized by a high number of mature neutrophils in peripheral blood (PB), a hypercellular bone marrow due to neutrophilic granulocyte proliferation and hepatosplenomegaly. None standard of care exist for CNL; most patients are palliated with hydroxyurea, interferons, splenic radiation or splenectomy.
In the past years CNL has been often confused with chronic myeloid leukemia (CML), atypical CML (aCML) or chronic myelomonocytic leukemia (CMML), however, this diagnosis has been more defined since the oncogenic mutations in the granulocyte colony-stimulating 3 factor receptor (CSF3R) gene were identified in approximately 83% of WHO-defined CNL patients.
CSF3R T618I mutation is now considered as a highly specific molecular marker for CNL that is sensitive to *in vitro* and *in vivo* inhibition by currently approved protein kinase inhibitors.
Aims: here we report a case of a 76-years old man with diagnosis of chronic neutrophilic leukemia, according to WHO criteria, successfully treated with ruxolitinib.
Methods: On May 2015 a 76 aged male patient presented at our Institution with fatigue, night sweats, neutrophilic leukocytosis (neutrophils 42.080/mmc, immature granulocytes <5%), and symptomatic splenomegaly (277x127x200 mm). Marrow biopsy was hypercellular (100%) with myeloid hyperplasia, mild myeloid dysplasia and profound erythropoietic hypoplasia; reticulin fibrosis was minimally present. Molecular profiling demonstrated no mutations of JAK2 or CALR and polymerase chain reaction (PCR) studies for t(9; 22) and BCR-ABL fusion, was negative.
The patient was initially treated with hydroxyurea with a provisional diagnosis of prefibrotic phase of primary myelofibrosis (PMF), but symptoms worsened and the therapy was interrupted after 9 months for progressive anemia (Hb 9,9 gr/dl) and thrombocytopenia (82.000/mmc); meanwhile polymerase chain reaction (PCR) studies revealed the presence of CSF3R T618I mutation, suggesting diagnosis of CNL. By taking into account the activity of ruxolitinib in overt PMF, we decided to start this drug. The initial dose was 5 mg twice daily with a gradual increase in the dose to 20 mg twice daily when platelet count became normal.
Results: on a follow-up of 6 months after initiation of ruxolitinib therapy, symptoms resolved, hemoglobin and platelet levels improved (PLT 186.000/mmc), leukocytosis persisted (WBC 24.600/mmc), and the patient achieved a dramatic reduction in spleen size (209x119x74 mm).
Summary/Conclusions: Current data suggest that constitutively active JAK-STAT signaling plays a central role in the pathogenesis of BCR-ABL1 –negative myeloproliferative neoplasms (MPNs); our experience suggests that ruxolitinib use in CNL patients can induce partial responses by improving marrow function (normalization of hemoglobin and platelets count), splenomegaly and symptoms.

Non-Hodgkin & Hodgkin lymphoma - Biology

PB2064

PERIPHERAL BLOOD CELL STUDY FROM PATIENTS WITH FOLLICULAR LYMPHOMA AND DIFFUSE LARGE B-CELL LYMPHOMA: WHAT SHOULD WE EXPECT?

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Background: Follicular lymphoma (FL) may evolve to diffuse large B-cell lymphoma (DLBCL) and interactions between neoplastic cells and immune tumour microenvironment have been involved in this process. However, the potential value of the peripheral blood study to identify FL patients at high risk of progression is less known.
Aims: To describe the peripheral blood findings of patients with FL and DLBCL at diagnosis, and to investigate whether a particular lymphoid distribution could be associated with aggressive disease.
Methods: The study (performed between September 2012 and January 2017) included 52 patients (50% female) with a median age of 70.5 years (71% >60 years). Patients were newly diagnosed with *in situ* FL (n=1), Grade 1,2 FL (n=12), Grade 3 FL (n=11), and DLBCL not otherwise specified (n=28). *In situ* FL and Grade 1,2 FL were grouped as low-grade FL. Most patients with FL (11/13 low-grade FL and 8/11 Grade 3 FL) had clinical stages III/IV. Patients with primary or secondary immunodeficiency and those who had already received corticosteroids or chemotherapy were excluded from this study. A whole blood sample was studied at diagnosis of lymphoma and prior to the start of therapy, using multicolour flow cytometry immunophenotyping and a standard stain-lyse-wash protocol. A single monoclonal antibody panel including reagents against CD19, CD20, CD22, kappa, lambda, CD3, CD4, CD8, CD56 and CD45 was used, and a minimum of 300,000 events were acquired on the flow cytometer. Results were expressed as the absolute number/ml of monocytes, lymphocytes, T cells, CD4, CD8 and NK cells. Polyclonal and monoclonal B lymphocytes were also identified.
Results: No difference in the distribution by sex or age was found between patients with FL and DLBCL. A low cell count in at least, one lymphocyte population was detected in 35/52 patients (67.3%); 100% of cases had a low number of polyclonal B cells (<100/ml).Comparison of low-grade FL, grade 3 FL and DLBCL did not show any statistically significant difference regarding monocytes, CD4, CD8 and total T cells. Low-grade FL and DLBCL showed the highest number of differences, involving lymphocytes (2571±2439 *versus* 1495±671, p=0.001), NK cells (381±312 *versus* 204±167, p=0.03), the CD4:CD8 ratio (1.54±0.49 *versus* 2.06±1.44, p=0.002), and circulating monoclonal B cells, for both percentage (15.2±23.23 *versus* 1.94±5.23, p<0.001) and absolute number (869±1758 *versus* 18.75±46.47, p<0.001). Grade 3 FL and DLBCL also showed a different CD4:CD8 ratio (1.16±0.45 *versus* 2.06±1.44, p=0.001), with a trend toward significance regarding CD4 T cells (413±184 *versus* 685±457, p=0.077). Grade 3 FL had a lower number of polyclonal B cells as compared to DLBCL (66±41 *versus* 105±102, p=0.048). The peripheral expression of monoclonal B cells was higher in low-grade FL than in grade 3 FL, in both percentage (15.2±23.23 *versus* 4.58±8.48, p=0.008) and number (869±1758 *versus* 43.36±69.91, p=0.002) of monoclonal B cells. The number of lymphocyte subpopulations (≤1 *versus* ≥2) with low-cell counts was higher in grade 3 FL than in low-grade FL (p=0.03).
Summary/Conclusions: The peripheral lymphocyte profile in patients with FL and DLBCL is heterogeneous, but B-lymphopenia and CD4:CD8 ratio deviations are frequent findings. Regardless of clinical stage, low-grade FL had more circulating lymphoma cells and preserved lymphocyte populations than grade 3 FL. Further studies are warranted to confirm these exploratory findings and determine their clinical implications.

PB2065

POTENTIALITY OF PDPK1 AS A THERAPEUTIC TARGET MOLECULE IN MANTLE CELL LYMPHOMA

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Background: The deregulated activation of a Ser/Thr kinase 3-phosphoinositide-dependent protein kinase 1 (PDPK1) has been shown to promote the disease progression in various solid cancers. In hematologic malignancies, we have recently identified that the constitutive activation of PDPK1 and its downstream kinase RSK2 (PDPK1/RSK2 signaling axis) plays pivotal roles in multiple myeloma (MM) pathophysiology by promoting myeloma cell survival and proliferation (Chinen Y, Cancer Res 2014; Shimura Y, Mol Cancer Ther 2012). Mantle cell lymphoma (MCL) is cytogenetically and molecularly characterized by chromosomal translocation t(11;14)(q13;q32) for deregulated cyclin D1 (CCND1) overexpression, and has remained as one of hard-to-treat subtypes of non-Hodgkin lymphomas (NHLs).
Aims: The development of novel therapeutics for MCL has been urgently needed, therefore, this study investigated the potency of PDPK1 as a therapeutic target molecule in MCL cells.
Methods: Four MCL-derived cell lines (MINO, Jeko-1, JVM-2 and Z138 cells), three diffuse large B-cell lymphoma (DLBCL)-derived cell lines (KPUM-MS3, KPUM-UH1 and A3/KAW cells) and a Burkitt lymphoma (BL)-derived cell line (Namalwa) were utilized in this study. Patient-derived biopsied specimens were obtained with informed consent and subjected to the immunohistochemical (IHC) staining of phospho (p-) PDPK1^{Ser241}. Cell proliferation was assessed by a modified MTT assay. Antibodies utilized for Western blotting was performed for evaluating protein expression levels of PDPK1, p-PDPK1^{Ser241}, p-RSK2^{Ser227}, and RSK2. BX-912, a specific inhibitor for PDPK1, was purchased from Selleckchem (USA). RNA interference of PDPK1 was performed by transfecting short hairpin RNA plasmids into MCL cell lines by means of nucleofection (Lonza, Switzerland). This study was approved by the institutional review board of our institute.
Results: By means of IHC examination, our study revealed that PDPK1 was activated through phosphorylation in tumor cells of all 7 MCL patient-derived specimens examined, and this was also the case in all 5 DLBCLs examined and in all 5 follicular lymphomas examined. These indicated that PDPK1 is generally active in various types of B-cell lymphoid neoplasms. The *in vitro* treatment with BX-912 for 48 hours resulted in the dose-dependent inhibition of cell proliferation in all four MCL cell lines (IC_{50} 0.9~2.5 mM), and this inhibitory effect of BX-912 was more profound in MCL cell lines compared with three DLBCL cell lines (IC_{50} 3.7~17.0 mM) and a BL cell line (IC_{50} 2.9 mM). In addition, the flow cytometric analysis revealed that the growth inhibition of MCL cells by PDPK1 blockade with BX-912 was at least partly mediated through the induction of apoptosis. As the molecular sequelae, PDPK1 blockade by BX-912 resulted in dephosphorylation of RSK2-NTKD, while AKT activity or CCND1 expression was unaltered by BX-912 treatment in MCL cells. By gene knockdown of PDPK1 by RNA interference using three different short hairpin RNAs, we further validated that the reduction of PDPK1 protein caused the inactivation of RSK2 and the growth inhibition in MCL cell lines. Finally, when combined with various agents those are utilized for the treatment of MCL, such as doxorubicin, etoposide, fludaraibine, bortezomib, or ABT263, BX-192 showed additive/synergistic growth inhibitory effects in MCL cell lines.
Summary/Conclusions: Collectively, our study suggested that PDPK1/RSK2 signaling axis is the potential therapeutic target in MCL.

PB2066

THE ACQUISITION OF RESISTANCE TO BENDAMUSTINE HYDROCHLORIDE INDUCES MULTIDRUG RESISTANCE IN A NOVEL MANTLE CELL LYMPHOMA-DERIVED CELL LINE KPUM-YY1

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Background: Bendamustine hydrochloride (BH) has been one of the most promising genotoxic moieties for mantle cell lymphoma (MCL), however, its mechanisms of action and the mechanisms for the acquisition of resistance to BH have not been fully clarified.
Aims: We tried to identify the underlying mechanisms for BH resistance to develop the strategy to overcome BH resistance.
Methods: This study was conducted in accordance with the Declaration of Helsinki and with the approval of the Institutional Review Board. Patient's sample was obtained along with the written informed consent. We firstly established a novel MCL-derived cell line, KPUM-YY1, from circulating lymphoma cells of a 77-year-old male patient with MCL. A BH-resistant subline of KPUM-YY1 (KPUM-YY1R) was established by continuous exposure to BH with gradual escalation of its concentration from 5 μM up to 50 μM for about 8 months. Cytogenetic analysis was performed by double color-fluorescence *in situ* hybridization and spectral karyotyping (SKY). The comparative gene expression profile (GEP) and the ingenuity canonical signal pathway analyses between of KPUM-YY1 and KUPM-YY1R were performed to identify the differential gene expression pattern along with the acquisition of BH resistance. Cell viability was evaluated by a modified MTT assay.
Results: SKY analysis revealed that both primary tumor cells and KPUM-YY1 had complex karyotype including three-way translocation t(8;14;11)(q24;q32;q13), involving the rearrangement of *cyclin D1*. The growth inhibitory IC_{50} to BH was 20 μM in KPUM-YY1 cells, while the cell proliferation was not inhibited by up to 60 μM of BH in KPUM-YY1R cells. When compared with the parental KPUM-YY1 cells, KPUM-YY1R cells showed the partial cross-resistance against doxorubicin, mafosfamide, melphalan, and vincristine. By GEP analyses, total of 472 genes were differentially expressed in KPUM-YY1R compared with KPUM-YY1 cells, including 312 upregulated more than 1.5-folds and 160 downregulated less than 0.67-folds in KPUM-YY1R cells. The ingenuity canonical signal pathway analysis based on the GEP results sug-

gested that KPUM-YY1R cells harbored the distinct gene expression patterns in *MDR1,* a gene for p-glycoprotein (P-gp) of drug transporter molecule, *MGST1*, a member of glutathione S-transferase (GST) families, and argininosuccinate synthetase 1 (*ASS1*), a rate-limiting enzyme for arginine biosynthesis. The upregulation of MDR1 (P-gp) and MGST1 were confirmed by Western blot or RT-PCR analysis in KPUM-YY1R compared with KPUM-YY1. Importantly, the addition of P-gp inhibitors, such as cyclosporine A, or GST inhibitors, such as ethacrynic acid, at least partly restored the sensitivity to BH in KPUM-YY1R cells, indicating the functional significance of the upregulation of MDR1 and MGST1 in the development of BH resistance in MCL. In addition, BH-resistance cells were also found to express decreased mRNA level of ASS1 which has been reported to play tumor suppressor roles and its loss has been associated with clinical aggressiveness in various cancers.
Summary/Conclusions: This study revealed that the multiple molecular mechanisms overlappingly underlie the development of BH resistance, therefore, the acquisition of BH resistance potentially leads multidrug resistance in MCL cells. The newly developed KPUM-YY1 cells and KPUM-YY1R cells deserve the identification of multiplex mechanisms underlying BH activity/resistance and the future development of strategy which overcomes the treatment refractoriness in MCL.

PB2067

COMPARISON OF OVERALL SURVIVAL ACCORDING TO BONE MARROW ASPIRATION RESULTS IN PATIENTS WITH NON-HODGKIN'S LYMPHOMA

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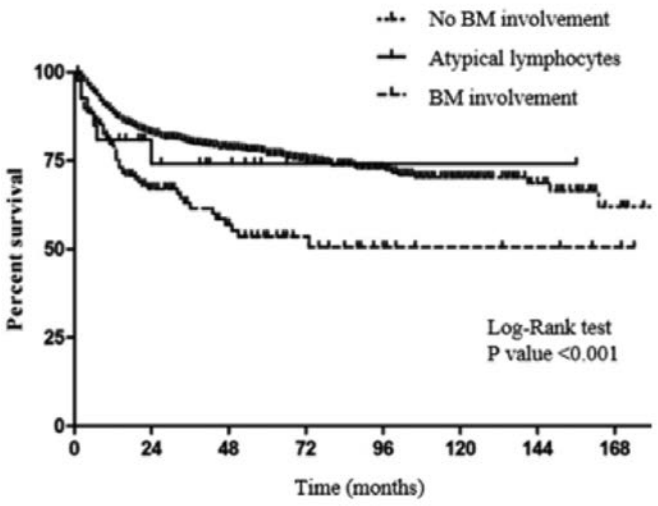
Background: Bone marrow (BM) biopsy with or without aspiration is usually included in the staging workup for patients with non-Hodgkin's lymphoma (NHL). According to the National Comprehensive Cancer Network guidelines, BM biopsy is mandatory for lymphoma, but aspiration is optional. Moreover, the role of BM aspiration is controversial. Other studies have shown that BM aspiration morphologically or flow cytometry is often inconsistent with biopsy and is less likely to detect lymphoma than biopsy. There are no clear guidelines regarding results that are positive in BM aspiration and negative in biopsy.
Aims: The aim of this study was to establish guidelines through a comparison of the overall survival (OS) of patients with NHL using morphological method.
Methods: We performed a retrospective analysis of BM involvement in patients with newly diagnosed NHL in the Korea University Hospital from January 1991 to December 2016. OS was compared according to the BM groups, which were divided into three groups: the group without BM involvement in both BM aspiration and biopsy, the group with atypical lymphocytes only in BM aspiration, and the group with BM involvement in biopsy regardless of BM aspiration results. Atypical lymphocytes were identified as positive in BM aspiration if they displayed cleaved nuclei, vacuolation, and granulation including lymphoid aggregates, leukemic presentation of mature B-cell neoplasm, and lymphoma associated hemophagocytic lymphohistiocytosis. Reactive changes, or relative lymphocytosis were excluded. OS was assessed using the Kaplan-Meier method, and the log-rank test was used for comparison between the groups. Multivariate analysis were performed using a Cox proportional hazards model.
Results: In total, the data of 1,773 patients, of which 391 patients had indolent NHL and 1,382 patients had aggressive NHL, were reviewed. Of the 1,773 patients, 1,148 (64.7%) yielded negative results on both BM aspiration and biopsy, 30 (1.7%) yielded positive results with atypical lymphocytes only in BM aspiration, and 190 (10.7%) yielded positive results on biopsy. Remaining 405 patients were excluded owing to inadequate results in BM aspiration and/or biopsy. Median follow-up duration was 37.62 months (range, 0-288).
At the time of Kaplan-Meier survival analysis, OS was significantly worse for patients with BM involvement in biopsy compared with those with no BM involvement in both BM aspiration and biopsy (2-year OS, 39.0% v 60.6%; log-rank P<0.001). However, there was no significant difference in OS between patients with atypical lymphocytes only in BM aspiration and those with no BM involvement in both BM aspiration and biopsy (2-year OS, 42.8% *vs* 60.6%; log-rank P=0.184). Patients with atypical lymphocytes only in BM aspiration also had no significant difference compared with those with BM involvement in biopsy (log-rank P=0.291; Figure 1).
Multivariate analysis was performed by adjusting survival related variables such as sex, age, lactate dehydrogenase, Ann Arbor stage, Eastern Cooperative Oncology Group performance status, number of extranodal sites, lymphoma characteristics (indolent *vs* aggressive), and transplantations. The classification according to BM involvement remained a significant prognostic factor for OS (P<0.001). However, in the subgroup analysis, the group with atypical lymphocytes only in BM aspiration showed no significant difference compared to the group without the BM involvement in both BM aspiration and biopsy (Odds ratio, 1.915; 95% confidence interval, 0.940–3.903; P=0.074). Therefore, the detection of atypical lymphocytes only in BM aspiration had no significant difference in the OS even when the relevant factors were corrected.
Summary/Conclusions: This study suggests that the detection of morphologically atypical lymphocytes only in BM aspiration, but not in biopsy, is not significant in predicting the OS of patients with NHL. Therefore, even if atypical lymphocytes are detected during BM aspiration in patients with NHL, it may not be sufficient to judge the BM involvement and predict the OS of these patients.



Kaplan–Meier curves of overall survival according to the BM groups.

Figure 1.

PB2068

***IN VIVO* IMAGING OF LUMINESCENT DIFFUSE LARGE B-CELL LYMPHOMA XENOGRAFTS COMBINED WITH MASS SPECTROMETRY IMAGING IDENTIFY SPECIFIC MOLECULAR ALTERATION DURING R-CHOP RELAPSE.**

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Background: Diffuse large B-cell lymphoma (DLBCL) is the most common B-cell non-Hodgkin's lymphoma (NHL) throughout the world, comprising 30–35% of all NHLs, with approximately 71 000 new cases and 19 000 deaths estimated for 2014. Currently, R-CHOP, a combination of immunotherapy (Rituximab, targeting the cell surface protein CD20 expressed by B cell lymphoma) and chemotherapy (Cyclophosphamide, doxorubicin, vincristine and prednisone), remains the most commonly used regimens for newly diagnosed advanced DLBCLs. However, as it is a biologically aggressive disease, up to one-third of patients will ultimately become refractory to initial therapy or relapse after treatment and display poor survival outcome, underlying the urgent need for novel therapeutic approaches based upon selective molecular targets. We are combining *in vivo* luminescent/fluorescent DLBCL xenograft models with mass spectrometry imaging (MSI) analysis to study the tumors characteristics during R-CHOP treatment and relapse. The *in vivo* imaging approach allows us to precisely quantify tumoral development and response to therapy, as well as to differentiate tumoral cells from the tumoral micro-environment. On the other hand, MSI technique provides information regarding analyte composition at an almost cellular level. Therefore, we can identify, localize the molecules, proteins, drugs or metabolites. 2 types of analysis are performed: i) comparison between primary untreated tumors and tumors relapsing from R-CHOP therapy. ii) study of the therapy resistant and sensitive areas of each tumor.
Aims: Our aim is to investigate and analyze the various chemical composition of DLBCL xenografts during tumoral development and R-CHOP treatment relapse, in order to identify yet uncharacterized targets that could become alternative targets for therapy.
Methods: 10 millions cells of a U2932 lymphoma cell line were xenografted into 60 athymic nude immuno-deficient mice . Tumoral growth was repeatedly quantified in a non-invasive manner based on tumors' luminescent signal using the *in vivo* imaging system (IVIS) Lumina II. R-CHOP treatment was applied to mice after primary tumoral growth. 2 types of samples are generated: i) untreated tumors, ii) tumors realpsing from R-CHOP
Mass spectrometry imaging is then used to analyze and compare the chemical and biological profiles of DLBCL xenografts at these stages of tumoral growth.
Results: *In vivo* imaging allows us not only to precisely assess primary tumor

development but more importantly, to monitor accurately response to R-CHOP and relapse from this therapy. The tumors at different stages of response to R-CHOP therapy are being analyzed and compared from lipidomics, metabolomics and proteomics point of view. Primary analysis indicate very distinctive metabolomics and lipidomic profiles between relapsed and non treated tumors.
Summary/Conclusions: Combining IVIS and MSI allow us for a better understanding of the disease and the treatment effects and the possible mechanisms allowing tumor cells to escape therapy. We are currently investigating in more details these different lipidomics, metabolomics or proteomics signatures between the different stages of DLBCL response to R-CHOP treatment in order to identify new candidates for alternative therapies.

PB2069

THE PROGNOSTIC ROLE OF INDOLEAMINE 2,3-DIOXYGENASE EXPRESSION IN HODGKIN'S LYMPHOMA.

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Background: Indoleamine 2,3-dioxygenase (IDO) is an inducible enzyme that catalyzes the initial and rate-limiting step in tryptophan along the kynurenine pathway. IDO is a key factor maintaining immune tolerance and expression and it correlates with poor clinical outcome in different types of cancer and hematological malignancies. It also plays a role in a lot of pathophysiological processes, such as antitumor and antimicrobial defense. IDO causes immunosuppression in the tumor microenvironment by tryptophan breakdown. Although, only several reviews have been made to evaluate IDO prognostic value and its expression value in hematological malignancies.
Aims: The aim of the study was to assess the impact of the IDO expression on clinical outcome in patients with Hodgkin's lymphoma (HL).
Methods: A total number of 35 patients with HL were included in the group (10 males and 25 females; median age: 17-60 years, range: 38.5 years). Early stages (I-II) and advanced stages (III-IV) were diagnosed in 48.5% (17/35) and 51.4% (18/35) of patients, respectively. B-symptoms had 37.1% (13/35) of patients at the time of diagnosis. Patients were treated with ABVD or BEACOPP (14/esc) and radiation therapy. The mRNA expression level of IDO was measured in pre-treatment tumor tissue specimens from HL patients using real-time qPCR analysis.
Results: For 35 patients with HL, the overall response rate after the first-line therapy was 88.6% (31/35). Progression of the disease during the therapy was observed in 11,4% of patients (4/35). Among the patients, who achieved a remission, 9 had relapses. In our study, only 20% (7/35) of HL patients were IDO-positive (IDO^+), while the majority of cases in the group (80%, 28/35) were IDO-negative (IDO^-). There were no significant differences in IDO expression between histological subtypes of HL. We also did not find any association between stage of disease and IDO expression in our study. Patients with the absence of IDO expression tended to have a better response to the 1st line chemotherapy comparing to patients with positive IDO expression. The overall response rate was achieved in 71.4% (5/7) of IDO+ cases and in 92.9% (26/28) of IDO- cases. The relapsed/refractory disease was more frequently found in HL cases with IDO^+compared IDO^-expression (28.5% (2/7) *versus* 7.1% (2/28), respectively, $p<0.05$). We did not register any death of patients in IDO- group, while one patient in IDO+ group died during the follow-up period (median duration – 37 months; range 10–65 months). ROC analysis revealed that the expression of IDO in the tumor is an important marker which is associated with clinical outcome of HL patients (Se=46.2%; Sp=95%; AUC=0.71, p=0.004).). The presence of IDO expression in pre-treated HL biopsies was associated with the reduced event-free survival (EFS) in HL patients. A 4-year EFS rate for IDO^+ HL patients was 50% compared with 73% for IDO-negative HL patients (p=0.002). The prognostic significance of IDO^+ expression in clinical outcome of HL (EFS) was also confirmed by multivariate analysis (HR=2.9; 95%CI 0.8-10.1, p=0.006).
Summary/Conclusions: On the base of the study, our findings suggest that IDO might be a promising marker for HL prognosis as well as represents an attractive target for HL immunotherapy in patients with poor outcome.

PB2070

SECONDARY CHROMOSOMAL ABNORMALITIES AND THEIR IMPACT ON TREATMENT OUTCOME IN PEDIATRIC BURKITT LEUKEMIA.

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Background: Burkitt leukemia (BL) constitutes around 13.5% of pediatric mature B-cell non Hodgkin's lymphoma. It is characterized by translocation involving the *MYC* gene to one of the immunoglobulin genes. The clinical significance of secondary chromosomal abnormalities associated with this characteristic translocation remains unknown.
Aims: We aim to analyze the impact of secondary chromosomal abnormalities on treatment outcome in pediatric Burkitt leukemia.
Methods: Patients with BL presenting to Children Cancer Hospital in Egypt-57357 (CCHE) from July 2007 till end of December 2015, were reviewed for karyotyping, *cMYC* status by FISH using break apart probes, and secondary chromosomal abnormalities. These results were correlated with survival analysis.
Results: Eighty-seven BL patients were diagnosed and treated during the study period according to the FAB/LMB 96 protocol. Majority were males (77.3%) and above 10 years of age at presentation (42%). Associated central nervous system involvement was diagnosed in 32.9% of the patients. LDH more than 2 times the upper limit was seen in 79.5%, and 52.3% of the patients suffered from tumor lysis syndrome at presentation. Informative karyotype for 66 patients demonstrated translocation of the *MYC* and *IGH* genes in 54 patients (86%) while translocation of the *IGK* and *IGL* were found in 2 (3%) and 7 (11%), respectively. Secondary chromosomal abnormalities were detected in 40 (60%) patients, with 5 or more abnormalities in 4 patients, 3 chromosomal abnormalities in 12 patients, and 2 chromosomal abnormalities in 20 patients. The most common secondary chromosomal abnormality was duplication of chromosome 1q which was found in 16 patients. Other secondary chromosomal abnormalities included structural abnormality of chromosome 14q other than MYC translocation (6 patients), chromosome 6q deletion (4 patients), chromosome 13q deletion (3 patients), marker chromosome (3 patients), loss of chromosome 17p(2 patients), isochromosome 9q (2 patients), translocation of chromosome 13, trisomy 13 and trisomy 9 in one patients each. Relapse or tumor progression on chemotherapy was seen in 16% of the whole group of patients. The 5 year OS was 57.7%, while 5 year EFS was 51.6%. When comparing incidence of relapse in relation to complex karyotype, we found that nine out of 16 (56.2%) patients having complex karyotype experienced relapse whereas relapse occurred in only 6 (12.5%) patients having non-complex karyotype (p-value= 0.005)
Summary/Conclusions: The frequency of secondary chromosomal abnormalities in our series is in concordance with other publications with duplication 1q being the most common. followed by deletion 6q, 13q, and 17p. Complex karyotype was significantly associated with higher incidence of relapse and poor outcome.

PB2071

IGVH SOMATIC MUTATION PROFILE AS PATHOGENETIC SIGNATURE IN SPLENIC MARGINAL ZONE LYMPHOMA AND SPLENIC DIFFUSE RED PULP LYMPHOMA

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Background:
Splenic lymphomas (SLs) are rare chronic lymphoproliferative neoplasms with a very indolent clinical course and a non-characteristic phenotype and karyotype. Splenic marginal zone lymphoma (SMZL), a specific type of small B-cell lymphoma and characterized by a peculiar morphology with micronodular pattern of infiltration, biphasic cytology, and the almost constant presence of marginal zone differentiation. Splenic diffuse red pulp lymphoma been introduced as a provisional entity but differential diagnosis with other SLs is needed to be clarified since the therapeutic approaches are distrinct.
Aims: The aim of our study to determine the immunoglobulin variable heavy chain (IgVH) gene usage and somatic mutation patterns in a series of SMZL and SDRP patients.
Methods: We studied 24 patients with SMZL, 40 patients with HCL and 10 patients with SDRPL. Diagnosis was based on standard WHO criteria. In all patients, the diagnosis was based on peripheral blood and BM findings. The baseline clinical and laboratory features as well as follow-up and outcome were recorded for every patient. Rearranged IgVH genes were amplified essentially in reactions that contained only one of the 5' leader region primers for the indicated 6 VH families and a 3'J primer. All PCR reactions were performed using appropriate positive and negative controls. The rearranged VH genes identified for each case seemed to represent functional rearrangements because no stop codons or crippling mutations were identified.
Results: A comparison of the VH genes to reported germline sequences in SMZL revealed that 6 cases used the VH3 family VH gene segments, 2 the VH4 family, 16 the VH1 family segments. The VH1 family genes V1-2 were used in 16 cases. In 4 out of 24 cases (16.67%), IgVH genes were in germline or near germline configuration, whereas in 20 cases (83.33%), IgVH genes were somatically mutated. We have shown no differences in clinical and laboratory characteristics, immunophenotype, outcome or overall survival were found between the mutated and unmutated cases of SMZL. A comparison of the VH genes to reported germline sequences in SDRPL revealed that five cases used the VH3 family VH gene segments and five the VH4 family, one of case with unmutaited IgVH genes.
Summary/Conclusions: Our analysis also showed the selective use of VH1 family genes in a high proportion of SMZL cases (66.67%), while VH4 and VH3 family genes were represented at a lower frequency (8.33% and 25%, respectively). The present study may revealed that SMZL and SDRPL derive from different cellular origin and may use in differential diagnosis.

PB2072

CELL OF ORIGIN ASSIGNMENT USING IMMUNOHISTOCHEMISTRY IS INFLUENCED BY BCL-2 EXPRESSION IN DLBCL PATIENTS TREATED WITH CHEMO-IMMUNOTHERAPY

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Background: Diffuse Large B-cell Lymphoma (DLBCL) is a heterogenous disease with variable clinical and pathologic presentations. Using gene expression profiling or Lymph2Cx assay, DLBCL can be assigned as germinal center (GCB) or non-germinal center (Non-GCB) subtype. However such assays remain cumbersome or unavailable for routine clinical care. Immunohistochemical (IHC) algorithms, such as the one proposed by Hans *et al.*, are easy to use tools but demonstrated variable concordance to gene expression profiling. Importantly, cell of origin (COO) assignment appears to influence overall survival (OS) but not progression free survival (PFS). Furthermore, antiapoptotic BCL-2 oncogene expression confers prognostic significance in GCB DLBCL but its significance in Non-GCB is unknown.
Aims: To examine the prognostic impact of cell of origin (COO) assignment in conjunction with BCL-2 expression in a cohort of DLBCL patients.
Methods: After due IRB approval, adult patients diagnosed with DLBCL and treated at our institution between 2010 – 2015 were identified. Clinical and pathologic variables were retrospectively abstracted. IHC expression was deemed positive if >30% of staining was observed. Cell of origin analysis was determined by the Hans criteria. All patients were treated with combinational chemotherapy containing rituximb. Patients who died prior to receiving therapy were excluded. Categorical and continuous variables were compared using Chi-squared and Wilcoxon tests, respectively. Time to end point analysis was computed using the method of Kaplan and Meier with log ranks. Relapse, progression or death was considered an event for PFS estimation. Analysis was computed using JMP software, version 11.
Results: A total of 122 patients were identified and analyzed. Median follow up of the cohort was 21.8 (1.47 – 107) months, during which OS was 73.5% and PFS was 59.9%. Stratified by IPI, 2-year OS was 85%, 76.3%, 72% and 49.5% for low, low-intermediate, high-intermediate and high risk patients, respectively (p=0.006). After stratifying patients to GCB and Non-GCB, baseline characteristics between the strata with regards to gender, age, stage, extranodal disease, lactate dehydrogenase (LDH), International Prognostic Index (IPI) and BCL-2 expression were not significantly different.
At 2-years, PFS was significantly higher for GCB *vs* Non-GCB at 72.5% *vs* 48.6%, respectively (p=0.008) but OS was similar at 77.6% *vs* 69.9% (p=0.2) (Figure 1). Interestingly, BCL-2 expression predicted OS irrespective of COO assignment. Patients with BCL-2 expression had a 2-year OS of 55.6% *vs* 56.2% for GCB and non-GCB, respectively. Whereas, patients without BCL-2 expression has a superior 2-year OS at 79.9% *vs* 78.3% for GCB and non-GCB, respectively (p=0.02).

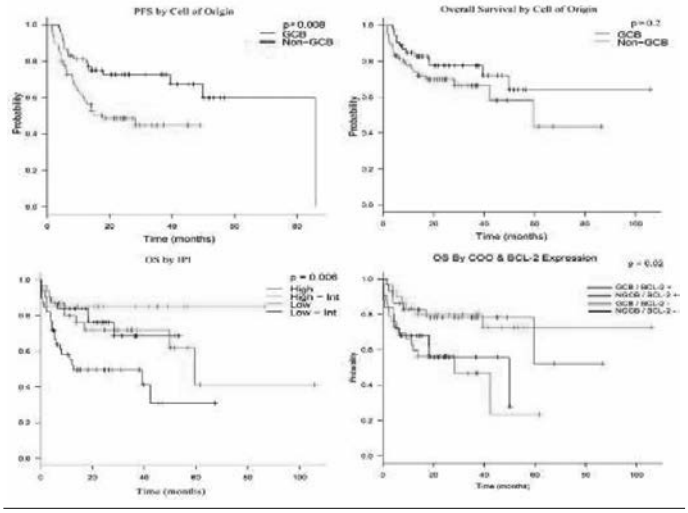

Figure 1.

Summary/Conclusions: COO assignment using IHC demonstrated superior PFS for GCB over non-GCB however this was mitigated by BCL-2 expression. This raises questions regarding the currently presumed pathogenesis of the different subtypes and how to utilize the currently available targeted therapies including BCL-2 inhibitors. These observations warrant further study.

PB2073

ARE DIFFERENCES BETWEEN PEDIATRIC EBV-ASSOCIATED LYMPHOMAS AND CARRIERS REGARDING LATENCY PROFILE AND MICROENVIRONMENT COMPOSITION INVOLVED IN LYMPHOMAGENESIS?

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Background: Epstein–Barr virus (EBV) infects more than 90% of the population worldwide. The virus has evolved to persist life-long in B-lymphocytes of infected individuals, but disruption of this tightly regulated B-cell infection could result in EBV-associated B cell lymphomas. In Argentina, primary infection is mostly subclinical and 90% of patients are seropositive by 3 years old. However, EBV presence is statistically associated with Hodgkin lymphoma (HL) and Diffuse Large B cell lymphoma (DLBCL) in patients younger than 10 years, suggesting a relationship between low age of EBV infection and B-cell lymphoma development in children from Argentina.
Aims: Given that viral latent proteins and microenvironment composition play a key role in tumor pathogenesis or control of viral infection, our aim was to compare this scenario in pediatric EBV-associated lymphomas derived from the germinal center (GC) and post-GC with the same areas in tonsils from pediatric EBV carriers, to investigate whether an alteration of microenvironment could be related to lymphomagenesis
Methods: Formalin fixed paraffin embedded (FFPE) pediatric biopsy samples from 26 DLBCL, 55 HL and 41 tonsils from EBV carriers were analyzed. Immunohistochemistry for LMP1, EBNA2, CD4, CD8, Foxp3 and GrB was performed, together with EBERs *in situ* hybridization, and positive cells were counted in the EBV+ milieu.
Results: Latency II pattern (LMP1+ EBNA2-) was predominant in HL (100%), DLBCL (55%), as well as in EBV+ CG in pediatric carriers (90%). CD4+ cell count displayed no differences between EBV+ and EBV- HL or DLBCL (p>0.05, Mann Whitney test), whereas statistically higher CD4+ cells were counted at the EBV+ GC in pediatric carriers (p=0.014, Mann Whitney test). On the other hand, CD8+ cells did not exhibit statistical differences neither in EBV-associated lymphomas nor in benign conditions at the CG, and the same was observed for Foxp3 regulatory cells (p>0.05, Mann Whitney test). In contrast, CD8+ cell count were statistically higher exclusively at EBV+ subepithelial region in tonsils, compared to EBV- counterpart (p=0.0039, Mann Whitney test). Finally, cytotoxic activity evaluated by GrB expression displayed a trend to higher mean in EBV+ DLBCL (p=0.057, Mann Whitney test) but no in HL. Concerning EBV, pediatric carriers did not shown differences in cytotoxic activity according to EBV presence at the CG (p>0.05, Mann Whitney test). In fact, GrB cytotoxic activity was prevalent only at the EBV+ subepithelial region (p= 0.0420, Mann Whitney test).
Summary/Conclusions: Latency II pattern prevails in both pediatric EBV-associated lymphomas and in EBV+ GC from carriers, indicating that LMP1expression may collaborate in the lymphomagenesis process at the GC in pediatric patients from our country. Cytotoxic activity against EBV infection may be only relevant in pediatric DLBCL, and in EBV+ subepithelial regions in pediatric carriers, whereas in EBV+ HL is not increased, in contrast to previously described. CD4+T helper cell response plays a key role at the GC region in EBV carriers, by participating directly as effectors cells, by helping to the overall immune response in the control of viral infection and restrict latency expression to type II pattern, and, ultimately, by limiting the cell outgrowth. Failure in this process may trigger malignant transformation in EBV-associated lymphomas.

PB2074

MICROARRAY EXPRESSION PROFILE OF LONG NONCODING RNAS IN GERMINAL CENTER-LIKE DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Long noncoding RNAs (lncRNAs) are constantly transcribed and involved in a variety of biological activities. The contributions of lncRNAs to the development of germinal center (GCB)-like diffuse large B-cell lymphoma (DLBCL) remain largely unknown.
Aims: The aim of this study was to investigate the expression profile of lncRNAs in human GCB DLBCL cell lines (OCI-ly1 and OCI-ly19) and normal B lymphocytes by microarray.
Methods: We used Arraystar Human LncRNA Microarray V3.0 for profiling of lncRNAs in our specimens.Sample labeling and array hybridization were performed according to the Agilent One-Color Microarray-Based Gene Expression Analysis protocol (Agilent Technology) with minor modifications. Quantitative

real-time polymerase chain reaction (qRT-PCR) was used to confirm the results of six upregulated and two downregulated lncRNAs. Bioinformatic analysis (gene ontology analysis, pathway analysis and network analysis) was performed to predict the biological functions and potential mechanisms of the differentially expressed lncRNAs in GCB DLBCL.
Results: We demonstrated that 21,539 lncRNAs were expressed in all samples analyzed, of which 1,648 lncRNAs were upregulated and 2,671 lncRNAs were downregulated in GCB DLBCL cell lines (OCI-ly1 and OCI-ly19) (≥2.0-fold, P<0.05). Pathway analysis indicated that 64 pathways corresponded to upregulated transcripts, and 62 pathways corresponded to downregulated transcripts (P<0.05). In addition, a lncRNA-mRNA co-expression network was constructed to identify potential target genes related to the 3 upregulated and 2 downregulated lncRNAs.
Summary/Conclusions: Our data suggested that lncRNAs may play an important role in the pathogenesis of GCB DLBCL, and profile of lncRNAs may be used as a potential biomarker in the diagnosis of DLBCL and predicting its clinical outcome.

PB2075

FLOW CYTOMETRY IN EVALUATION OF EXTRANODAL LYMPHOMA PRESENTING AT UNUSUAL LOCATIONS COMPARED TO NODAL LYMPHOMAS

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Background: : Immunophenotyping is a fundamental step in the diagnosis of hematolymphoid neoplasms. Lymphomas arising at extranodal sites can present significant diagnostic challenges due to their morphological diversity. In recent years flow cytometry (FCM) has proven useful in the evaluation of nodal and extranodal lymphoproliferative disorders on samples obtained by surgical specimens or fine needle aspiration cytology (FNAC). For years FNAC has been used for initial evaluation of suspected hematolymphoid neoplasms. Flow cytometry can additionally help in identifying B or T cell nature of neoplastic cells, clonality in case of B-cell neoplasms and any aberrant phenotype. The possibility of detecting CD20 status can help in initiating targeted therapy without undergoing tissue biopsy to do so. FNA cytology with Flow cytometry can serve as a replacement for open biopsy and may help in eliminating the need for more invasive procedures. In this study FCM analysis on cytological specimens, including nodal and extranodal mass from GIT, Thyroid, Kidney, Breast, Tonsil, cerebrospinal fluid and ascitic fluid, was performed.
Aims: The aim of our study was to evaluate the efficacy of flow cytometer for the evaluation of extranodal and nodal lymphomas on 40 patients.
Methods: The currant study was prospectively conducted on 40 patients with a clinical suspicion of hematolymphoid neoplasms. Samples for flowcytometric immunophenotyping (FCI) were obtained by fine needle aspiration (FNA) or by tissue scraping along with samples for cytomorphological, histological and immunohistochemical (IHC) evaluation. Samples collected in Isotone were submitted for FCI on 5-color Beckman Coulter FC-500, using a set of mature and immature antigens markers for lymphoid cells. Results of FCI on cytological specimens along with cytological findings were compared with histological and IHC diagnosis.
Results: Flowcytometric immunophenotyping conducted on extranodal sites included total 10/40 (25%) cases out of which most common site was GIT (4 cases) followed by CNS (3 cases), Kidney (1 case), Thyroid (1 case), Breast (1 case), and Tonsil (1 case). Definite diagnosis using only FCI could be obtained in 25/40 (62.5%) cases in which 6/10 (60%) cases was conducted on exranodal and 19/30 (63%) cases on nodal tissue samples. The remaining 15 cases which could not be categorized by FCI included Hodgkin lymphoma (6 cases), inadequate cellularity (5 cases), Tuberculosis (2 cases), ALCL (1 case), Mantle cell lymphmoma (1 case) and Ewing's/PNET (1 case). Combining FCI with cytological findings definite diagnosis could be found in 33/40 (82%) cases, compared to 38/40 (95%) cases by histological and IHC examination. As per World Health Organization (WHO) classification of tumors of the hematopoietic and lymphoid tissues 2008 most common lymphoma at extranodal site was DLBCL followed by acute lymphoblastic leulemia/lymphoma. Whereas at nodal site most common lymphoma was DLBCL followed by Hodgkin's lymphoma. The estimated diagnostic sensitivity of FCI alone was 60.5% with 50% specificity, similar for both extranodal and nodal tissue samples. Whereas after combing FCI with cytological findings sensitivity and specificity was found to be 78.4% and 100% respectively. Immunophenotyping of lymphoblastic leukemia/lymphoma by FCM on cytological specimens was found to be in 100% concordance with FCI on peripheral blood/bone marrow aspirates.
Summary/Conclusions: Flowcytometric immunophenotyping along with fine needle aspiration cytology offer a rapid, simple and minimally invasive procedure for the detection of hematolymphoid neoplastic cells in solid tissue especially at extranodal sites. Flow cytometry alone may not consistently provides a definite diagnosis of lymphoma subtypes but can be very helpful in diagnosing extranodal lymphoma and nodal lymphoblastic leukemia/lymphoma.
KEYWORDS: Flow cytometry, extranodal lymphoma

PB2076

POSSIBLE ROLE OF FLOW CYTOMETRY TO CHARACTERIZE INFILTRATING CD4 CELLS IN THE MICRO ENVIRONMENT OF LYMPHOMA TISSUE SAMPLES

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Background: In our previous work (Di Gaetano et al, Ann Haematol, 2014) we analyzed by flow cytometry (FC) the rich infiltrated characterizing the microenvironment of Hodgkin lymphoma (HL), mainly comprised of CD4 T lymphocytes . We confirmed that the majority of these CD4 T expressing the activation markers (CD38) but lose the CD26 and we suggested to identify the subset CD4+CD26-CD38+ to identify the non-neoplastic cellular pattern in HL. A subset connectable to regulatory T (Treg) cells, because the low expression of CD26 (DPP4) added to the presence of CD39 (NTPDase) may be responsible for the generation of adenosine, which plays a major role in Treg-mediated immunosuppression
Aims: We wanted to test if this subset may also characterize T infiltrating lymphocytes the lymph nodes of Non-Hodgkin's lymphomas (NHL) and to verify the expressions of the two enzymatic markers (CD26 and CD39) in microenvironments of HL and NHL analyzed by FC
Methods: In 2016 we analyze by FC in lymph nodes of 6 HL and in 32 NHL (12 DLBCL, 10 FL, 5 SLL, 3 MZL, 2 MCL) the CD4 T subset testing the expression of CD26, CD38, CD39.
Results: In CD4 T HL, CD39 is expressed in 44% of the subset and the increased presence (50%) of CD4+CD26-CD38+ cells is confirmed. Compared with HL, the cells of DLBCL are not statistically (t Student test) different: CD38 (64 *vs* 55; p=0,39), CD26-CD38+ (50 *vs* 46; p=0,66), CD39 (44 *vs* 59; p=0,15). While HL and FL cells are significantly different: CD38 (64 *vs* 23; p <0,05), CD26-CD38+ (50 *vs* 18; p <0,05), CD39 (44 *vs* 23; p <0,05). The other three types of NHL, few in number, show a tendency to a significant difference compared with DLBCL.
Summary/Conclusions: The our data show the phenotipic variations in the microenvironements of different types of lymphoma emphasizing of DLBCL the similarity with HL and the difference with FL and other NHL.They also suggest a link between a activated environment (CD38+) and a high CD39, which, in addition to a low CD26, could enhance the generation of adenosine and, therefore, an increased immune suppressive activity. The profile by FC of CD4 T infiltrating can characterize lymphomas in its environment indicating also signals and biological mechanisms representative of possible therapeutic target

PB2077

TREG CD4 PHENOTYPE IN THE PERIPHERAL BLOOD OF LYMPHOMAS

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Background: The T regulatory (Treg) cells down-regulate antitumor responses by several distinct mechanisms. One is the adenosinergic pathway which, through ectonucleotidases, sequentially converts ATP to AMP and generates immunosuppressive adenosine. Several studies have demonstrated in humans the overexpression of CD39 (NTPDase) and low adenosine deaminase (ADA) levels, the enzyme responsible for adenosine breakdown, and of CD26, a surface-bound ADA associated glycoprotein. In our previous work (Di Gaetano et al, Ann Haematol, 2014) we analysed by flow cytometry (FC) the T CD4 lymphocytes of solid biopsies, the surrounding neoplastic cells in Hodgkin lymphoma (HL) lymph nodes (LN) and we demonstrated the presence of an activated profile (CD38+) with a reduction of CD26 (CD4+CD26-CD38+). We also confirmed a link of this subset with an overexpression of CD39.
Aims: By using the same FC technique we wanted to explore if, as in the lymph nodes, CD4+CD26-CD38+ subset and high levels of CD39 might characterize the peripheral blood (PB) of HL at diagnosis and possibly to distinguish them from those of B Non-Hodgkin lymphomas (B-NHL).
Methods: We have analysed by FC the PB of 16 healthy controls (HC), 10 HL and 22 NHL testing within T CD4 cells the expression of CD26, CD38 and CD39.
Results: In HC CD26-CD38+ cells were 2.6% of all T CD4 and 5.5% expressed CD39. Compared with HC, the subset CD4+CD26-CD38+ of HL was statistically different (2.6 *vs* 17; p <0.05) as well as in B-NHL (2.6 *vs* 12.9; p <0.05). The expression of CD39 between HC and HL was not different (5 *vs* 9.8; p=0.1), while it was statistically significant between HC and NHL (5 *vs* 19.5; p <0.05).
Summary/Conclusions: Our results may suggest that T CD4 profile in the PB can characterize the patients with HL and B-NHL and this could be probably variable according to the type of neoplasm. The significant presence of CD4+CD26-CD38+ subset in PB of HL and B-NHL would seem to suggest that the low expression/reduction of CD26 of ADA activity may indicate the Treg-mediated immunosuppression. Interesting is the diversity of NHL showing increased CD39 expression on T CD4 lymphocytes probably connected with

the clone of B lymphocytes involved in cancer. This may support that leukemic cells may contribute to create and to characterize an immune-subversive environment and to facilitate immune escape mechanisms. FC analysis of CD26 and CD39, markers likely connected with the adenosinergic pathway, in PB can represent effective parameters to determine and characterize the Treg CD4 in different types of lymphoma and could serve as targets in the follow-up of HL and B-NHL.

PB2078

BCL-2 AND KI-67 AS INDEPENDENT PREDICTORS OF POOR-RISK IPI GROUP OF PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Diffuse large B cell lymphoma (DLBCL) is heterogeneous disease ina terms of clinical behaviour, morphology, phenotype and genetics. Gene expression profiling has made a distinction between two entities germinal center B-phenotype (GCB), activated B-center phenotype (ABC). Use of immunohistochemical algorithms for identification of these phenotypes has been translated into clinically feasible approach defining groups as GCB, non-GCB. These algorithms do not provide completely accurate prognostic information so the International Prognostic Index (IPI) which identifies poor- and good-risk patients with diffuse large B cell lymphoma (DLBCL) is still part of all current diagnostic guidelines; however, the majority of patients have an intermediate IPI, with an uncertain prognosis.
Aims: In this study, we investigated the impact of bcl-2, bcl-6, CD10, MUM1 and Ki-67 on IPI as well as impact of GCB and non-GCB subclassification according to Hans and Muris algorithm on IPI risk stratification.
Methods: We have analyzed 50 patients with DLBCL for the expression of bcl-2, bcl-6, CD10, MUM1 and Ki-67 Patients were divided into two groups, the non-GCB, GCB group or favorable group 1 and unfavorable group 2, according to Hans's algorithm and Muris's algorithm. Clinical-pathological, biochemical parameters of disease have been correlated with subgroups of DLBCL and biomarkers individually. The impact of the expression of bcl-2, bcl-6, CD10, MUM1 and Ki67 on IPI-highest score in multiple regression analysis, afterwards in regression equation and variance analyse.
Results: Group with GCB phenotype (defined by expression of bcl-2, bcl-6, CD10 and MUM1) according to Hans's and Muris's algorithm showed positive correlation with good-risk patients identified by IPI. Multiple regressional analysis proved impact of biomarkers on IPI. Following this analysis, bcl2 i Ki67 are independent predictors of poor-risk IPI group of patients, (bcl-2: p 0,0107, Ki67: p 0,0377). The value of F-ratio 2,9845 proves that there is a linear connection between models including all variables bcl-2, bcl-6, CD10,MUM1 and variable depended on the value (IPI)(p 0,0210). The mutual impact of bcl-2, bcl-6, MUM1, Ki67 is significantly related to poor-risk IPI patients.
Summary/Conclusions: Multiple regressional analysis proved impact of biomarkers on IPI. Ki67 and bcl-2 are independent predictors of poor-risk IPI group of patients. Sequential addition of bcl-2 expression, Ki67 and GCB phenotype into the IPI significantly improves risk stratification in DLBCL. These finding can be part of treatment strategies that should be considered in future trials.

PB2079

COMPARATIVE PATHOLOGIC ANALYSIS OF MEDIASTINAL B-CELL LYMPHOMAS: EXPRESSION OF P63 BEST DIFFERENTIATES PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA FROM CLASSICAL HODGKIN LYMPHOMA

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Background: Mature B-cell lymphomas of the mediastinum include primary mediastinal large B-cell Lymphoma (PMLBCL), classic Hodgkin Lymphoma (CHL), B-cell lymphoma unclassifiable, with features intermediate between diffuse large B-cell lymphoma and CHL (gray zone Lymphoma) and others. PMLBCL and mediastinal CHL, mostly nodular sclerosis (NS) share many clinicopathologic characteristics, however, therapeutic options and responses are quite different.
Aims: We aimed to find distinctive histolologic or immunohistochemical findings to better differentiate PMLBCL and CHL of the mediastinum.
Methods: A total of 32 cases of mediastinal B-cell lymphomas consisting of PMLBCL (N=16), CHL (N=13), and gray zone lymphoma (N=3) were collected from 6 university hospitals from Korea. Immunohistochemistry (IHC) for various cell lineage markers and EBV *in situ* hybridization were performed to confirm the diagnosis, and additionally, expression of P63, GATA3 and cyclinE was investigated.
Results: Most clinical features were overlapped between PMLBCL and CHL except more frequent disease progression and mortality in PMLBCL ($p<0.05$). In pathologic review, presence of epithelioid granuloma favored CHL ($p=0.078$), whereas fine reticulated fibrosis was unique for PMLBCL ($p<0.001$). By IHC, P63 was predominantly positive in PMLBCL (15/16) than CHL (2/11) with the highest diagnostic power ($p<0.001$). GATA3 was expressed in the majority of CHL (9/12) compared with PMLBCL (0/16) ($p<0.001$). Expression of cyclinE was rarely found in a minor population of PMLBCL.
Summary/Conclusions: Expression of P63 in the tumor cells, even focal, is the most helpful feature to distinguish PMLBCL from mediastinal CHL. Additional diagnostic markers include GATA3 in CHL and reticular fibrosis in PMLBCL.

PB2080

CASTLEMAN'S DISEASE: HISTOLOGICAL SUBTYPES AND MICROVESSEL DENSITY

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Background: Castleman`s disease (CD) is a rare non-clonal lymphoproliferative disorder. Most of the cases are characterized by increased vascularity in the affected tissue. The disease falls into two major histological variants: plasma cell type and hyaline vascular type. However, the correlation between microvessel density and the subtype of the disease has not been established yet.
Aims: To investigate the association between microvessel density and histological type of CD.
Methods: Twenty-eight lymph nodes from patients diagnosed with CD were used for the study. The age of the patients ranged from 24 to 65 years, 14 were male and 14 were female. Three nodes without evidence of metastasis removed for breast cancer were used as controls. The diagnosis of hyaline vascular CD was based on overall preserved immunoarchitecture with typical angio-follicular hyperplasia, circular arrangement of mantle cells around hyalinized germinal centers ("onion skin" pattern). The plasma cell type of CD was confirmed by presence of perifollicular sheets of CD138+ plasma cells. Vessels were labeled with CD34 immunostain. Slides were scanned by the whole slide digital Panoramic scanner. Percentage of blood vessel area (vessel density index) was calculated using Panoramic Viewer software, statistical analysis was conducted with Student's t-test.
Results: The plasma cell variant of CD was diagnosed in 8 patients, the hyaline vascular variant – in 20 patients. In control group vessels occupied 10±1,0% of the area. In patients with plasma cell variant percentage of blood vessel area was increased to 15.1±1.4% ($p<0,05$). Patients with hyaline vascular CD were divided into 2 groups depending on the vessel density index. In 15 patients (75%) percentage of vessel area was 6.8±2.3%, which was somewhat lower than in patients with plasma cell variant (not statistically significant). In 5 patients (25%) with hyaline vascular CD, the percentage of vessel area was higher - 12,3±1,5% ($p<0.05$) and did not differ from levels in patients with plasma cell variant.
Summary/Conclusions: The highest index of vessel density in the lymph nodes with CD was observed in plasma cell variant. In hyaline vascular variant, the index was characterized by significant variability, which could reflect the heterogeneity of this type of the disease. Increased density of blood vessels in the lymphoid tissue may be considered as a possible target for angiogenesis inhibitors, especially in patients with progressive disease.

PB2081

PROGNOSTIC SIGNIFICANCE OF IMMUNOHISTOCHEMICAL MARKERS IN R-CHOP TREATED DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS

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Background: Despite its clinical, morphological and molecular heterogeneity, diffuse large B-cell lymphoma (DLBCL) is the most frequent lymphoid malig-

nancy in adults. The role of immunophenotype variability for the therapeutic outcome has long been the cornerstone for DLBCL management strategy.
Aims: To evaluate the immunophenotypic characteristics of DLBCL and the prognostic significance of specific biomarkers such as bcl2, bcl6, CD 10 and MUM1, in a population-based cohort of patients treated with R-CHOP.
Methods: We performed a retrospective assessment of all cases of DLBCL diagnosed at our institution between 2005-2013. The immunohistochemical expression patterns of all DLBCL patients were analyzed and correlated with the therapeutic response to R-CHOP regimen.
Results: The study included 101 patients diagnosed with DLBCL, with a median age at diagnosis of 57.1 years (19-90 years) and male/female ratio of 1.3/1. Ninety-one patients were eligible for R-CHOP treatment. The median follow-up was 41 months. Out of the 90 cases analyzed by immunohistochemistry CD 10, BCL2, BCL6 and MUM1 expression was found in 17.6%, 50.5%, 72.7% and 81.8% of cases, respectively. Negative expression for CD10, as well as positive expression of BCL2 were adverse prognostic factors for 3-years overall survival (OS) and disease free survival (DFS) (OS for bcl2: 72.3 *vs* 89.7, p<0.05, OS for CD10: 84.1 *vs* 75.1, p<0.05). BCL6 and MUM1 expressions, however, did influence neither OS nor DFS.
Summary/Conclusions: This study confirms the prognostic value of a multimarker assessment which includes bcl2, bcl6, CD 10 and MUM1 expression for patients R-CHOP therapy.

Other Non-malignant hematopoietic disorders

PB2082

LYMPHOID NEOPLASMS: A REALLY IMPORTANT TRIGGER IN HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

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Background: Triggered by several conditions Hemophagocytic lymphohistiocytosis (HLH) is an unusual, aggressive and life-threatening dysfunction caused by an excessive immune activation. It has become more recognized over the past decade. HLH was first described in 1939 by Scott and Robb-Smith, next case was reported in 1952 by Farquhar and Claireaux describing two infant siblings with progressive and lethal cytopenias, hepatosplenomegaly, and fevers with autopsy showing hemophagocytosis. A lengthy and unstoppable activation of antigen-presenting cells (macrophages, histiocytes) and CD8+ T and NK cells is characteristic. This condition leads to an important hyperinflammatory situation and organ damage including splenomegaly, fever, cytopenia, hypertrigliceridemia and/or coagulapathy. Histiocyte Society (HS) criteria have been applied for diagnosing HLH, however not all of them are usually showed at the presentation. This disease can be described in two different scenarios: primary (usually in children, genetic, and known as familial form) and secondary (acquired). It can be triggered by a large variety of events that disrupt inmune homeostasis. When we talk about triggers, we can divide them in two broad categories, those that cause inmune activation and those that lead to inmune deficiency. Lymphoid neoplasms can be both.
Aims: Due to the lack of publications about HLH secondary to Lymphoid Neoplasms (LN), we would like to analyze the casuistry of our hospital and making a comparison with the current literature.
Methods: We conducted a retrospective analysis through medical files of all patients with suspected diagnosis of HLH between 1994 and 2017 in our in patient ward. Clinical features, age, diagnostic criteria proposed by the HS, etiology, treatment and evolution were analyzed. In our study 18 out of 50 patients met the requested criteria for HLH diagnosis.
Results: We report 10 LN secondary cases (4 males, 6 females). The median age at diagnosis was 60,5 years, ranged between 46 and 80 years. In all of them, but in one, who presented long-term pancytopenia, symptoms were developed very fast. The most frequent causes of consultation were cytopenia and general syndrome. In two of them HLH was diagnosed with LN relapse, in one patient during a transformation from a low-grade B-cell lymphoma to DLBCL (Diffuse large B-cell lymphoma), in 6 of them we diagnosed LN and HLH concomitantly, and in the last one coinciding with a Richter Syndrome. Four of 10 were secondary to T-cell neoplasm. All patients met 5 o more HS diagnostic criteria. In only 3 of them HLH was healed. One patient is still in remission. Nine died, 7 of them due to HLH complications. Treatment was chemotherapy (depending on their LN) in almost all of them. Fluctuations were detected among activity HLH parameters due to LN response. Detailed characteristics of patients are shown in Table 1.

Table 1.

Case	Age (Yrs)	Sex	BCC (PMN/Hb/Plt) (x10e/uL/gr/dL /x10e/uL)	LDH (U/L)	Trg (mg/dL)	Fibri (mg/dL)	Ferritin (ng/dL)	PS	Fever	Adeno-pat	Splenomegaly	Trigger	Treatment	Evolution	Cause of death HLH	Diagnostic Criteria
1	46	M	1,5/8,8/25	ND	344	124	1695	2	Yes	Yes	No	Non agressive Lymphoproliferative Sy	HLH 2004 (CSAVP16-Dexametason)	Fever stop, LDH improvement . Traumatic brain hemorrhage, death.	Yes	6
2	77	F	5,1/7,1/35	1389	177	395	nd	ND	No	Yes	Yes	High grade T-Lymphoma	Metilprednisolone high dose.	Multiorgan failure, death.	Yes	5
3	54	F	0,7/9,6/2	ND	234	ND	ND	ND	Yes	No	Yes	Peripheral T-Lymphoma	CHOP x8	Complete remission after Chemo. Healed of HLH an LN.	Yes	5
4	76	M	5,0/11,1/42	1073	282	550	ND	ND	Yes	Yes	Yes	DLBCL	Support treatment	Sectic shock, death.	Yes	5
5	80	F	6,8/7,4/13	293	739	388	1650	3	Yes	Yes	Yes	Richter Sy, P53+	Inmunoglobulin+Dexametasone. R-CHOPx6	HLH complete remission, death due to LN relapse.	No	6
6	52	M	0,7/6,6/21	503	333	114	3742	3	Yes	Yes	Yes	Relapsed T-Lymphoma	HLH 2004 without Cyclosporine a.	HLH complete remission, death due to LN relapse.	No	7
7	49	F	4,3/10,6/47	466	642	55	1282	3	Yes	No	Yes	T-Lymphoma, SLE, EBV associated Lymphoproliferative Sy	Dexametasone +Inmunoglobulin +Cyclosporine a	Respiratory and liver failure. Death.	Yes	5
8	47	M	3,3/81/35	200	245	490	1300	1	Yes	Yes	Yes	DLBCL relapse	R-ESHAP x 2	HLH complete remission, death due to LN relapse.	No	5
9	78	F	1,7/8/61	750	600	ND	16400	2	Yes	No	Yes	DLBCL relapse	HLH 2004	Inicial HLH reocvery, aggravation because febrile	Yes	6
10	67	F	4,2/8/98	643	251	235	1015	3	Yes	Yes	Yes	Non agressive Lymphoproliferative Sy to DLBCL Transformation	R-CHOP x 3	HLH complete remission, death due to infection after chemo.	No	5

Summary/Conclusions: HLH triggered by LN is diagnosed in older patients than other causes secondary HLH (46-80 *vs* 4-84 y/o in our center), we think this is because in our experience there are not children or Young adult in HLH due to LN group. We would like to highligt that although LN is a very commom HLH trigger there are a few works describing them in the literature, that is why we would like to spread our experience. We would like to emphasize in he importance of an early diagnosis. Despite being a serious disease, it is still underdiagnosed, reaching the diagnosis most of the times after seeing hemophagocytic phenomena in bone marrow biopsy. Agreeing with literature, main consulting reasons are similar to our series. Correlation between neoplastic activity and inmune activation, as well as test and facts which could predice evolution should be more studied. Finally we would like to address the necessity of considering this possibility in the face of a patient with fever which does not respond to antibiotics and has not clarified citopenia, as well as the importance of conducting cheap and very profitable test such as ferritin or tryglycerides level when symptoms or clinical features of lymphoid neoplasm are not concordant with the expected evolution.

PB2083

MARIH, A NATIONAL NETWORK FOR RARE IMMUNOHEMATOLOGICAL DISORDERS

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Background: Health networks focused on rare diseases were created following a call for proposals from the French Ministry of Health in the summer of 2013. The main objective of these networks is to facilitate and to coordinate the actions being implemented by all actors involved in treating rare diseases. Of the 23 national networks identified in 2014 in France, the network for rare immunohematological rare diseases "MaRIH" brings together national reference centres and recognized centres of expertise as well as patients' associations involved in treating those pathologies, on behalf of scientific medical societies.
Aims: Improving care, communication and training, pushing forward research development and epidemiological surveillance.
Methods: MaRIH brings together people involved in those medical pathologies: 8 national reference centres, 5 centres of expertise, more than 50 diagnosis and/or research laboratories, 9 patients' associations on behalf of 7 scientific societies.
Results: The main missions of this network are to improve the care, the research and to educate professionals, patients as well to disseminate more information to the general public on these rare diseases. *Improving care*: Thanks to its visibility (events, leaflets, website), MaRIH should help primary care doctors to more quickly diagnose and therefore provide faster and appropriate treatment based on best practice recommendations at the national level (PNDS) as well as international guidelines. The network will also be setting up new multidisciplinary meetings for specific immunological and hematological rare disorders through MaRIH centres so physicians in France or in other countries can have easily an expert opinion for their patients. At the same time, improving the child-adult transition was identified by the steering committee as a top priority. *Communication and training*: MaRIH is involved in organizing many multidisciplinary events in France to improve the visibility of the centres and to provide education on these rare diseases. The 1st annual conference of the network took place on June 25th 2015 and the third one is planned on June 1rst 2017 in Paris. Moreover, a patient's day meeting was organised on January 30th 2016 in Paris to inform on the update status of research on their disease as well as to help patients in daily common problems (sport, psychology, transfusion…). *Pushing forward research development and epidemiological surveillance*: the network has appointed a research project manager for its scientific and strategic committee to support, provide stability for and add value to research centre activities. The research project manager watch out for calls for tender, set-up of new registers and continually monitor the regulations for retrospective and prospective studies, both in France and at the international level. Furthermore, MaRIH supported successfully the application of several of its members for European reference networks (Figure 1).

Figure 1.

Summary/Conclusions: The creation of these new networks allows strengthening the links between the various actors involved in the field to improve care and answer transversal questions. In this way, MaRIH pilot concerted actions to all its members around immunohematological rare diseases by: 1- increasing the visibility of the actors on the web or during events. The MaRIH website includes all the informations of the members as well as recommendations and events (www.marih.fr). 2- communication and training. MaRIH organizes two annual events, one for patients and another one for professionals. Moreover, MaRIH sends clinical cases by email to professionals and produce an annual webcast. 3- pushing forward research development and epidemiological surveillance. Thanks to his research project manager, MaRIH facilitates the submission and the set-up of new registries or clinical studies. In the future, MaRIH will continue and futher develop all these actions, in close collaboration with the French Ministry of health.

PB2084

CLINICAL FEATURES AND ETIOLOGY OF PATIENTS WITH THROMBOTIC MICROANGIOPATHIES

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Background: Thrombotic microangiopathy (TMA) is a heterogeneous group of disease that has a fatal pattern of endothelial damage. TMA can be found in association with diverse clinical conditions such as carcinoma metastasis, malignant hypertension, infections, and TTP (thrombotic thrombocytopenic purpura). TTP is a rare, life-threatening multisystem disease, characterized by microangiopathic hemolytic anemia, thrombocytopenia, fever, renal dysfunction, and neurological disorders.
Aims: The purpose of this study is to evaluate the etiology associated with TMA.
Methods: All of the six TMA patients who were newly admitted to our clinic in two months period were enrolled in this study. Effectiveness, response, adverse effects and safety of plasmapheresis were evaluated using laboratory and clinical findings. (See Table 1).
Results: First patient presented with cachexia, thrombocytopenia, and TMA. He did not respond to plasmapheresis and corticosteroid treatment. We diagnosed carcinoma metastasis and liver metastasis, respectively, through bone marrow biopsy and PET(positron emission tomography). We thusly ascertained that TMA was due to carcinoma unknown primary. The second patient presented with general neurological findings like Guillain-Barre Syndrome and paraplegia with renal failure, thrombocytopenia, and TMA. After PLEX and corticosteroid treatment, laboratory and neurological clinical recovery were observed after one month. The third patient had chronic obstructive pulmonary disease and pneumonia in anamnesis. who presented with anemia, thrombocytopenia, fever and pneumonia findings. We conducted PLEX therapy. On the 8th day of PLEX, the patient had anaphylaxis, we performed cardio pulmonary resuscitation. The fourth patient

presented with acute renal failure with malign hypertension. We performed hemodialysis together with PLEX treatment. Because his diagnosis was acute renal failure, malign hypertension, and TMA. The fifth patient presented with epistaxis and sepsis. He had chronic TTP diagnosis from two years ago. We diagnosed the patient as relapse TTP. Early treatment against infection and PLEX increased his platelet counts as early as the second day of treatment. The sixth patient presented with a fever that had been going on for five days. We treated the patient with PLEX together with the corticosteroid. Because his ADAMTS13 level was very low and he had 35% schistocytes.

Table 1.

Features and Findings of Patients

Patient number	Etiology	Age	Gender	Hemoglobin (g/dl)	Platelet	LDH (U/L)	Total Bilirubin (mg/dL)	ADAMTS13 Activity	Shigella Toxin&Shiga toxin-producing Escherichia Coli	Schistocyte Count (%)
1(M.Y)	Carcinoma Metastasis	60	Male	5,6	17000	1063	2,65	89,92	-*	5
2(A.Ç.)	Idiopatic	62	Male	7,8	13000	355	1,21	28,35	-*	20
3(M.G.)	Viral Infection	60	Male	7,9	16000	1308	2,66	58,79	-*	10
4(C.B.)	Malign Hypertension	36	Male	7,6	88000	455	0,81	96,26	-*	4
5(A.K.)	Sepsis	56	Male	10,6	67000	282	0,82	0,2	-*	26
6(A.Y.)	Bacterial Infection	52	Male	9,2	30000	755	1,2	0,49	-*	25

*: negative test

Summary/Conclusions: We diagnosed our first patient with carcinoma unknown primary, who did not respond to PLEX and corticosteroid treatment. The results we received for that patient indicate that PLEX with corticosteroid treatment alone, remain ineffective in cancer-related TMA patients. Etiology of our second patients TMA was idiopathic. His clinical and laboratory findings improved rapidly in response to PLEX and pulse corticosteroid treatment. One viral infection induced TMA patient had anaphylactic reaction receiving his 8th PLEX. Allergic reactions should always be kept in mind when administering PLEX. One patient with TMA and malign hypertension-induced renal failure was successfully treated with PLEX, hemodialysis and antihypertensive treatment. We successfully treated our bacterial infection and sepsis-induced TTP patients with PLEX and antibiotic administration. In second TMA patient, we coupled PLEX with high dosage corticosteroid treatment even though he had an infection. For he had high schistocyte count and atypical neurological findings.ADAMTS 13 activity may only be a guide for diagnosis of TTP, but it is unreliable for a definitive one. In conclusion, diagnosis of TTP and other TMAs is difficult. Etiology, clinical features, laboratory findings should all be taken into account when diagnosing TMA. While it is established that ADAM TS13 deficiency is the major cause in acquired TTP, finding the etiology of other TMAs is determinant for a successful treatment of the latter.

PB2085

HAEMOLYSIS AS SCREENING TEST IN LYSOSOMAL STORAGE DISEASES

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Background: Lysosomal storage disorders (LSDs) are a group of rare inherited metabolic diseases, whose clinical hallmark is organomegaly among others, due to progressive accumulation of several non-catalyzed products inside the lysosomes. This storage leads to intracellular oxidative stress status triggering oxidized metabolites production as oxysterols, which are related to apoptosis and cellular eriptosis, as well as haemolysis dysregulation.
Aims: To evaluate the link between LSDs and haemolysis and if it could be used as a screening test in LSDs.
Methods: The osmotic resistance test (ORT) was evaluated in 150 samples including controls, LSDs carriers (LSDs-C) and LSDs patients (LSDs-P). Briefly, the blood was mixed with different concentrations of sodium chloride solution (NaCl) and the haemoglobin released was quantified by spectrophotometry. The raw data was normalized using isotonic solution (0.9% NaCl). The statistical analysis (non-parametric tests and ROC curves), was computed by IBM SPSS statistics v22 software and all statistical tests will be considered and taken as bilateral significance level α=0.05.
Results: The analysis shown that haemolysis at 0.48% of NaCl allow us to sort out controls *vs* LSDs-C/LSDs-P (AUC=0.726) whereas no significant differences were observed between LSDs-C and LSDs-P (p-value>0.05).
Summary/Conclusions: According to our results the ORT test is an useful screening test in LSDs.

PB2086

CLINICAL SIGNIFICANCE OF ELEVATED SERUM COBALAMIN (VITAMIN B12) LEVELS

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Background: Hypercobalaminemia is a frequent but underestimated abnormality. Elevated serum cobalamin levels may be a sign of a wide range of diseases like solid neoplasms, haematological disorders like myeloproliferative disorders, chronic myelogeneous leukemia, promyelocytic leukemia, polycythemia vera, hypereosinophilic syndrome as well as liver and kidney diseases.
Aims: We aimed to evaluate the underlying disorders of the patients with high cobalamine levels (>1000 pmol/l) between 01.02.2016- 01.02.2017 in Hacettepe University Pediatric Hematology Department.
Methods: We investigated the patient records of the patients examined between 01.02.2016- 01.02.2017 in our department and included the patients with serum cobalamine levels higher than 1000 pmol/l. We excluded the patients who are taking Vitamin B12 supplement.
Results: There were 46 patients with serum cobalamine levels higher than 1000 pmol/l out of 14367 patients seen between 01.02.2016- 01.02.2017 in our department. The reason to check the cobalamin levels were anemia, neutropenia and thrombocytopenia in most of the patients. Only 2 patients were referred to our department because of hypercobalaminemia. The underlying disorders were found to be leukemia in 3 patient (Acute lymphoblastic leukemia (ALL) n:1, acute myeloblastic leukemia (AML) n:1 , large granular lymphocytic leukemia (LGLL) n:1), myelodisplastic syndrome (MDS) in 2 patients, isolated thrombocytopenia in 4 patients, isolated neutropenia in 7 patients, bicytopenia in 4 patients and aplastic anemia in 2 patients, cobalamine metabolism defects in 10 patients, hypereosinophilia in 2 patients, polisitemia in 1 patient, cystic fibrosis in 1 patient, HIV in 1 patient, FMF (familial mediterrenian fever) in 1 patient, chronic kidney failure in 2 patients, sickle cell anemia in 1 patient, factor 7 deficiency in 1 patient, thrombosis in 1 patient and epistaxis in 1 patient.
Summary/Conclusions: An observed elevation of cobalamin merits the a full diagnostic work up to assess the presence of an early diagnostic marker of these diseases. When we look at the patients except hematological neoplasm and cytopenias, most of the underlying reasons is associated with inflamation and infection, cobalamin was found to be elevated as an acute fase reactant. A certain approach is needed whether to determine the potential indications to search for high serum cobalamin levels and to determine the practical clinical strategy when elevated cobalamin levels discovered.

PB2087

THE HEMATOLOGIC FINDINGS OF INHERITED METABOLIC DISEASE; THEY ARE MORE THAN EXPECTED

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Background: Inherited metabolic diseases are pathological conditions that generally develop as a result of impairment of the production or breakdown of protein, carbohydrate and fatty acids. Hematological problems are some of the most frequently observed findings of inherited metabolic diseases. These may be seen together with other systemic findings or sometimes as the first and only diagnostic finding of disease. Early determination of hematological findings has a positive effect on the prognosis of metabolic diseases.
Aims: The aim of this study is to evaluate the incidence of hematological findings in inherited metabolic diseases since there are a few studies about the true incidence in literature.
Methods: Three hundred eighteen patients who were being followed-up within the previous 6 months at Gazi University Department of Pediatric Nutrition and Metabolism, Turkey, were included in the study. Patients' hematological findings were taken from Department of Pediatric Nutrition and Metabolism and hospital data-processing records. Since patients were in different age groups, hematological findings were compared with normal values for each patient's age group. The hematological findings were classified under seven main groups; anemia of chronic disease, iron deficiency anemia, vitamin B12 deficiency anemia, hemophagocytosis, leukocytosis and thrombocytosis. Metabolic diseases were classified according to the textbook of Inborn Metabolic Diseases: Diagnosis and Treatment.
Results: Nine hundred twenty-two hematological examinations of the 318 patients were included to the study, and 282 hematological findings were determined, 127 anemia of chronic disease, 80 iron deficiency anemia, 56 cytopenia and four vitamin B12 deficiency anemia. Leukocytosis (n=1), thrombocytosis (n=5) and hemophagocytosis (n=9) were also observed.
Summary/Conclusions: It was determined that although anemia of chronic disease and nutritional anemia are the most common hematological findings, these may be diagnosed late, while neutropenia, thrombocytopenia, pancytopenia and hemostasis disorders may be diagnosed earlier. Metabolic diseases must be considered in the evaluation of cytopenias, particularly in cases with an atypical cause that are resistant to treatment and have additional accompanying findings. Our study is the most comprehensive one in the liter-

ature, and we think it would positively contribute to the monitoring and prognosis of congenital metabolic diseases.

PB2088

HEMATOTOXIC EFFECTS OF GENERIC TRIAZOLE FUNGICIDES TEBUCONAZOLE ON WISTAR HANNOVER RATS

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Background: Pesticides are extensively used in agriculture today. Fungicides based on derivatives of triazole are the most widespread all over the world. Tebuconazole (TB) is one of the most frequently used substance of this group. Literature review confirms that triazole fungicides have the ability to cause different hematotoxic effects.
Aims: Since 2007-2016 years we have investigated 10 test-substances of generic tebuconazoles (purity up to 97%) from different manufacturers with purpose to assess their hematotoxic action on males Wistar Han rats peripheral blood in the subchronic 90-days oral toxicity study (according to SOP and OECD 408 reccomendations in compliance with GLP).
Methods: The Wistar Han males were randomly allotted to four groups. The input controls of peripheral blood parameters were conducted after a period of animals acclimatization. The goal was to evaluate the physiological state of the Wistar Han rats and the blood picture before treatment. Doses 0; 10; 50; 200 mg/kg/bw/day were defined and were the same in all studies. Blood samples for hematological measurements were examined at 4, 9, 13 weeks after TB exposure in the same groups of animals throughout the experiment. Hemoglobin (HGB) concentration, hematocrit (HCT), total amount of erytrocytes (RBC), leukocytes (WBC) and platelets (PLT), mean corpuscular hemoglobin (MCH) were evaluated.
Results: As a result, all generic TBs on high toxic doses level (200 mg/kg/bw/day) had shown the tendencies for quantitative hematological changes. TBs mainly provoked the significant decrease of HGB concentration and RBC count on 4th and 9th weeks of exposure. Morphological changes of RBC (anisocytosis) were seen too. It means that generic TBs had anemic effect. In general, changes of hematological parameters were not principally significant and did not differ from control values at 13th weeks of experiments, except two TB's, which had shown significant decrease of HGB. Also some of generic TBs lead to decrease (leukopenia) or increase (leukocytosis) of leukocytes count in peripheral blood. In case of generic pesticides, the presence of impurities can demonstrate various hematotoxic action. Also the adverse effects on peripheral blood of males Wistar Han rats were observed at a dose of 50 mg/kg/bw/day and demonstrated the lesions of red blood. But abovementioned changes were not so clearly expressed. Any adverse hematotoxic effects at 10 mg/kg/bw/day dose were not observed in all studies.
Summary/Conclusions: As a conclusion, due to our results the triazole fungicides generic tebuconazoles have hematotoxic action. They induce anemia in Wistar Han rats and quantitative white blood cells changes. Today it is very important to investigate the hazardous effects of pesticides on the blood system.

PB2089

WHAT WE CAN DO TO MAKE A STANDARDIZATION AND HARMONIZATION OF APTT?

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Background: Transfusion enhanced many kind of complication in patients therefore peri-operative coagulation monitoring was suggested before the surgical procedure to protect the patients against bleeding. Also there are some diseases which had bleeding events in surgical procedure or spontaneously. We should detect these kind of diseases and we should examine the correct measure of active parsiel thromboplastin time (APTT) before surgical procedure by detecting the mild ormoderate deficiencies of plasma factor levels and by eliminating the lupus antıcougulant from plasma. This was caused to make the importance of APTT reagents.
Aims: We tried to show the importance of APTT reagents and how to reach the correct measure of APTT in this study.
Methods: We are planning to examine 300 patients, 109 of 300 patients were included as yet. Patient's APTT levels were calculated ACL-TOP analyzer by using three different reagents.First reagent was HemosILAPTT-SP which was sensitive against both plasma factors and lupus anticoagulant. It contains mix collodial silica and normal range of APTT- SP was 25.4-36.9 s. Second reagent was HemosIL SynthASil-SS which was sensitive against only plasma factors.It contains silica and normal range of APTT-SS was 25.1-36.5 s. The third reagent was HemosIL SynthA Fox-SF which was sensitive against only lupus anticoagulant. It contains ellagic asid and normal range of APTT-SF was 21.5-30.4 s.
Results: Forthy-five of 109 patients had normal level of APTT by measuring three types of reagents. Seventeen of 109 patients had long level of APTT by measuring three types of reagents. Twenty of 109 patients had long levelof APTT by measuring Hemosil synhAsil-SS reagent and had normal level with Hemosil SynthAFox-SF and Hemosil APTT-SP. Seven of 109 patients had long level of APTT by measuring Hemosil synhAsil- SS reagent and Hemosil APTT-SP reagent and had normal level of APTT with Hemosil SynthAFox-SF. Twenty of 109 patients had long level of APTT by measuring Hemosil synhAsil-SS reagent and Hemosil SynthA Fox-SF reagent and had normal level of APTT with Hemosil APTT-SP.
Summary/Conclusions: Haemostasis is a complex physiological cascade which was began at the endothelium injury. Many kinds of complex prosedures occured after this injury. Beacuse of this complex cascade pathway, bleeding is not occured every kind of APTT level. Bleeding events usually occured at <%30 level of plasma factors but mild or moderate plasma factor levels can caused bleeing. Therefor sensitivite of APTT reagents are very important. Every kind of APTT reagent do not have equal sensitivite against plasma factor levels, phospholipid composition and concentration in thromboplastin reagent. Several studies suggested that range of APTT should be determinate according to the devices and reagents and also several studies comparied APTT reagents which was included silica, ellagic asid and phospholipids by composed of syntethic or animal orginated and several studies determined a target level of APTT for looking at the plasma factors levels. If we want to get a correct result of APTT, ranges of APTT must be determine according to reagents which was used in APTT devices and APTT reagents must be sensitive against borderlines cases who had a mild or moderate low levels of factors and the presence lupus antıcouglant. We need further studies to make a standardization and harmonization of APTT.

PB2090

ACQUIRED PURE RED CELL APLASIA IN AN ADOLESCENT: COULD IT BE ANYTHING ELSE?

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Background: Pure red cell aplasia (PRCA) is a syndrome characterised by normocytic (sometimes macrocytic), normochromic anemia with severe reticulocytopenia and marked reduction or absence of erythroid precursors from the bone marrow. PRCA may be congenital, in the form of Diamond-Blackfan Anemia, or acquired which is rather rare in childhood. An immune mechanism interrupting erythroid differentiation is responsible in primary autoimmune PRCA, on the other hand secondary acquired PRCA may be associated with autoimmune/collagen vascular disorders, infections, lymphoproliferative disorders, hematological malignancies. solid tumors and drugs.
Aims: Here we report a 16-year-old male with acquired pure red cell aplasia who was treated successfully with steroids and cyclosporine after elimination of the secondary causes.
Methods: Case: An 16-year-old boy presented with a history of pallor and fatigue noticed three months prior to admission. He had been diagnosed with immune thrombocytopenia when he was 5 years old and had been in remission since that time. There was no history of blood transfusion, chronic illness or any other medication. His physical examination revealed pallor and a 2/6 systolic murmur with no other abnormalities. Complete blood count revealed severe macrocytic anemia and reticulocytopenia with hemoglobin:2.2 g/dL, hematocrit: 6.2%, mean corpuscular volume:108.7 fL, red blood cell: $0.57x10^{12}$/L, reticulocyte: 0.2% and mild leukopenia and lymphopenia. Peripheral blood smear showed macrocytic red cells with occasional tear drop cells. Stool for occult blood was negative. The direct and indirect antiglobulin tests were negative Serum bilirubin, LDH, haptoglobulin, liver function tests and renal function tests were in normal limits. Hemoglobin F was 2.9%. Bone marrow aspiration showed red cell hypoplasia, without dysplasia or giant pronormoblasts and normal myeloid and megakaryocytic series. A high resolution computed tomography of chest ruled out thymoma. Serum immunoglobulins revealed low IgA with normal IgG and IgM levels. Anti-nuclear antibody was negative but anti-dsDNA was positive. Parvovirus B19 DNA and other serologic markers including antibodies to HIV and hepatitis A, B and C were all negative. He was transfused with erythrocytes and discharged with a hemoglobin value of 7.2 g/dL. On his follow-up, hemoglobin levels were observed to decrease again. A diagnosis of primary acquired PRCA was considered and prednisone with a dose of 1 mg/kg/day and cyclosporine with a dose of 6 mg/kg/day were started to maintain through levels of 150-250 ng/mL. His hemoglobin level gradually increased and reached to 12 g/dL and leukopenia and reticulocytopenia resolved completely. Prednisone was tapered after 4 weeks and stopped. He is still on cyclosporine treatment and has been transfusion free with stable hemoglobin levels in the second month of his treatment.
Results: Primary acquired PRCA is very rare in childhood, secondary causes must be elliminated for definitive diagnosis. Our patient was found to have lymphopenia, low immunglobulin A level and positive antidsDNA in further investigations, yet these results are not sufficient for a specific diagnosis like common variable immune deficiency or systemic lupus erythematosus. Therefore we considered primary acquired PRCA as the most possible diagnosis and started immunosuppresive therapy; his clinical follow-up will probably give us further details about the underlying disease.

Summary/Conclusions: Immunosupressive therapy including cyclosporine with or without steroid has been reported as the most effective treatment in primary acquired PRCA. Consistently, we had a dramatic response to immunosuppressive therapy in our patient.

PB2091

APLASTIC ANEMIA IN CHILDHOOD: A TEN YEARS' SINGLE CENTER EXPERIENCE

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Background: Aplastic anemia in childhood is a rare, life-threating disorder, characterized by peripheral blood pancytopenia and a hypocellular bone marrow without signs of dysplasia or fibrosis. Acquired aplastic anemia needs to be distinguished from inherited bone marrow failure syndromes or myelodysplastic syndromes.
Aims: The aim of this study is to assess the clinical and laboratory findings at the time of diagnosis, the treatment approach and the outcome of children with aplastic anemia treated in our department during the past decade.
Methods: This retrospective study evaluated 9 children with aplastic anemia, who were treated and followed up in the Pediatric Department of AHEPA, during the period 2006-2016.
Results: We identified 9 children with aplastic anemia. The patients' population included 6 (66.7%) males and the mean age at admission was 9.7 years. At the time of diagnosis, the average neutrophil count was 750/mm^3, the Hb count was 8.4mg/dl and platelets count was 8770/mm^3. In all of our cases aplastic anemia was acquired, expect one case of Fanconi anemia. Predisposing risk factors (including drugs exposure, viral infections, chemicals) were identified in 4 patients. Among the 9 studied patients, 3 (33.3%) had moderate-non severe, 2 (22.2%) had severe and 4 (44.5%) had very severe aplastic anemia. All of the patients received immunosuppressive therapy (consisting of antithymocyte globulin, cyslosporine A and steroids), 2 remained transfusion independent, 4 underwent bone marrow transplantation- 2 from a matched related donor and 2 from a matched unrelated donor. One patient with refractory disease received, as an alternative first line therapy, eltrombopag. Complete response was achieved in 22.2%, partial response was achieved in 22.2%, relapse occurred in 11.1% and 44.5% of the patients had refractory disease. The overall survival was 77.8%.
Summary/Conclusions: A remarkable progress has been made during the past decades in the understanding of pathogenesis and management of children with aplastic anemia. Bone marrow transplantation from a matched related donor is the recommended first line therapy resulting in an excellent survival rate that exceeds 90%. In the future the development of targeted strategies for patients and the enhancement of supportive care will further improve outcome and diminish the disease's late complications.

PB2092

CAUSES OF IRON DEFICIENCY ANEMIA IN THE HEMATOLOGY CLINIC – SINGLE CENTER EXPERIENCE

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Background: Iron deficiency anemia (IDA) is the common nutritional deficiency worldwide. The studies concerning various causes of IDA in adult men are rare, although it is assumed that chronic gastrointestinal blood accounts for the majority.
Aims: Of the study is to evaluate retrospectively adult men with IDA that were hospitalized in our Hematology Clinic.
Methods: Two hundred fifteen male with IDA were enlisted at this study from January 2005 to december 2015. Anemia was defined as Hg <13g/dL using the WHO criteria. IDA was considered present if serum ferritin was 15 ng/mL combined with serum iron concentration <30ug/dL with a transferrin saturation of <10%. Complete physical examination, the history of the disease and fecal occult blood test (FOBT) of three spontaneously passed stools was done in all patients. All patients had complete blood count, serum and total iron binding capacity, and a serum ferritin level. Most patients underwent esophagogastroduodenoscopy (EGD). Colonoscopy was performed if lesion that caused IDA was not found, and/or FOBT was positive.The abdominal CT scan were performed according to clinician's recomandation together with other tests related with blood lost.
Results: The median age was 62 (range 32 to 86) years old. 168 of 215 (78.13%) men with IDA had symptoms such as fatigue, dizzines, or digestive complaints. The history of prior gastrectomy, hemorrhoid, that probably had caused IDA were reported in 32 (14,88%), 43 (20.0%), patients, respectively. FOBT was positive in only 65 (30.23%) subjects. 170 (79.06%) patients underwent EGD. The most common findings from EGD were gastritis (48 patients) and peptic ulcer (39 patients). Seventy eight (36.27%) patients were found to have upper gastrointestinal disorders (20 patients with erosive gastritis, 19 gastric ulcer, 16 duodenal ulcer, 23 gastric cancer. Eighty-nine (41.39%) patients underwent colonoscopy. That showed 44 clinically important lesions that probably caused IDA ; colon cancer in 17 (7.90%) patients, colon polyp in 10 (4.65%) patients and hemorrhoid in 17 (7,90%) patients. Concerning malignant lesions which are responsible for IDA, the malignant lesions were found more frequent in patients older than 50 years accounting for 20.45% (27/132 patients) and patients younger than 50 years 17.80% (13/73 patients).
Summary/Conclusions: This study demonstrated that gastrointestinal blood loss is the main cause of IDA in adult men, and that there is a high rate of malignancy in men older than 50 years.

PB2093

IMPACTS OF CLINICAL AND BIOCHEMICAL PARAMETERS ON KEY HEMATOLOGICAL INDICES IN ADULTS: A COHORT STUDY

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Background: Studies in Caucasians have shown that values of hematological parameters could be affected by a wide variety of factors, most notably age and gender. However, parallel work in different ethnical populations, especially from Asia-Pacific region, is lacking. Importantly, it remains largely unknown whether some fundamental variables such as nutritional status, lipid profile, and hepatitis infection (either HBV or HCV) also affect the variation of values in hemogram.
Aims: Therefore, we conceptualize this study to explore through several key parameters regarding their potential impacts on the changes of hemogram.
Methods: Adult individuals aged 18 or older from several adjacent villages in Yun-Lin County, located in the central part of western Taiwan, who came to our hospital for annual health exams were screened for the current study. The work, retrospective in nature, was approved by institutional IRB. Information on age, gender, hemogram, levels of total cholesterol (TC), triglyceride (TG), apolipoprotein B (Apo B) as well as albumin, and results of serological testing for hepatitis B and C infection, was obtained from a centralized digital data base. All the clinical data, after given a coding number for each case, were encrypted and provided to the investigators without identifiable personal information. We analyzed the impacts of various parameters on several key hematological indices.
Results: Overall, 26,497 individuals were included in the current analysis after excluding those with hemogram data fell outside of normal range. Carriers of either hepatitis B (HBV) or C (HCV) who had abnormal liver function (defined by elevated levels of aspartate transaminase or alanine transaminase) were excluded as well. Age, gender, and serum levels of TC, Apo B, and albumin all significantly impacted most key hematological profiles. As the levels of TC and Apo B correlated well with each other (correlation coefficient r=0.82211, p <0.0001, Pearson's correlation), we did not incorporate TC in our multi-variate analysis. Several key variables were found to influence some hematological indices in the multi-variable regression model. Increasing age and male gender negatively affected the platelet count, whereas higher Apo B level was associated with elevated platelet count. Surprisingly, hepatitis C carriers with normal hepatic function had slightly higher platelet number than non-HCV carriers. Gender and serum albumin level were the major determinants of variation in hemoglobin level. Total white cell count increased with male gender and elevating Apo B level but was inversely correlated with change in age and serum albumin level (Table 1).

Table 1.

Summary/Conclusions: The hematological indices are influenced by a wide variety of factors, especially age, gender, and serum level of Apo B. As age,

Apo B, white cell count, and platelet count all impose risk of thromboembolism, further work exploring the interactions and impacts of these parameters on the development of cardiovascular diseases should be mandatory.

PB2094

UNUSUAL DISTRIBUTION OF INTERLEUKIN-10 C-592A GENE POLYMORPHISM IN PATIENTS WITH PRIMARY IMMUNE THROMBOCYTOPENIA FROM NORTH-WESTERN RUSSIA

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Background: Primary immune thrombocytopenia (ITP) is a rare hematological disease with unknown etiology. It is characterized by heterogeneity of the laboratory parameters as well as the features of clinical manifestation. DNA polymorphism of several cytokine genes has been suggested to modulate the risk of ITP development or/and treatment response in distinct population groups. There is no data on the prevalence of cytokine gene polymorphisms in ITP patients from the North-Western region of Russia (NWR).
Aims: To establish the features of genotypes distribution for several cytokine promoter gene polymorphisms in ITP patients from NWR.
Methods: A total of 68 patients (59 women and 9 men) with chronic primary ITP were involved in the study. The median age of the group was 57 years (range: 24-77). The mean duration of ITP was 7 years (2-48). In 19 (32.2%) women, ITP was diagnosed before 30 years old; 26 (38.2%) patients (5 men and 21 women) were diagnosed at age 30-50 years; 23 (33.8%) patients (4 men and 19 women) developed ITP after 50 years old. The control group consisted of 240 healthy persons originated from NWR. Nucleotide variations in the genes coding for interleukin (IL)-1b (-31T/C), IL-6 (-174G/C), IL-10 (-592C/A) and tumor-necrosis factor alpha (TNFA -308 G/A) were discriminated by PCR and subsequent restriction analysis (PCR-RFLP). Intergroup differences in genotype frequencies were assessed by Fisher's exact method. Odds ratios (OR), their 95% confidence intervals (CI) and p-values were calculated by using the GraphPad Prism 5.0 software.
Results: The frequency of the IL-10 -592CC genotype was slightly increased in the ITP group when compared to controls (65.7% *vs* 54.0% respectively; OR=1.6, 95% CI: 0.9-3.1, p=0.15). Interestingly, this variant of the IL-10 gene was more prevalent among women than men with ITP (71.2% *vs* 25.0% respectively; OR=7.4, 95% CI: 1.4-40.5, p=0.016). When compared to controls, the IL-10 -592CC genotype was significantly overrepresented in the group of women with ITP (71.2% *vs* 54.0%; OR=2.1, 95% CI: 1.1-4.2, p=0.044). On the contrary, in the group of affected men we observed the increase of persons who had IL-10 -592A allele (75.0% *vs* 46.0% in control group; OR=3.5, 95% CI: 0.7-18.3, p=0.15). Genotype frequencies for other studied genes were similar between the patients and control group as well as between women and men with ITP. We have also found almost 2-fold increase of the IL-1b -31CC frequency in women diagnosed before 30 years old compared to other patients (15.8% *vs* 8.2% respectively; OR=2.1, 95% CI: 0.4-10.5, p=0.39). The presence of the TNFA -308A allele was more often seen in patients diagnosed before 50 years old (26.7% *vs* 8.7% in other ITP patients; OR=3.8, 95% CI: 0.8-18.8, p=0.12).
Summary/Conclusions: We suggest that the IL-10 -592CC genotype is associated with increased risk of ITP in women from NWR. On the other hand, the IL-10 -592A allele could be involved in pathogenesis of ITP in men. Further studies are needed to clarify the significance of TNFA and IL-1b gene polymorphism in ITP development.

Platelets disorders

PB2095

COMBINED TREATMENT OF AZATHIOPRINE AND ROMIPLOSTIM IN PATIENTS ITP REFRACTORY TO STEROIDS OR THROMBOPOIETIN ANALOGS

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Background: More than 70% of patients with Immune Primary Thrombocytopenia (ITP) respond to steroids, but 40 to 70% relapse in the first year follow-up. The use of romiplostim in this group is effective, although 9% failure has been described. In recent literature, there are clinical cases and small series describing the potentiating effect of combined treatment with thrombopoietin analogues and immunosuppresive drugs such as steroids, cyclophosphamide and rituximab. We have not found references to the combined use of azathioprine (AZA) and romiplostim (ROM).
Aims: To describe our experience in the combined use of azathioprine and romiplostim as a rescue treatment in patients with acute or newly diagnosed ITP refractory to corticosteroids or corticosteroid-dependence and refractary to maximal doses of romiplostim monotherapy.
Methods: We analyzed patients with newly diagnosed or persistent ITP, with corticosteroid-dependence or refractory to steroids and refractary to romiplostim, both in monotherapy. We have considered refractoriness to steroids not reaching platelets higher than $30x10^9$/L. Corticosteroid-dependece as the need for ongoing or repeated doses administration of corticosteroids for at least 2 months to maintain a platelet count at or above 30 $X10^9$/L and/or to avoid bleeding. We considered refractoriness to romiplostim not get platelets greater than $30x10^9$/L with 10mcg/kg/week for at least 3 consecutive weeks. All patients have been diagnosed in a single center with the same physician responsible for the treatment and follow-up. The initial doses of AZA was 100mg/days (2mg/kg/day) and ROM 10mcg/kg/week. Patients have been evaluated every week untill platelets were higher than $30x10^9$/L for to consecutive weeks, after this they were reviewed monthly.
Results: We treated 4 patients (75% female) with a median age at diagnosis of ITP of 53 years old (RIQ, 20-61 years). Treatments received prior to the use of the combination of AZA and ROM were polyspecific immunoglobulins (Ig), steroids (dexamethasone, prednisone) and romiplostim. Responses to steroids and romiplostin in monotherapy were: • Median dexamethasone cycles (40mg/days x 4 days) was 2.5 (2-4 cycles, IQR). The initial dose of prednisone was 1-2mg/kg/days with a median treatment day of 31.5 days (28-60 days, IQR). The type of response to steroids was PR with corticode-pendence in one patient, 3 patients NR. • Median time from ITP diagnosis and romiplostim indication was 9.5 weeks (7-48 weeks, IQR). Median platelets count at the start of romiplostim was $6x10^9$/L ($2-13x10^9$/L, IQR). The median platelet count achieved at maximal doses of romiplostim for at least 2 consecutive weeks was $10x10^9$/L ($3-19x10^9$/L, IQR). Once established the refractoriness to romiplostim, we manteined ROM 10mcg/kg/week and AZA was iniciated at 100mg/day. The median time from romiplostim indication to the association with azathioprine was 9.8 weeks (5.5 to 15 weeks, IQR). The median time to response after initiation of combination of AZA and ROM was 21 days (15-35 days, IQR). The types of response were: • One patient did not respond after 60 days of combined treatment. • 1 patient with RC maintains for 7 months in the absence of active treatment. The combined was necesary during 8 months. • 2 CRs still undergoing combined dose reduction (current dose romiplostim 2mcg/kg/week and azathioprine 50mg /d). Median platelets from onset of dose reduction $169x10^9$/L ($128-176x10^9$/L, IQR). Duration of RC, 7 and 14 months. Non adverse events have been described in combination treatment.
Summary/Conclusions: The use of azathioprine and romiplostim in combination could be a safe and effective alternative in subjects refractory to steroids or corticosteroid-dependence and thrombopoietin analogs alone. More studies are needed to clarify the mechanism of complementation between the two drugs.

PB2096

AGONIST-INDUCED PLATELET REACTIVITY CORRELATES WITH BLEEDING IN HEMATO-ONCOLOGICAL PATIENTS

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Background: Prophylactic platelet transfusions are administered to prevent bleeding in hemato-oncological patients. However, bleeding still occurs, despite these transfusions. This practice is costly and not without risk. Better predictors of bleeding are needed and flow cytometric evaluation of platelet function might aid the clinician in identifying patients at risk of bleeding. This evaluation can be performed within the hour and is not hampered by low platelet count.

Aims: Our objective was to assess a possible correlation between bleeding and platelet function in thrombocytopenic hemato-oncological patients.
Methods: Inclusion was possible for admitted hemato-oncology patients aged 18 years and above after written informed consent. Furthermore, an expected need for platelet transfusions was necessary. Bleeding was graded according to the WHO bleeding scale. Platelet reactivity to stimulation by either adenosine diphosphate (ADP), crosslinked collagen-related peptide (CRP-xL), PAR1- or PAR4-activating peptide (AP) was measured using flow cytometry.
Results: A total of 114 evaluations were available from 21 consecutive patients. Platelet reactivity in response to stimulation by all four studied agonists was inversely correlated with significant bleeding. Odds Ratio's (OR) for bleeding were 0.28 for every unit increase in median fluorescence intensity (MFI) [95% Confidence interval (CI) 0.11-0.73] for ADP; 0.59 [0.40-0.87] for CRP-xL; 0.59 [0.37-0.94] for PAR1-AP and 0.43 [0.23-0.79] for PAR4-AP. The platelet count was not correlated with bleeding (OR 0.99 [0.96-1.02]).
Summary/Conclusions: Agonist-induced platelet reactivity was significantly correlated to bleeding. Platelet function testing could provide a basis for a personalized transfusion regimen, in which platelet transfusions are limited to those at risk of bleeding.

PB2097

TUMOR NECROSIS FACTOR-A AND TUMOR NECROSIS FACTOR-B SINGLE NUCLEOTIDE POLYMORPHISM AND CHRONICITY IN EGYPTIAN PEDIATRIC PATIENTS WITH IMMUNE THROMBOCYTOPENIA

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Background: Although the etiology of immune thrombocytopenic purpura (ITP) remains unclear, both genetic and environmental factors may contribute to the development of disease. Tumor necrosis factor alpha & beta (TNF-α and TNF-β) are important pro-inflammatory cytokines that play a role in regulation of cell differentiation, proliferation and death, as well as in inflammation, innate and adaptive immune responses, and have been implicated in a wide variety of human diseases. We hypothesized that inflammatory cytokine genes polymorphisms (TNF-α and TNF-β) in ITP pediatric patients may play a fundamental role in pathogenesis of the chronic course of the disease and this might be the base for future specific immunomodulatory therapies for chronic ITP (cITP) in children.
Aims: The current case-control study aimed at detecting TNF-α (-308 G/A) and TNF-β (+252 A/G) genes polymorphism in Egyptian children with cITP and studying their possible association with chronic evolution of the disease.
Methods: The current study included 80 Egyptian cITP patients at Pediatric Hematology Unit, Cairo University (mean age 7.08±3.64 years) and 100 matched unrelated healthy controls. Genotyping was performed using polymerase chain reaction restriction fragment length polymorphism technique (PCR-RFLP).
Results: TNF-α genotyping revealed that wild G/G, heterozygous G/A and homozygous A/A genotypes among cITP patients were 81.2%, 15% and 3.8% respectively *versus* 79%, 20% and 1% in control group, while TNF-β wild A/A, heterozygous A/G and homozygous G/G genotypes among cITP patients were 55%, 40% and 5% respectively *versus* 60%, 28% and 12% in control group, with no statistically significant difference between both groups. Patients having homozygous TNF-α genotype showed statistically significant higher mean age, longer disease duration & lower mean platelet count (p=0.005, 0.024 and 0.008 respectively). TNF-α polymorphism was more frequent among unresponsive patients compared to responsive patients with statistically significant difference. Calculated risk estimation revealed that combined genes polymorphism conferred three fold increased risk of development of cITP (OR=3.491, 95% CI: 1.235-9.869, p=0.015).
Summary/Conclusions: We hereby report a strong association between combined polymorphisms of both TNF-α & TNF-β genes and susceptibility to chronicity of ITP in Egyptian children. Further studies for gene polymorphisms which could affect the pathogenesis of ITP and facilitate the development of new therapeutic modalities are recommended.

PB2098

PROGNOSTIC FACTORS IN PRIMARY IMMUNE THROMBOCYTOPENIA OF CHILDHOOD

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Background: Primary immune thrombocytopenia (ITP) is an immune disorder with varied course. According the duration of the disease, it is distinguished in newly diagnosed (<3 months), persistent (3-12 months) and chronic (>12 months). International studies have highlighted prognostic factors for each form of ITP in children, but similar studies have yet to be been performed in Greece.
Aims: The evaluation of clinical and laboratory parameters and the identification of prognostic markers for the three forms of the disease in children with ITP from an academic reference center in Greece.
Methods: This retrospective study included 57 children with ITP in the past 13 years, aged 1-16 years (median age 5.2). The following data were recorded: age, gender, preceding infection, bleeding type, duration of symptoms and platelet count at the diagnosis, treatment, disease course and immunological markers and comparison was made among the three types of ITP.
Results: 39 children had newly diagnosed, 4 had persistent and 14 had chronic disease. Due to the small number of children with persistent form they were incorporated in the group of children with newly diagnosed ITP. In chronic ITP children are more likely be above 10 years of age (p=0.015)and to have gradual initiation of the disease (p=0.001) compared with newly diagnosed/persistent group (57% *vs* 21% and 79% *vs* 9%, respectively). Recent history of infection was found mainly in newly diagnosed/persistent group (79% *vs* 21%, p=0.013). Platelet count below 10 x 10^9/L at diagnosis was found more frequently in newly diagnosed/persistent group (79% *vs* 36%, p=0.01). Similar, but not statistically significant difference, was found with mucosal bleedings (70% *vs* 50%, p=0.81). Children with newly diagnosed/persistent disease had less frequently impaired immunological markers (12% *vs* 65%, p <0.003) and received more frequent intravenous gamma globulin and/or corticosteroids (p>0.05). None of the children exhibited severe spontaneous bleeding.
Summary/Conclusions: Even though ITP in children is usually a self-limited disease, with rare serious bleeding complications, the newly diagnosed/persistent and the chronic form of the disease are characterized by different predictive parameters that can be used in clinical practice.

PB2099

CANCER-ASSOCIATED IMMUNE THROMBOCYTOPENIA

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Background: Cases of cancer-associated immune thrombocytopenia (IT) have been reported recently, but there are few reports and case series that describe clinical features and response to treatment.
Aims: We report our experience of 10 years at a single hospital in Spain, in patients with IT concurrent with neoplasia.
Methods: We identified the patients by data search of hospital records from 2006 to 2016, with diagnosis of IT with previous diagnosis of cancer, not related with chemotherapy or radiotherapy, not suggestive of bone marrow infiltration, drug-induced, infection of disseminated intravascular coagulation. For the diagnosis, the examination of bone marrow was not mandatory.
Results: The two most common cancers associated with IT were bladder and lung neoplams, but the occurrence of prior cancer (third part of patients) was not uncommon. The IT can appear at any stages of cancer, and it is mainly detected at the first two years after the diagnosis when the patient have been in acceptable antitumoral response. They usually manifest with very low platelet count <20.000, but not always with evident clinical bleeding. The response to therapy was fast and complete with corticoids (usually in the first week) in the majority of patients, but some cases require the combination second line with immunoglobulins or thrombopoietin receptor agonists, and in the follow-up, the response was persistent without recurrence in the first year post-treatment (Table 1).

Table 1.

Patients with immune thrombocytopenia diagnosed concurrently with cancer

PATIENT	GENDER	AGE	TYPE OF CANCER	STAGE	PREVIOUS CANCER	TIME OF STARTING	BLEEDING PHENOTYPE	NADIR PLATELETS /mm3	TREATMENT	OUTCOME OF IT	TIME OF RESPONSE
1	female	47	BREAST	4	no	SECOND YEAR	without bleeding	10.000	CT	CR	2 days
2	male	60	LUNG	4	no	FIRSTYEAR	gastrointestinal	2.200	CT+IVIG	CR	20 days
3	female	52	LUNG	3	breast	FIRSTYEAR	without bleeding	50.000	CT+TRA	CR	20 days
4	male	77	BLADDER	2	no	SECOND YEAR	epistaxis	2.600	CT	CR	5 days
5	male	80	LUNG	3	no	CONCURRENT	gastrointestinal	30.000	CT	CR	4 days
6	male	88	BLADDER	2	prostate	FIRSTYEAR	without bleeding	17.000	CT	CR	20 days
7	male	55	COLON	4	no	SECOND YEAR	gastrointestinal	1.000	CT	CR	2 days
8	female	86	ENDOMETRIU	1	no	FIRSTYEAR	without bleeding	16.000	CT	CR	4 days
9	female	78	BLADDER	1	breast	FIRSTYEAR	gastrointestinal	2.100	CT+TRA	CR	30 days

Abbreviations: PC: previous cancer; CT: corticosteroids; IVIG: intravenous immunoglobulin; TRA: thrombopoietin receptor agonists; IT: immune thrombocytopenia CR: Complete response (continuous platelets count >30,000 by treatment)

Summary/Conclusions: The CAIT is a rare hematological paraneoplastic syndrome that occur in solid tumors, usually associated to low platelet count but without life-threatening bleeding, requiring therapy with corticosteroids as first line, and generally related with a benign clinical course with a rapid and persistent response.

PB2100

THE ROLE OF MEAN PLATELET VOLUME IN NEONATAL SEPSIS: A RETROSPECTIVE CASE CONTROL STUDY IN A LEVEL III NEONATAL INTENSIVE CARE UNIT

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Background: Sepsis is a relatively common diagnosis in the neonatal period. Apart from blood cultures which are the gold standard, C-reactive protein (CRP), total white blood cell count (WBC) and the ratio of immature to mature neutrophils (I:T) are considered to be useful markers of sepsis in the neonatal period. There are a few studies that show that mean platelet volume (MPV) is elevated in infectious disease processes.
Aims: The aim of this study was to investigate whether mean platelet volume is increased in neonates with sepsis.
Methods: Only term neonates were included in the study. Exclusion criteria included: (a) Any neonate born with a genetic defect, (b) Any neonate with suspected immunodeficiency, (c) Any neonate requiring surgery in the post-natal period, (d) Neonates admitted to NICU for hyperbilirubinemia, (e) Neonates requiring extensive resuscitation at birth resulting in documented Hypoxic Ischemic Encephalopathy or requiring transfer to a Regional Perinatal Center. Medical records were reviewed from March 2015 to June 2016 and a total of 114 eligible neonates were included in the study and they were divided into 2 groups: 39 neonates with clinical sepsis (as defined by either culture positivity and/or clinical features plus treatment with antibiotics exceeding 48 hours) and 75 healthy controls (as defined by neonates in whom antibiotics were never started or discontinued when cultures were negative for 48 hours and the absence of clinical features of sepsis). Total white blood cell count, C-reactive protein, immature to total neutrophil count and mean platelet volume drawn on two occasions (first within 24 hours and the second between 24 to 48 hours after delivery) were compared between the two groups.
Results: There was no statistically significant difference in the mean platelet volume between the study group and the control group (p value 0.9 in the first 24 hours and p value of 1 in the 24-48 hour sample). There was however, a statistically significant difference between immature to total neutrophil count and C-reactive protein on both samples (p value <0.0001) (Table 1).

Table 1.

Two-sample t-test for characteristics in the two groups

	Mean (se)		
Measurements	Study	Control	p-value
Age	39.2 (0.18)	39.1 (0.13)	0.4065
Weight (gm)	3409.0 (93.36)	3398.4 (529.70)	0.9156

Chi-Square Test for Gender Frequencies in the two groups

	n (%)		
Gender	Study	Control	p-value
female	31 (41.33%)	13 (33.33%)	0.4052
Male	44 (58.67%)	26 (66.67%)	

Comparison of markers between control and study group

WBC1	14.3 (0.89)	14.5 (0.46)	0.2817
WBC2	20.4 (1.27)	17.9 (0.57)	0.1249
IT1	0.2 (0.03)	0.1 (0.01)	<0.0001*
IT2	0.1 (0.02)	0 (0.01)	<0.0001*
MPV1	8.7 (0.16)	8.7 (0.09)	0.9074
MPV2	8.9 (0.14)	8.8 (0.08)	1.0
CRP1	6.6 (1.98)	0.5 (0.21)	<0.0001*
CRP2	26.4 (4.17)	2.6 (0.44)	<0.0001*

*Used a two-sample t-test. The others used a Mann-Whitney t-test.

Summary/Conclusions: In our study there was no statistically significant difference in the mean platelet volume values between neonates with sepsis and healthy controls. C-reactive protein and immature to total neutrophil count continue to be reliable markers of neonatal sepsis.

PB2101

IS PLATELET TRANSFUSION WARRANTED IN PATIENTS WITH ACUTE TTP REQUIRING CENTRAL VENOUS CATHETER INSERTION?
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Background: Thrombotic thrombocytopenic purpura (TTP) has a high mortality rate. The cornerstone of management is plasma exchange (PE) which usually requires urgent insertion of a central venous catheter. Patients often have a platelet count of <50x10^9/L at presentation however, National BCSH Guidance advises against platelet transfusion in TTP due to the perceived high aggregability state and reports of associated fatal thrombosis. The risk of thrombocytopaenia related haemorrhage however creates anxiety and dilemma for the team responsible for line insertion and may lead to delays or unnecessary platelet transfusion.
Aims: The aim of the study is determine the average platelet count at time of line insertion and to see if any bleeding complications are observed.
Methods: We retrospectively reviewed all central venous catheter lines inserted in patients presenting to a regional TTP Centre over a 4-year period from 2012-2016.
Results: A total of 48 patients confirmed to have TTP with an ADAMTS13 <5% underwent line insertion: 94 central venous catheter lines were inserted: 40% femoral, 60%–internal jugular vein. The median number of lines inserted per patient episode was 3, with a range of 1-5. Median presenting platelet count for first line insertion was 25x10^9/L (IQR 9-26 x10^9/L). 70% of lines were inserted by critical care and the remaining 30% by interventional radiology. Platelet transfusion was not administered pre line insertion in any case. No significant bleeding complications were documented during or after line insertion. 5 patients had 'excessive oozing at the insertion site' documented, within the first 24 hours of insertion, for which no intervention was required. There were no deaths related to line insertion.
Summary/Conclusions: In conclusion, this study shows no significant bleeding risk associated with central venous catheter insertion in thrombocytopenic patients presenting with TTP. The results support guidance against prophylactic platelet transfusion in this setting and provide reassurance for teams tasked with central line insertion in this critically unwell patient group.

PB2102

LONG-TERM EFFICACY AND SAFETY OF THROMBOPOIETIN AGONISTS IN ADULT REFRACTORY CHRONIC IMMUNE THROMBOCYTOPENIA
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Background: Management of chronic immune thrombocytopenia (cITP) aims not only to increase and maintain platelet counts in safe levels, but also to improve quality of life. Thrombopoietin agonists eltrombopag and romiplostim have been approved in refractory ITP. The lack of randomized studies allows only for real-world data comparison on the two agents.
Aims: In the present study we evaluate and compare long-term efficacy and safety of etrombopag and romiplostim in clinical practice and assess the switching feasibility between the two agonists.
Methods: Treatment with thrombopoietin agonists was initiated in 20 adult patients (pts) with refractory cITP between June 2011-2016. Patients resistant or intolerant to the first agonist switched to the second one. Complete response (CR) was defined as a platelet count of ≥100x10^9/L.
Results: Eltrombopag was administered in 15 pts, 6 male:9 female with a median age of 46 years (19-76 yrs) for 13 months (1.4-54 mo). Patients had received a median of 1 previous treatment (range 1-7): corticosteroids (15/15), intravenous immunoglobulin (5/15), rituximab (2/15), vincristine (1/15), cyclosporine (2/15), romiplostim (2/15), danazol (1/15) and splenectomy (1/15). Before eltrombopag treatment, the majority (8/15) showed grade 4 (WHO) thrombocytopenia. Initial dose was 50 mg and increased to 75 mg daily in 3/15 pts and in combination with corticosteroids that were gradually tapered by the 5th week in 12/15. Median platelets value by the 2nd week of administration was 140x10^9/L (5-450 x10^9/L); whereas, by the 4th week increased to 185x10^9/L (16-500x10^9/L). At the end of follow-up, all patients but one achieved CR with median platelets of 145x10^9/L (60-400 x10^9/L). Regarding adverse events, 1/15 pt presented hemolytic anemia, 1/15 pt hepatotoxicity grade 2 with episodes of thrombocytopenia grade 4 and 1/15 pt pulmonary embolism during the second month of treatment. The latter 2 pts switched to romiplostim. Romiplostim was administered in 9 pts, 4 male:5 female aged 44 years (33-63 yrs) for 40.7 months (22.4-60.1). They had received a median of 3 previous treatments (range 1-8): corticosteroids (9/9), intravenous immunoglobulin (6/9), rituximab (6/9), vincristine (2/9), cyclosporine (2/9), eltrombopag (2/9), danazol (1/9) and splenectomy (2/9). The majority (5/9) presented thrombocytopenia grade 4 before romiplostim. Median platelets number by the 2nd week of administration was 50x10^9/L (8-140 x10^9/L); whereas, by the 4th week increased to 115x10^9/L (20-400x10^9/L). At the end of follow-up, 6/9 patients achieved CR with median platelets at 145x10^9/L (110-400x10^9/L). All patients received concomitant steroid treatment that was gradually tapered and stopped in 6/9 pts. 2/9 pts switched to eltrombopag due to thrombocytopenia grade 3 and 1/9 pt to danazol and low-dose steroids achieving CR. No adverse events associated with romiplostim treatment were reported.
No significant differences were found between the 2 treatment groups. All 4 patients that switched to the other agonist achieved CR without adverse events.
Summary/Conclusions: Our real-world data suggest that both eltrombopag and romiplostim are safe, well tolerated and highly effective in refractory cITP and furthermore, switching to another agonist is safe and effective. Future studies will determine predisposing factors for adverse events and more accurate classification of patients that will allow for better treatment guidance.

PB2103

VITAMIN D RECEPTOR GENE POLYMORPHISMS IN ADULT PRIMARY IMMUNE THROMBOCYTOPENIA
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Background: Recently, several studies have demonstrated the role of vitamin

D receptor (VDR) polymorphisms in the development of autoimmune diseases. Vitamin D affect both innate and adaptive immune responses that have been blamed in immune thrombocytopenia (ITP) pathogenesis.
Aims: The aim of this study is to assess the association of vitamin D receptor gene polymorphism *BsmI* in cases of adult primary immune thrombocytopenia.
Methods: Vitamin D receptor polymorphism *BsmI* (rs1544410) was detected by Polymerase Chain Reaction followed by Restriction Fragment Length Polymorphism (PCR–RFLP). Deoxyribonucleic acid (DNA) samples were extracted from peripheral blood of 40 ITP patients and 60 geographically and ethnically matched healthy controls.
Results: Statistically significant difference was found in the *BsmI* polymorphism between ITP patients and controls ($\chi^2 = 8.77$, P value=0.01). The *BsmI* polymorphism *B* allele was higher in ITP group than that in controls but in statistically insignificant difference ($\chi^2 = 2.125$, P = 0.145). *bb* genotype played a protective role in ITP incidence.
Summary/Conclusions: This is the first published report on VDR gene polymorphisms in adult primary ITP patients. The *BsmI* genotype was associated with increased risk for ITP incidence with no obvious effect on bleeding severity, platelet count nor site of bleeding.

PB2104

A SURVEY OF THE TREATMENT OF THE PREVENTION OF NAIT IN THE UK AND IRELAND

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Background: Neonatal alloimmune thrombocytopenia, (NAIT) is caused by maternal antibodies generated against alloantigens carried on fetal platelets, which cross the placenta and induce destruction of platelets in the fetus. In most cases the maternal immunisation is triggered by exposure to fetal blood at delivery. As a result, the clinical presentation tends to be more severe in subsequent pregnancies. Recent studies and guidelines have suggested that intravenous immunoglobulin (IVIG) with or without steroids can significantly reduce the severity of thrombocytopenia in subsequent pregnancies.
Aims: We set out to establish if there is consistency in the management of the prevention of NAIT across Ireland and the United Kingdom (UK).
Methods: A survey was set up on Survey Monkey and all members of the UK-Ireland Haematology group were contacted by email with a link to the survey in January 2015. In total 90 individual Specialists were contacted across 70 centres.
Results: 30 responses were received to the following questions. Who manages the prevention of NAIT in your centre? 34% of respondents stated that it was managed jointly by haematologist/feto-maternal specialists, with 26% responding it was overseen solely by haematologists and 40% solely by feto-maternal specialists. Secondly what risk stratification each respondent used to decide risk of NAIT in the current pregnancy? 82% stated that they took into account multiple risk factors but 18% stratified risk based only on the outcome of previous pregnancy. Thirdly how many groups do you define after risk stratification? 60% identified 3 strata of risk (standard, high and very high) with 40% classifying two risk groups (standard *versus* high risk). Fourthly respondents outlined their management of a standard risk group defined as confirmed thrombocytopenia with antibody. 43% give IVIG 1g/kg weekly from 20 weeks, 28% give 1g/kg from 20 weeks increasing to 2g/kg at 32 weeks with 6% starting 1g/Kg IVIG from 24 weeks. 23% referred to feto-maternal specialist to decide IVIG . Just 20% give 0.5mg/kg of steroids from 20 or 32 weeks. For high risk pregnancies defined as confirmed antibody positive with previous intracranial haemorrhage (ICH) after 28 weeks: 36% of centres give IVIG 1g/kg from 20 weeks, 36% give 1g/kg from 20 weeks increasing to 2g/kg at 32 weeks with 14% giving 2g/kg from 20 weeks and 14% initiating at 12 weeks. 40% gave 0.5mg/kg of steroids varying from 12-32 weeks starting. 60% of centres use a very high risk protocol (ICH before 28 weeks) with more intensive IVIG starting at 12 or 20 weeks with steroids of variable intensity and duration. Finally respondents were questioned whether there was a planned delivery time and method for the pregnancy? 58% plan a delivery at 38 weeks with no specific delivery mode. 18% plan delivery at 38 weeks by caesarean section, 8% plan a caesarean section but with no set time and 16% have no specific protocol plan for delivery.
Summary/Conclusions: The results of this survey reveal that the optimal medical management for the prevention of NAIT, namely the medication, dosage and schedule vary widely reflecting the lack of good evidence to guide centres in this very challenging area. Based on this survey we plan with our colleagues in UKOS a prospective study of treatment and outcomes.

PB2105

THE EVALUATION OF REACTIVE OXYGEN SPECIES IN CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA AND HELICOBACTER PYLORI INFECTION *VERSUS* CHRONIC ITP WITHOUT HELICOBACTER PYLORI INFECTION

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Background: Chronic idiopathic thrombocytopenic purpura (ITP) is an acquired disease characterized by a low platelet count caused by an immunological peripheral platelet destruction or a decreased platelet production. Several studies have shown increased reactive oxygen species (ROS) levels in chronic ITP and also a possible association between Helicobacter pylori (H. pylori) infection and immunological peripheral platelet destruction.
Aims: To evaluate whether patients with chronic ITP and H. pylori infection exhibit higher ROS levels compared to patients with chronic ITP and no H. pylori infection and whether there are statiscally significant differences between the two groups.
Methods: We studied 29 patients with chronic ITP (median age 39 years) hospitalized in the Clinic of Hematology, Filantropia City Hospital, Craiova, Romania, between 2014 and 2016 (informed consent obtained). All patients were diagnosed with ITP, other causes of thrombocytopenia having been ruled out by bone marrow aspiration. The patients were divided in two groups: patients with ITP and H. pylori infection (group A) and patients with chronic ITP without H. pylori infection (group B). Serological tests (ELISA) were used to indicate the presence of a H. pylori infection and reactive oxygen species were evaluated by FORT (Free Oxygen Radicals testing) test from a single drop of capillary blood, at the time of diagnosis, before the administration of any drug (the normal value of FORT is considered less than 2.3 mmol/L H_2O_2), using a CR3000 analyzer (Callegari SpA, Parma, Italy). The differences between the two groups were assessed using the Student T-test and a p-value of less than 0.05 was considered statistically significant.
Results: Group A consisted of 11 patients positive for H. pylori, whereas group B included 18 patients with no H. pylori infection. ROS levels, measured by the FORT test, were elevated in both groups (between 2,8 – 3.6 mmol/L H_2O_2). However, statistically significant differences were found in favour of group A, with higher ROS values than group B. The A group also associated lower platelet counts and more patients pertaining to this group relapsed in comparison to group B.
Summary/Conclusions: In chronic ITP, increased levels of ROS are associated with elevated autoantibody production. Autoantibodies are involved in platelet destruction via highly a immunogenic activity. On the other hand, association of H. pylori infection, via chronic inflammation, led to a supplementary increase in ROS levels and increased platelet destruction.

PB2106

IMMUNE THROMBOCYTOPENIA AND PREGNANCY: A SPANISH CASE SERIES OF 270 PREGNANCIES IN PRIMARY ITP.

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Background: Effect of pregnancy on the course of primary immune thrombocytopenia (ITP) is not well known. Besides, due to the lack of clinical assays, evidence about outcome predictors of pregnants and neonates born to mothers with ITP is scarce.
Aims: To evaluate management and results of pregnancy and delivery on pregnant ITP women and on their offspring.
Methods: All women diagnosed of primary ITP (according to international consensus criteria) from 2011 to 2016 in 23 Spanish Hematology Departments who had at least one pregnancy after ITP onset were included in this registry.
Results: We included 270 primary ITP pregnancies from 184 women. At pregnancy diagnosis, we observed a majority of chronic ITP cases (71.4%). At ITP diagnosis, median age of our case-series was 23 years (IQR, 19-29) and median platelet count was 18 x 10^9/l (IQR, 6-35). Median time from ITP diagnosis to pregnancy was 167 months (IQR, 0-366). Median number of pregnancies prior to ITP diagnosis were 1 (IQR, 0-2) with 1 pregnancy (IQR, 1-2) after ITP diagnosis as a median.

50.8% of women received corticosteroids, immunoglobulins (IVIG) (16.9%), rituximab (6.8%) and/or splenectomy (8.4%) as ITP treatments between or before new pregnancies. On the other hand, 26.4% of women needed treatment for ITP during pregnancy, mainly steroids (13.5%) and IVIG (10.2%).
The median platelet-count nadir during pregnancy was 74 x 10^9/l (IQR, 36-172). 127 (47%) pregnancies suffered from non-haemostatic platelet levels (less than 50 x 10^9/l) with 73 (27.0%) women who achieved less than 30 x 10^9/l. 56 (20.7%) women exhibited hemorrhagic symptoms, being 30 (11.1%) of them severe bleedings.
Regarding type of delivery, this was vaginal in 63.4% of pregnancies and cesarean sections 30.5%. Median platelet count at delivery was 110 x 10^9/l (IQR, 76-181). 43 patients (23.4%) experienced 57 bleeding episodes.
We only observed 48 cases (20.4%) of neonatal thrombocytopenia among 235 living newborns.
Summary/Conclusions: Our results are comparable to previously reported studies. No severe bleeding complications during pregnancy and/or delivery were observed in our case series. Rate of neonatal thrombocytopenia, and therefore, newborn bleeding is low.

PB2107

ANALYSIS OF THE DEMOGRAPHIC, CLINICAL, LABORATORY AND TREATMENT-RELATED DATA OF ITP PATIENTS IN GREECE BASED ON THE NATIONAL ITP REGISTRY OF THE HELLENIC SOCIETY OF HAEMATOLOGY

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Background: Immune thrombocytopenia (ITP) consists of various acquired disorders caused by autoantibodies against platelets resulting in increased platelet destruction and impaired thrombopoiesis. ITP is characterized as primary when an underling etiology cannot be identified and secondary when a certain etiology exists. Data concerning ITP characteristics at a national level are limited.
Aims: The purpose of the study was to access systematically the demographic, clinical, laboratory and treatment-related data of ITP in Greece based on the national database (ITP registry) operated and supported by the Hellenic Society of Haematology.
Methods: Patient data were collected over 2013-2016. The data source is a unique database initiated and managed by the Haematology Department of the University of Crete (UoC) and supported by the Center of Information and Communications Technologies of the UoC. The registry has been configured for national and regional base usage considering hospitals as the core unit. A certified researcher/administrator has access to a platform where he/she can record and study patients' data. The entire project has been developed using the robust open source tools of operating systems and Relational Data Base Management System (RDBMS) packages.
Results: We analyzed data from 696 adult ITP patients registered from 14 different hospitals from all parts of Greece. The median age at diagnosis was 53 years (range 15-97 years). Two peaks were observed at the age of 19-30 and 71-80 years. There was a female (60.89%) *versus* male (39.1%) predominance with higher frequency of females in younger (19-30 years) and of males in older (71-80 years) ages. Females appeared with more severe thrombocytopenia. The median platelet count at diagnosis was 15x10^3/ml. The majority of patients presented with hemorrhagic symptoms (70.9%). The main manifestations were cutaneous bleeding (64%), oral cavity bleeding (20.9%), epistaxis (8.9%), menorrhagia (7.8%) and gastrointestinal bleeding (5.5%). 430 patients (61.78%) had primary ITP and 266 (38.22%) secondary ITP. Among these secondary ITP cases, 44.22% were related to infectious agents, 25.74% to drugs, 17.17% to collagen vascular diseases and 12.87% to cancer. Patients with positive ANA antibodies with no evidence of any underlying diseases were included in the primary ITP group. The main patient comorbidities were hypertension (22.64%), thyroid disease (12.32%) and cardiovascular disease (10.17%). Treatment was given in 620 patients at diagnosis. Specifically, 577 (93%) patients were treated with cortocosteroids, 322 (51.9%) with intravenous IgG, 265 (42.7%) with both, and 112 (18%) received other treatments including rituximab (4.8%), anti-D immunoglobulin (4%) and thrombopoietin receptor agonists (4%). The majority of the patients (85%) responded to the initial treatment. Follow-up data for more than one year are currently available in 259 patients (133 with persistent ITP and 126 with chronic ITP). Splenectomy has been performed in 59/696 patients (8.47%).
Summary/Conclusions: Primary ITP is more frequent than secondary ITP in Greece, the disease displays two peaks at the ages of 19-30 and 71-80 years, presents a female predominance and high frequency of hemorrhagic symptoms. Treatment is mainly based on corticosteroids and/or intravenous IgG. Registration and follow-up of larger number of patients and evaluation of response to various treatments are anticipated to extend our knowledge on the pathophysiology and natural history of ITP and may also reveal peculiarities at local level.

PB2108

PRESENTING SYMPTOMS AFFECT OUTCOME IN IMMUNE MEDIATED THROMBOTIC THROMBOCYTOPENIC PURPURA

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Background: Whilst immune mediated Thrombotic Thrombocytopenic Purpura (TTP) has classically been suspected by the presence of a pentad of symptoms (microangiopathic haemolytic anaemia, fever, disturbed neurological function, renal failure, thrombocytopenia), the limitations of this have long been recognized and a wide variety of symptoms are seen on initial presentation.
Aims: A retrospective review of the significance of specific symptoms and their duration on mortality.
Methods: A retrospective review of all consecutive admissions to a single tertiary center between 2009 and 2015. Only patients who required plasma exchange were included. Patients' symptoms and their duration were reviewed in addition to presenting anti-ADAMTS13 IgG antibody levels and ADAMTS13 antigen levels, both of which have previously been found to have prognostic significance.
Results: 106 patients (68% female) were included with a median age of 48. 58% were Caucasian and 19.8% Afro-Caribbean. The mortality rate was 7.4% (n=8). 47% of patients had neurological symptoms on presentation, 24% reported a bleeding history and 12% a recent infection. The most common presenting symptoms were headache (27.4%), bleeding (24%) spontaneous bruising/petechial rashes (19.8%), speech disturbances (encompassing expressive/receptive dysphasia, aphasia, dysarthia and slurred speech, 19.8%) and TIA or stroke like symptoms encompassing hemiplegia or facial weakness/droop (16%). The highest rates of mortality were seen in patients who experienced loss of consciousness (mortality 33.3%), abdominal pain (mortality 22.2%) and heavy bleeding (mortality 16.7%). The anti-ADAMTS13 IgG level was not however significantly higher in these symptoms when compared to others (Table 1) suggesting microangiopathic thrombosis location plays an important role in TTP prognosis. The median duration of symptoms prior to presentation was 7 days (range 1-60 days). 8.5% of patients were asymptomatic, all relapsed TTP. Patients in the highest quartile for symptom duration (>10 days) had significantly higher anti-ADAMTS13 IgG antibody level than those in the lowest quartile for symptom duration (<2 days) (65% *vs* 26%, p=0.002) and may have increased mortality (symptoms <2 days mortality 7.4%, symptoms >10 days 14.3%, p=0.19).

Table 1.

Symptom (n)	Mortality	Antibody (NR: <6%)	Antigen (NR: 74-134%)
LOC (6)	33.3%	44%	2.9%
Abdominal pain (9)	22.2%	50%	3.3%
Heavy bleeding (18)	16.7%	28%	2.9%
Headache (29)	3%	44%	4.5%
Speech Disturbance (21)	4.8%	48%	4.2%
Tia/stroke symptoms (17)	5.9%	42%	4.8%
Pyrexia (16)	12.5%	32%	5.7%
Lethargy (15)	13.3%	58%	7%
Spontaneous Bruising (15)	6.7%	56%	6.4%
Confusion (10)	0%	48%	4.2%
SOB (10)	10%	45%	4.1%

Summary/Conclusions: Whilst there is little difference in the anti-ADAMTS13 IgG antibody and ADAMTS13 levels seen with difference symptoms, there is a wide disparity in terms of mortality suggesting the effect of microangiopathic thrombosis differs by location. Abdominal pain, not previously recognized as a significant symptom in TTP, seems to be a poor prognostic indicator although this should be interpreted with caution given the sample size. Anti-ADAMTS13 IgG antibody level increases with symptom duration and this may lead to increased mortality.

PB2109

NOVEL TECHNIQUES FOR MONITORING GALNZMANN THROMBASTENIA PATIENT UNDERGOING SURGICAL INTERVENTIONS

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Background: Glanzmann thrombasthenia (GT) patients undergoing surgical procedures are often treated by platelet transfusion. However many GT patients who have been previously exposed to platelets may form antibodies either against the missing αIIbβ3 antigen or directed against MHC-class molecules thus hampering the efficacy of care. Due to the rarity of disease there is paucity of data regarding platelet transfusion protocols during the perioperative period. We herein describe our experience with monitoring the proportion of donor platelets following transfusion, and their contribution to whole blood clot formation.
Aims: To describe the use of flow cytometry (FC) analysis in order to detect donor transfused platelets in A GT patient undergoing a minor surgical procedure and to assess the correlation between FC analysis and the results of Rotational thromboelastography(ROTEM).
Methods: A nine year old female patient with GT underwent teeth extraction. The Patient received platelet transfusion around the procedure. Complete blood counts, ROTEM, FC to detect the number of donor platelets and their ADP dependent activation, were sampled and followed till 7 days post teeth extraction.
Results: Prior to teeth extraction upon injection of local anesthetics patient developed a buccal hematoma probably owing to local blood vessel penetration. The patient did not experience any post extraction bleeding. Hematoma was absorbed within several days. Post transfusion platelets FC demonstrated 20.6%% donor platelets equivalent to 55,620 donor platelets. Platelets activation was determined following ADP addition by examination CD62 antigen expression. Seven days post platelet transfusion FC demonstrated 2.6% equivalent to 8,658 donor plantlets. The decline in the number of active platelets was associated with a reduced clot firmness (MCF) and lower α-angle as assessed by ROTEM (Figure 1).

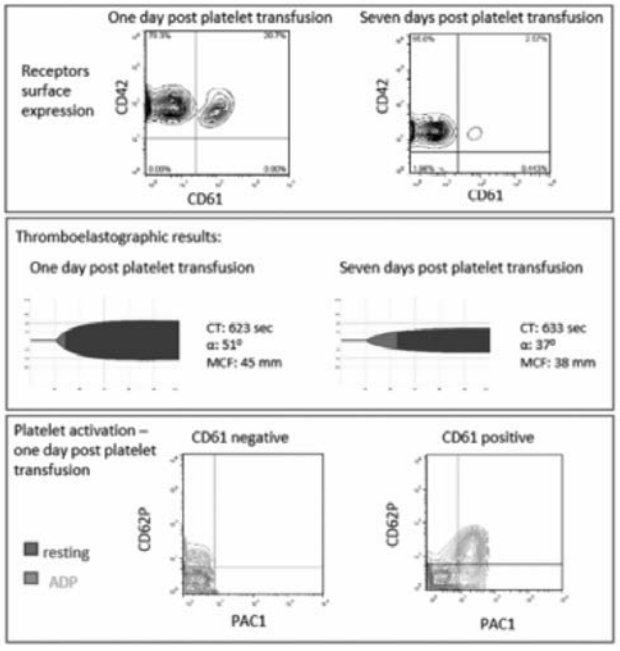

Figure 1.

Summary/Conclusions: Clinical decision making in patients with GT may be aided by application of novel techniques, evaluating the number of active donor platelets and actual clot formation. This data may help making more knowledgeable decisions as for the need for further platelet transfusion or for the need for rFVIIa. Thus leading to improved monitoring and better patients' care.

PB2110

CAN HISTOCHEMICAL C-MPL POSITIVITY IN BONE MARROW BE A PREDICTOR FOR SPLENECTOMY IN IMMUNE THROMBOCYTOPENIA?

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Background: Splenectomy is used as the second line therapy in patients with immune thrombocytopenia (ITP). However, there is no parameter predicting splenectomy decision.
Aims: Aim of the present study was to evaluate immune histochemical Cloned Myeloid Leukemia Virus (c-mpl) positivity in bone marrow specimens of ITP patients with or without splenectomy indications.
Methods: Bone marrow specimens were taken from 24 patients who were diagnosed with ITP and who had splenectomy (15 female, 9 male, mean age 50±16) before splenectomy and 30 patients who were diagnosed with ITP but did not have splenectomy (15 female, 15 male, mean age 52±19). c-mpl staining was carried out retrospectively. Immunohistochemical (IHC) staining using Avidin-Biotin complex system (ABC) was conducted. For IHC, dissections prepared from blocks were taken onto poly-L-lysine coated slides (MicroSlides Snowcoat X-tra, Surgipath, Richmond, IL, USA) and kept in an incubator at 37 °C overnight. Dissections were treated with IHC c-mpl (Santa Cruz/sc-13187) stain. Cytoplasmic and nuclear staining was observed in megakaryocytes using IHC c-MPL and vitamin D. Evaluation was made based on the intensity of the staining; *i.e.* negative (0), weak (1+), moderate (2+) and strong (3+) (1). All patients who had splenectomy were in chronic phase of the disease. The present study was supported as a Scientific Research Project by Adnan Menderes University (TPF-15027).
Results: c-mpl positivity was statistically significant in patient group who did not have splenectomy (Table 1). In patient group who had splenectomy, c-mpl was not associated with refractory status.

Table 1. c-mpl positivity in patients group who had and did not have splenectomy.

	with splenectomy(n)	without splenectomy (n)	p value
c-mpl positivity(n)	1	12	‹0.001
c-mpl negativity(n)	23	18	‹0.001
Total (n)	24	30	‹0.001

Summary/Conclusions: Status of c-mpl in ITP is ambiguous. Significant level of positivity in patient group who did not have splenectomy might the useful for splenectomy indication.

PB2111

CLINICAL SIGNIFICANCE OF IMMATURE PLATELET FRACTION MEASUREMENT IN THROMBOCYTOPENIC DISORDERS DURING PREGNANCY

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Background: Thrombocytopenia is the second most common hematologic abnormality during pregnancy and is usually a benign condition. The challenge to the clinician is to weigh the risks of maternal and fetal bleeding complications against the benefits of diagnostic tests and interventions. This condition can also be associated with several diseases, either pregnancy specific or not, such as preeclampsia, HELLP syndrome, or idiopathic thrombocytopenic purpura (ITP). The differential diagnosis between ITP and gestational thrombocytopenia is clinically important with regard to the fetus, due to the risk of neonatal thrombocytopenia. The immature platelet fraction (IPF) is young cells that have recently been released into the circulation, and are considered indicators of bone marrow recovery. They contain a higher concentration of RNA than mature platelets. Measure of immature platelet fraction (IPF) has been suggested as a less invasive and early diagnostic test in the study of thrombocytopenic disorders. Immature platelet fraction can be currently measured by fully automated hematology analyzers providing clinical utility for diagnosing and monitoring thrombocytopenia.
Aims: The aim of this is to know whether IPF can be a useful parameter in pregnant women with thrombocytopenia to predict the potential risk of bleeding.
Methods: Pregnant women with thrombocytopenia were selected (2015-2016); a total of 25 patients (mean age: 33 yrs, range 19-43 yrs) were examined with platelet count <100.000 platelets/μL. Venous whole-blood samples were collected into Vacutainer EDTA-K2E tubes (Becton Dickinson and Company, Plymouth, UK). Complete blood counts and immature platelet fraction (%IPF) were immediately analyzed within 2 h of blood withdrawal by Sysmex XN20 system (Sysmex Corporation, Kobe, Japan). Novel PLT-F channel uses fluorescent light and stains platelets specifically with Oxazine Dye (Fluorescent Fluorocell). Bleeding complication has been collected in order to know if there is related to%IPF.
Results: Mean platelet count was 73.000 platelets/μL (range of 69-91) and IPF mean was 11% (2,5-23,4). Lab test Hemoglobin shows a mean of 95,17 g/L [range of 45-132] (in no-bleeding group was 105,8 g/L whereas in bleeding-group was 86,14 g/L p=0,0768) p=0,07. IPF% was <10% in 11, which means a 44% of the patients. 14 patients bleed during or after labor, 56% among all the patients in this study. Related to this group, 11 patients had IPF <10%; 3 of bleeding patients showed an IPF >10%. All pregnant women with an IPF <10% (11/11) bleed as a complication. Pregnant women with thrombocytopenia and a IPF <10% has a higher risk of bleeding during and/or after labor compared with pregnant women with a IFP>10% (Fisher 12,41, P<0,001). 5 (20,83%) patients among all of them were under treatment (earlier or during labor): 3 (12,5%) with steroids and 2 (8,33%) with other methods.

Summary/Conclusions: Thrombocytopenia is a potential risk of bleeding during the labor. A high IPF indicates either consumptive or recovering thrombocytopenic disorders, such as immune thrombocytopenic purpura, while low IPF is characteristic of bone marrow suppression states. Although not directly used in clinical decision making, the reference range is critical to the introduction of new parameters and the interpretation of laboratory results. Our results suggest that IPF is an easy laboratory parameter to be measured and a level <10% might be an independent bleeding factor which can be useful for detecting high risk pregnant patients. It should be corroborated in further studies.

PB2112

DOES EARLY RESPONSE TO FIRST LINE CORTICOSTEROID THERAPY PREDICT REQUIREMENT FOR SECOND LINE THERAPY IN IMMUNE THROMBOCYTOPENIA?

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Background: Immune thrombocytopenia (ITP) is an acquired,immune-mediated disease that is characterized by increased destruction of platelets by autoantibodies. ITP is characterized by mucocutaneous bleeding. Rarely, life-threatening bleeding such as central nervous system bleeding can occur. Typically, patients have isolated thrombocytopenia. The diagnosis of ITP is one of exclusion. Corticosteroids are chosen as a first-line therapy for adult patients who require treatment. Responses to first line therapy with corticosteroids is about 80% with approximately 20% to 30% long term complete remission. Most patients finally relapse, requiring second-line therapy.
Aims: Our aim was to investigate potential effects of early platelet response to corticosteroid therapy on achieving long term complete remission.
Methods: We retrospectively evaluated 43 ITP patients who were followed-up at our institution. All patients' thrombocyte counts were below 30 x10^9/L at diagnosis. All patients received initially methylprednisolone(MP) 1 mg/kg/day. For patients who responded with platelet count ≥150 x10^9/L methylprednisolone was tapered over 3 months. Those who were unresponsive to MP or relapsed after a complete response, were treated with second line therapies that splenectomy or medical treatment agents. The platelet counts of the patients on day 0, 3 and 7 were evaluated by complete blood counts and were confirmed with peripheral smears examination. Effect of the platelet counts on day 3 and 7 were compared in terms of second line therapy requirement or not. A platelet count of >30x10^9/L on day 3 and >100x10^9/L on day 7 was considered as a complete response. Vaccination against encapsulated organisms was given and imaging was done to detect accessory spleen before splenectomy.
Results: Baseline characteristics of the cohort of 43 patients with an initial diagnosis of ITP are shown in Table 1. The mean age at diagnosis was 51 years (18-84) with female/male : 25/18. All patients presented with severe thrombocytopenia (platelet counts below 30.0x 10^9/L). Most patients presented with mucocutaneous bleeding (n=39), only three patients had genitourinary or gastrointestinal tract bleeding and one patient was asymptomatic. Bone marrow aspiration and biopsy was done in 14 (%32.6) patients due to various reasons mainly, failure to respond to ITP treatment (7 patients) and advanced age (7 patients). On third and seventh day of MP therapy, median platelet counts were 30x10^9/L (2,0 x10^9/L -150 x10^9/L) and 100 x10^9/L (1,0 x10^9/L-347 x10^9/L), respectively. When platelet counts on the 3rd and 7th day in each patient were compared, a significant association was found in correlation analysis (*p<0.05*). 21 patients (%48.8) required second line therapy which were splenectomy (76.2%) or medical treatment (23.8%). Medical therapy consisted of rituximab, eltrombopag, danazol. There was a statistically significant difference between the patients with platelet count below and over 30x10^9/L on 3rd day of the MP therapy in terms of requirement for a second line therapy. (*p=0.04*). On the other hand, when 7th day was taken into consideration, there was not a statistically significant difference when cut off was taken as 100 x10^9/L (*p=0.09*) or 50 x10^9/L (p=0.06).
Summary/Conclusions: In the era of novel therapies used as second line, predicting the prognosis of an ITP patient at diagnosis has been a challenge. If disease related factors at diagnosis can be identified, then patients can be rescued from long term corticosteroid exposure and its adverse effects. Early response of platelet counts after starting corticosteroids seems to predict long-term complete remission. Expanding the study population may contribute more to our findings.

PB2113

THE IMPACT OF FC GAMMA RECEPTOR IIA AND IIIA GENE POLYMORPHISMS ON THE THERAPEUTIC RESPONSE OF RITUXIMAB IN EGYPTIAN ADULT IMMUNE THROMBOCYTOPENIC PURPURA

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Background: Chronic primary immune thrombocytopenia (ITP) is an acquired autoimmune disease characterized by enhanced clearance of platelets and impaired platelet production. Corticosteroid is the ministry line of treatment of ITP, patients who fail to respond to steroid (steroid resistant) or who relapse (steroid dependant) face the options of treatment with second line including anti CD-20 monoclonal antibody rituximab. Rituximab is a chimeric IgG1 monoclonal antibody (mAbs). The major mechanism of action of rituximab is the antibody-dependent cellular cytotoxicity (ADCC), ADCC effectiveness is influenced by process of activation of effector cells via their immunoglobulin G fragment C receptors (FcγRs). Fcγ receptors show distinct affinity to bind to IgG subtype specificities. Differential response to rituximab has been reported to correlate with specific polymorphisms in two of FcγR genes: FcγRIIa (H131R) and FcγRIIIa (V158F) in some diseases.
Aims: To clarify the effect of FcγRIIa-131 R/H and FcγRIIIa-158 V/F genes polymorphism on the response to rituximab in ITP patients.
Methods: We studied the frequency of the FCγRIIa (H131R) and FcγRIIIa (V158F) gene polymorphisms, in 100 chronic ITP patients; divided into 2 equal groups, first group received rituximb (375 mg /m2 per dose weekly for four weeks) and the other group received non-mabthera second line therapy. A polymerase chain reaction-restriction fragment length polymorphism assay [PCR-RFLP] was used to detect FcγRIIa-131 R/H and FcγRIIIa-158 V/F genes polymorphism. Evaluation of platelets counts was assessed initially before starting second line therapy then weekly for 3 months. At the end of third month the response to second line therapy was considered according to the following criteria; complete response (CR) PLT >100×10^9/L., partial Response (R), PLT=30-100×10^9/L , no response (NR), PLT<30×10^9/L.
Results: Regarding FcγRIIa gene (H131R) polymorphism distribution in the 100 patients; 28 patients (28%) had wild HH genotype, 41 patients (41%) have heterozygous genotype (HR) and 31patients (31%) have homozygous mutant genotype (RR). In our study, the 100 ITP patients included showed wild type of FcγRIIIa (V158F) gene polymorphism. By the end of month 3 of the second line therapy, 43/100 patients (43%) achieved CR, 37/100 patients (37%) achieved PR and 20/100 patients (20%) achieved NR. Among the 50 patients who treated with Rituximab; 18 patients (36%) achieved CR, 19 patients (38%) achieved PR and 13 patients (26%) achieved NR. Out of the 18 patients who achieved CR, 8/18 patients (44.4%) carried FcγRIIa RR genotype and 7/ 18 patients (38.9%) carried FcγRIIa HR, compared to 3/18 patients (16.7%) carried FcγRIIa HH genotype. However it was not statistically significant. Among the 13 patients who achieved NR, lowest rate was patients carried FcγRIIa RR genotype 3/13 patients (23.1%) compared to HR (38.5%) and HH (38.5%) genotypes. However it is not statistically significant. The mean value of platelet count at end of week 1, Week 2 and Week 3 of rituximab therapy show statistically significant differences (P value 0.001) being higher in patients achieved CR than who achieved PR or NR.
Summary/Conclusions: The higher platelet count achieved early (end of week 1, 2, and 3) after rituximab is suggestive for a better response later (at end of M3). FcγRIIa RR genotype is predictive for better response to rituximab in ITP patients.

PB2114

IMMUNE THROMBOCYTOPENIA. EGYPTIAN EXPERIENCE WITH STUDY OF IL-17,TGFB, IL-35 AND IL-12 CYTOKINES IN CHRONIC AND PERSISTENT IMMUNE THROMBOCYTOPENIA PATIENTS

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Background: The role of T cells in the pathophysiology of immune thrombocytopenia (ITP) is heterogeneous and complex.It has been studied in active and reactive ITP but not to same extend in chronic and persistent type.
Aims: In this study we review the demographic features of 150 immune thrombocytopenic Egyptian patients and for cases who were chronic and persistent with negative both autoimmune screen and virology for hepatitis B and C
Methods: We measured IL-12, IL-35, IL-17 and TGF-β by ELISA to assess role of subtypes of T cells in the pathophysiology of ITP.
Results: Our results revealed Chronic and persistent cases who fulfilled the criteria for cytokine assay were 45 cases with a mean (± SD) age of 31.60±8.78 years. Thirty two patients were presented by skin manifestations (71.1%).Eight patients presented with mucous bleeding (17.8%) and five patients presented by combined skin and mucous membrane bleeding (11.1%). Comparison between the cases studied and control groups revealed statistically significant lower platelet count in cases rather than the control. While the four measured cytokines were statistically significant higher in cases rather than the control. Correlation between platelet count and the level of cytokines was statistically insignificant. All cases were under treatment by low dose corticosteroid in addition to another immunesuppression medication. No correlation between measured cytokines and platelet count.
Summary/Conclusions: The higher expression of IL-12 and IL-35 is due to persistently higher TH1 activity which explain continuity of the disease.while the higher expression of Treg cytokines (IL-17 and TGF-B) may be explained by effect of immune suppression use or up regulation of their receptors on Treg cells which have resistance to their activity. In chronic ITP, the level of Tcell cytokines can't predict the course of disease.

PB2115

SWITCH OF TPO-MIMETICS IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA: FLORENCE MONOCENTRIC EXPERIENCE

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Background: Primary immune thrombocytopenia (ITP) is an immune-mediated condition characterized by isolated thrombocytopenia, with peripheral blood platelet count of <100.000/µl in the absence of an identifiable underlying cause of thrombocytopenia. Clinical studies in patients with ITP demonstrated that thrombopoietin (TPO) mimetics increase platelet production and can outpace platelet destruction.
Aims: We evalueted patients treated with both TPO-mimetics.
Methods: From November 2008 and February 2017, 65 patients were treated with TPO-mimetics with a median follow up of 29 months (1-96): 39 patients underwent therapy with Romiplostim and 26 to Eltrombopag. In our study we evaluated 18 patients who received both therapies: among patients treated at first with Romiplostim, 10 patients (9F; 1 M) switched to Eltrombopag and 8 patients (3 M; 5 F) switched from Eltrombopag to Romiplostim. In the group of 10 patients treated at first with Romiplostim, 5 patients started Eltrombopag because were no responders, 3 for loss of response and 2 patients because of adverse events. In the group of 8 patients at first treated with Eltrombopag, 4 patients didn't obtain any response with Eltrombopag and switched to Romiplostim, 1 patient underwent to Romiplostim for loss of response and 3 patients because of adverse events.
Results: Among patients switched from Romiplostim to Eltrombopag, 2 achieved complete response, 4 response and 4 were no responders; among patients switched from Eltrombopag to Romiplostim, 4 obtained complete response, 3 response, 1 was no responder.
Summary/Conclusions: Romiplostim and Eltrombopag stimulate the TPO-R but have different mechanisms of action, therefore, in our limited experience switching from one thrombopoietic receptoragonist to the other could be beneficial in clinical practice for patients with severe chronic immune thrombocytopenia who failed to respond or experienced adverse events to the first treatment.

PB2116

COEXISTENCE OF GLANZMANN'S THROMBASTHENIA AND MAPLE SYRUP URINE DISEASE: IMPLICATIONS FOR HEMOSTATIC MANAGEMENT

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Background: In Oman, autosomal recessive disorders are relatively commoner than western communities due to the high prevalence of inter-tribal marriage. Unfortunately, some patients have got more than one autosomal recessive genetic disorder, owing to complex consanguinity which might further complicate proper management plans.
Aims: To report an interesting case of combined Glanzmann's thrombasthenia and MSUD, and to review the existing data of platelet function disorders in Oman.
Methods: Case report and retrospective data analysis of all cases with confirmed or suspected platelet function disorders in Pediatric Hematology Unit, Sultan Qaboos University Hospital, Muscat, Oman from January 2006 till December 2016.
Results: We report a 4 year-old girl who is a known case of MSUD. Her parents are double first cousins (from both maternal and paternal sides). At the age of 3 months, she required Gastrostomy tube (G-tube) insertion. Preoperatively, full blood count and coagulation screen were perfectly normal. Unfortunately, she developed profuse bleeding at the site of G-tube insertion, followed by massive hematemesis. The patient received multiple blood products, but bleeding didn't stop. As an emergency measure, recombinant activated factor VII (rFVIIa) was given and resulted in cessation of bleeding. Platelet aggregation studies revealed defective aggregation with ADP, arachidonic acid, collagen and epinephrine which is consistent with Glanzmann's thrombasthenia. The diagnosis was further confirmed by platelet flow cytometry which showed no activity with CD41 and CD61, indicating absent GpIIb/IIIa complex. The patient experienced a severe bleeding phenotype, which is further complicated by multiple coexisting factors, including the recurrent episodes of metabolic crises which provoked worsening of platelet function, the development of platelet refractoriness at the age of 1 year , and the need for recurrent invasive procedures such as G-tube and central line insertion. Currently, the bleeding episodes are managed by rFVIIa at a dose of 120-180 µg /kg/dose. Excluding von Willebrand disease, we have 38 cases of confirmed or suspected platelet function disorders in our center, including 15 cases with Glanzmann's thrombasthenia, 7 cases with Bernard-Soulier syndrome, 5 cases with May–Hegglin anomaly and 11 cases of suspected, yet unconfirmed platelet storage pool deficiency.
Summary/Conclusions: In conclusion, children with platelet function disorders still have plenty of unmet needs, ranging from deficient accurate diagnostic facilities to the lack of agreed upon consensus management guidelines. The coexistence of another hereditary disorder may result in mutual management difficulties of both diseases. In developing countries, proper registry is needed to establish optimum care of such rare disorders.

PB2117

ASSESSMENT OF PLATELET REACTIVITY TO ASPIRIN AND CLOPIDOGREL WITH POINT-OF-CARE VERIFYNOW® ASSAY AND TWO ALTERNATIVE METHODS IN PATIENTS WITH CEREBRAL ANEURYSMS TREATED WITH ENDOVASCULAR PROCEDURES

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Background: Stent thrombosis and hemorrhage are the main complications after endovascular procedures for cerebral aneurysm treatment. Identifying an optimal pre-procedure response to antiplatelet therapy is essential to guarantee a successful result. A high variability in the individual responses to the antiagregant effect of aspirin and, specially, with clopidogrel has been reported. The VerifyNow® System (Accumetrics, San Diego, CA, USA) performs a turbidimetric-based optical detection of induced platelet aggregation in response to major antiplatelet agents (P2Y12 inhibitors, aspirin, GP IIb/IIIa inhibitors).
Aims: 1) To measure the antiplatelet effect of aspirin and clopidogrel with the point-of-care VerifyNow® assay in patients with brain aneurysms before undergoing endovascular treatment. 2) To compare the results with two alternative methods: impedance aggregometry. and PFA-100.
Methods: 38 patients with cerebral aneurysms, scheduled for elective endovascular procedure, were included in the study. All of them had started taking aspirin at a dose of 100 mg daily and clopidogrel at a dose of 75 mg daily 7 to 10 days before testing aspirin and clopidogrel sensitivity. The following functional tests were performed in all of them before the procedure: 1) VerifyNow® assay: Aspirin Reaction Units (ARU) <550 and P2Y12 Unit Reaction Units (PRU) <208 were considered to be good response to aspirin and clopidogrel respectively. PRU <85 was considered hyper-response to clopidogrel. 2) Impedance aggregometry from whole blood (Multiplate® analyzer, Roche Diagnostics, Mannheim, Germany): arachidonic acid (AA), adenosine diphosphate (ADP) and thrombin receptor activating peptide (TRAP) were used as agonists. TRAP was used to determine baseline platelet function. Aggregation with AA <40 U and aggregation with ADP <47 U were considered good responses to aspirin and clopidogrel respectively. 3) PFA-100: an overall assessment of platelet function was performed using epinephrine-collagen (COL/EPI) and ADP-collagen (COL/ADP) cartridges. Although COL/ADP is not an appropriate method to evaluate the effect of tienopyridines, we performed it to analyze whether hyper-responders to clopidogrel detected by VerifyNow® were also identified with PFA-100.
Results: The results of platelet function testing with three different methods are summarized in Table 1. None of the patients showed thrombocytopenia. Good response to aspirin was observed in 84.21%, 97.36% and 93.75% of the patients using VerifyNow®, Multiplate® and PFA-100 respectively. Good response to clopidogrel was detected in 86.84%, 38.88% and 62.5% of the patients using VerifyNow®, Multiplate® and PFA-100 respectively. VerifyNow® identified 6 (15.78%) aspirin-resistant patients. However, PFA-100 and Multiplate® assays showed a significant aspirin-mediated platelet dysfunction in 5 of them. Low response to clopidogrel was detected by Verifynow® in 5 (13.15%) patients consistent with Multiplate® results. Verifynow® identified 10 patients with excessive response, but only 2 of these results were reproduced by Multiplate® or COL/ADP. Multiplate® detected 19 patients (50%) with suboptimal response to clopidogrel, although these results did not correlate with those obtained by VerifyNow®.

Table 1.

	Platelet Count	Verify Now		PFA-100		Platelet aggregation		
	NV: 150-450x10³/µL	ASP	P2Y12	COL/EPI sec	COL/ADP sec	AA (0.5 M/m)	ADP (6.5 µM)	TRAP(32 µM)
Patient number		NV <550 ARU	NV <208 PRU	NV: 94-155	NV: 71-119	NV: 55-117	NV: 79-141	NV: 92-152
1	190000	444	216			4	59	110
2	304000	384	39	283	280	11	54	100
3	272000	430	187	>300	68	15	77	136
4	275000	384	32	289	285	17	17	
5	305000	387	166	>300	>300	18	83	
6	243000	350	250			28	57	134
7	318000	403	186	>300	69	10	44	
8	210000	560	165	218	139	18	67	
9	268000	562	165	>300	>300	6	15	130
10	268000	350	6	>300	>300	9	54	109
11	171000	441	220	>300	88	17	67	
12	383000	385	44	287		22	39	112
13	183000	391	184			29	72	127
14	140000	594	155	>300	108	56	56	90
15	304000	379	90			39	69	153
16	278000	350	36	259	58	26	48	164
17	319000	350	49	>300		22	45	153
18	343000	382	32	279	283	30	46	109
19	171000	384	3	>300	>300	18	33	133
20	208000	496	168	>300	80	19	47	126
21	190000	562	126	>300		22	78	120
22	156000	383	157	293		8	38	96
23	210000	378	144	>300	123	33	70	142
24	157000	377	183	>300	76	9	43	141
25	261000	378	138	275	277	26	65	122
26	282000	485	151	>300	124	22	38	99
27	216000	466	194	199	245	15	16	63
28	255000	449	61	143	84	19	27	105
29	194000	584	261	300	231	22	73	157
30	224000	562	195	116	>300	9	56	100
31	320000	405	187	>300	>300	46	135	167
32	352000	557	39	>300	>300	18	43	146
33	261000	467	96	>300	290	30	57	142
34	271000	405	210					
35	220000	394	90	285	300	31	103	159
36	191000	384	185	283	110	31	50	133
37	344000	448	149					
38	240000	527	109	150	300	15	33	98

Summary/Conclusions: The effect of aspirin can be accurately measured by platelet aggregation and PFA-100 (with COL/EPI); however, VerifyNow® seems to identify a higher number of poor responders. Multiplate® assay using only

ADP is not good enough to detect clopidogrel-mediated platelet dysfunction since it is not specific for the P2Y12 receptor. The addition of PGE1 to the ADP test may increase its sensitivity. VerifyNow® assay seems to overestimate the effect of clopidogrel, since hyper-response data are not reproduced by other techniques. According to our results, a high interindividual variability in response to clopidogrel is observed.

PB2118

THROMBOPOIETIN-RECEPTOR AGONISTS IN ITP - EXPERIENCE OF A CENTER

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Background: Thrombopoietin-receptor agonists (TRA), romiplostim and eltrombopag, are part of the treatment of chronic immune thrombocytopenia (ITP), resistant to first line therapy (corticosteroids and/or immunoglobulins) and with a significant bleeding risk. Both are approved for adult patients, but only eltrombopag was approved for pediatric use. When used before splenectomy, these treatments may serve as a bridge for surgery or even postpone/avoid the procedure.
Aims: In this report, we aim to evaluate the response to TRA treatment in patients with ITP and associated side effects in our center.
Methods: Inclusion criteria: patients with ITP resistant to first line treatment. Patients characteristics, response to TRA, clinical evolution and adverse effects were evaluated by retrospective analysis.
Results: Thirty-eight patients with ITP were included: 31.4% (12) were male and the median age at diagnosis was 38 years. 44.7% (17) had relapsed/resistant disease after splenectomy and 13.2% (5) were treated with a TRA as a bridge for this procedure. Sixteen (42.1%) of ITP patients were treated with romiplostim: 12 patients (75%) had a response to treatment, and 4 (25%) were resistant. In 11 of these patients, romiplostim was replaced by eltrombopag, either because of resistant disease, or more convenient administration (oral therapy). Thirty-three (86.8%) patients were treated with eltrombopag (5 pediatric cases): 27 patients (81.8%) responded while 6 patients had resistant disease (3 of these were HIV positive). The response rate was higher in patients with previous splenectomy (91.7% with romiplostim and 92.9% with eltrombopag) compared to those with no previous splenectomy (25% with romiplostim and 73.7% with eltrombopag). Six patients maintained response after treatment suspension (5 treated with eltrombopag and 1 treated with romiplostim). Generally, both treatments were well tolerated, with only one case of eltrombopag suspension because of a thromboembolic event.
Summary/Conclusions: In the current study, both TRA were effective in the treatment of ITP resistant to several lines of treatment, with similar response rates. As described in the literature, the response rate was higher in patients with previous splenectomy, and some cases maintained response after treatment suspension. The toxicity profile was acceptable. However, there are some concerns about their safety in long term therapy, namely the development of myelofibrosis, cytogenetic abnormalities and malignant evolution. Consequently, there is an urgent need for prospective studies to define the optimal period of treatment and surveillance, especially in pediatric patients. In our center, the median time of treatment with eltrombopag for all patients was 5.5 months (range between 1 to 34 months) and with romiplostim was 12 months (range between 1.5 to 85 months). The duration of treatment with eltrombopag in children and adolescents was around 6 months.

PB2119

THE EVALUATION OF REACTIVE OXYGEN SPECIES IN ESSENTIAL THROMBOCYTEMIA AND CORRELATION WITH JAK2V617F MUTATION

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Background: Essential thrombocytemia (ET) is a clonal disorder of the hematopoietic stem cells characterized by excessive myeloid proliferation, with predominant megakaryocytic expansion and a potential of transformation to acute myeloid leukemia. 50 to 60% of ET cases present a JAK2V617F mutation. 5% to 10% of JAK2V16F negative ET patients have MPL mutations at codon 515 and 50% to 70% of ET patients with non-mutated JAK2 and MPL (double-negative) carry mutations at exon 9 of CALR. Genomic instability in ET may be associated with an increased level of reactive oxygen species (ROS) which also leads to DNA damage. Hematopoietic stem cells of JAK2V617F positive murine models have higher ROS levels than found in normal mice (Marty et al, 2013).
Aims: To evaluate ROS levels in patients with ET and to observe if JAK2V617F positive cases associate higher ROS levels compared to patients without JAK2V617F mutation.
Methods: We studied 23 patients with ET admitted to the Clinic of Hematology, Filantropia City Hospital, Craiova, Romania, diagnosed with ET according to the 2008 revised WHO criteria (informed consent obtained). All analysis were performed after diagnosis and before the start of therapy. The JAK2V617F mutation was detected by allele specific polymerase chain reaction (PCR) testing. ROS levels were detected by flow-cytometry using a Cy Flow Space Sysmex flow-cytometer and a DCFDA Cellular ROS Detection Assay Kit. Studied parameters were compared both to healthy controls and to each other. Exclusion criteria were presence of any condition associated with an increased oxidative status (alcohol consumption, smoking, diabetes mellitus, hyperlipidemia, chronic renal failure, human immunodeficiency, cirrhosis, and active infection), use of antioxidants or iron supplementation. Data analysis was performed using Flow Max software. The differences between the two groups were assessed using the Student T-test and a p-value of less than 0.05 was considered statistically significant.
Results: The study group involved 12 females and 11 males, with a median age of 48 years. All patients had increased ROS levels at diagnosis compared to healthy controls. Eleven patients had JAK2V617F mutation and twelve were JAK2V617F mutation negative. Significantly higher ROS levels were found in JAK2-positive patients compared to JAK2-negative patients.
Summary/Conclusions: In our study, patients with ET had increased ROS levels. Cases with JAK2V617F mutation associated higher ROS levels compared to those without JAK2V617F mutation. In our future research, we will focus on the follow-up of these patients for a period of four years and we will try to observe if increased ROS levels enhanced genomic instability and transformation to acute myeloid leukemia.

PB2120

VARIATIONS IN PARAMETRES OF PLATELET COUNT AND PLATELET VOLUME ACCORDING TO GESTATIONAL AGE

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Background: Reference ranges of haematological parameters in preterm infants are limited.
In hematological evaluation not only platelet (PLT) counts but also 3 important platelet volume parametres (mean platelet volume [MPV], platelet distribution width [PDW], plateletcrit [PCT]) are also taken into consideration.
Aims: We wanted to investigate the impact of gestational age by determining variations in platelet volume parametres according to gestational weeks.
Methods: Medical records were prospectively reviewed in preterm infants admitted to Firat University Hospital from January 2001 to December 2007. Study group consisted of only one-hour-old newborns delivered in the clinics of Department of Gynecology, and Obstetrics of our hospital. The exclusion criteria included those with maternal history of antepartum haemorrhage, chorioamnionitis, fever, sepsis, preeclampsia and hypertension; and perinatal history of twin-to-twin transfusion syndrome, feto-maternal transfusion, injury and infection. A hundred and ninety-three newborns with apparent health problems were excluded from our study. Study group comprised 398 preterm infants born between 26-37 gestational weeks, and 63 healthy term (38 gestational weeks) infants. Blood samples from all cases were obtained within the first hours after birth. Blood samples were placed into tubes with EDTA, and analyzed in ADVIA 120® (Japan) hematology analyzer using suitable kits. Data were expressed as mean±standard deviation. Platelet counts, and volume were indicated for each gestational week, and groups of 24-31, 32-36, 37, and 38 weeks. One-way analysis of variance (ANOVA) was used for statistical analysis, and $p<0.05$ was accepted as the level of statistical significance.We established the reference ranges of platelet and platelet index in Turkish preterm and term infants. Platelet counts, and platelet volumes continually change as gestational age increases. Increases in platelet counts, and PCT, while decreases in MPV and PDW were detected. The gestational age-related changes in PLT patterns may reflect maturation of platelet regulation.
Results: Platelet counts increased beginning from the 26^{th} up to 28^{th}weeks. They did not change between 29^{th} and 33^{rd}weeks, while their levels rised again conspicuosly between 34^{th} and 37^{th} weeks. At 38^{th} week a dramatic increase occurred at 38^{th} week. MPV, and PDW values slightly decreased, while PCT values increased dependent on the gestational age. When we classified newborns in groups of 24^{th}-31^{th}, 32^{th}-36^{th}, and 37^{th}, and 38^{th} weeks, prominent, and statistically significant variations were observed between groups.
Summary/Conclusions: We established the reference ranges of platelet and platelet index in Turkish preterm and term infants. Platelet counts, and platelet volumes continually change as gestational age increases. Increases in platelet counts, and PCT, while decreases in MPV and PDW were detected. The gestational age-related changes in PLT patterns may reflect maturation of platelet regulation.

PB2121

RISK OF LUPUS AFTER PRIMARY IMMUNE THROMBOCYTOPENIC PURPURA: A 14 YEAR SINGLE CENTER EXPERIENCE

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Background: Primary immune thrombocytopenia (ITP) is an autoimmune disorder characterized by immune-mediated platelet destruction and suppressed platelet production. ITP may occur concurrently or precede the occurrence of SLE, which would have great diagnostic significance. ITP may also be the first early sign of the disease. Few studies have addressed the risk of systemic lupus erythematosus (SLE) after ITP.
Aims: To estimate the risk of SLE after ITP in adult Jordanian patients
Methods: All patients diagnosed with ITP and with a platelet count <100×10^9/L, between September 2002 and January 2017 were included in the study. Patients were retrospectively reviewed for diagnosis of SLE, and inclusion criteria included only those patients who had initial ANA screen at the time of the first presentation of ITP .All patents with the diagnosis of SLE at the time and before the presentation of primary ITP were excluded from the study.
Results: This study included a total of 58 patients (43 females and 15 males) who were followed up for a period of 14 years. Their age at the baseline ranged from 16 to 65 years with a mean (SD) of 31.2 (13.3). ANA was positive in 11 (19.0%) patients. Over the period of follow up, 9 (15.5%) patients developed lupus. The incidence was 13.3% among males and 16.3% among females, with no significant difference (p-value=0.786). There was significant association between ANA and lupus in both genders. Only one patient with negative ANA and 81.8% of patients with positive ANA developed lupus (P<0.005.
Summary/Conclusions: SLE developed in patients with primary ITP in with initial positive ANA titer at presentation. The results suggest that patients with initial positive ANA are at risk for development SLE. Thus, follow up after primary ITP diagnosis with positive ANA titer is of great importance as the risk of SLE is significant

PB2122

TREATMENT OF REFRACTORY IMMUNE THROMBOCYTOPENIA WITH THROMBOPOIETIN RECEPTOR AGONISTS: OUR EXPERIENCE IN CHILDHOOD

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Background: Immune thrombocytopenia (ITP) is an autoimmune disease in which antibodies develop against platelets (plts) and dysregulation of cellular immunity result in premature destruction of plts and impaired plt production. For most affected children, ITP is a self-limiting disease. Approximately, 10% of all ITP patients eventually develop refractory ITP (RITP). Thrombopoietin receptor agonists (TPO-RA) stimulate thrombopoiesis and are an alternative to Rituximab (Rtx) and splenectomy.
Aims: We present 3 different children with RITP treated with TPO-RA.

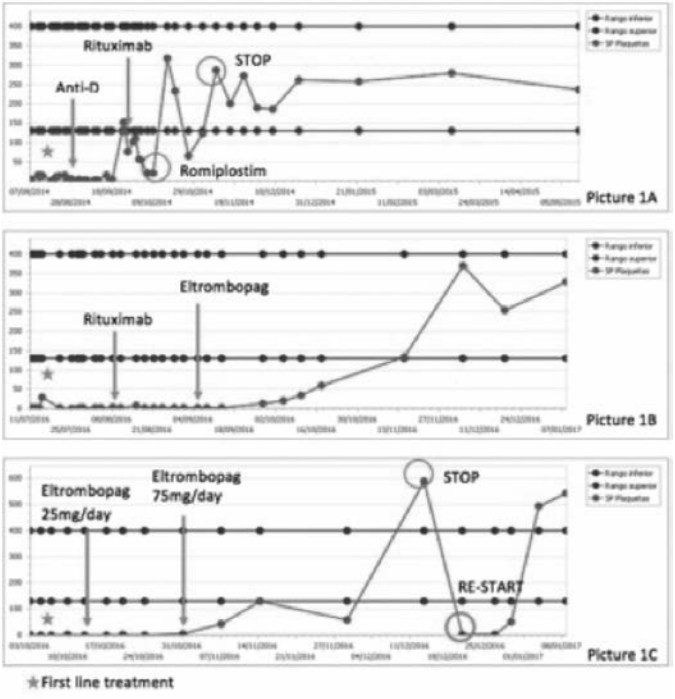

Figure 1.

Methods: CASE 1. A 5-year-old girl admitted to the hospital due to ITP with mucocutaneous bleeding. She was refractory to corticoids, immune globulin (Ig) and anti-D Ig. Rtx was started. After the 3rd dose, she responded temporarily along with fever, renal insufficiency and arterial hypertension, probably related to Ig A deficiency, not previously diagnosed. Romiplostim was indicated, reaching complete remission after 2 doses and it was stopped after the 4th dose, without any adverse reaction. Nowadays, plt count remains within normal limits (Figure 1A). CASE 2. A 5-year-old boy was diagnosed of ITP with cutaneous bleeding. He received treatment with prednisone and Ig with short response. Rtx was indicated; after 4th dose, severe thrombocytopenia and cutaneous bleeding persisted. Eltrombopag was started with response after 6 weeks of treatment (Figure 1B) and bleeding symptoms recovery. CASE 3. A 4-years-old boy with RITP was referred to our hospital. We decided to initiate treatment with Eltrombopag. He developed response after 4 weeks of treatment with a dose of 75mg/24h. Six weeks later, he presented 600,000plts/µL, so the drug was stopped. We observed a quick descent in plts levels and Eltrombopag was restarted with progressive response (Figure 1C).
Results: In all cases, splenectomy was avoided due to long-term risk of sepsis, as well as immunosuppressive agents like RTX in 3rd case. In 1st case, TPO-RA was able to stop with sustained response as described in some publications.
Summary/Conclusions: In our experience, TPO-RA appear to be efficacy and well tolerated in children.

PB2123

INVESTIGATION OF PLATELET FUNCTIONS IN PSEUDOTHROMBOCYTOPENIA

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Background: Pseudothrombocytopenia (pseudoTCP)), is incorrectly detection of low platelet counts in automatic blood counter devices and is most frequently caused by ethylene diamine tetra-aseticacid (EDTA) induced platelet clumping and *in vitro* agglutination. Therefore, pseudoTCP which accounts 15-30 of thrombocytopenic admissions, actually is not associated with a bleeding tendency. This situation may be detected with a careful investigation of peripheral blood smears (PBS) by experienced clinicians but in centers which does not have these facilities; misleading of worried patients through advanced centers or even unnecessery treatments with steroids and platelet transfusions often occurs.
Aims: In theory, formation of platelet clusters in the presence of EDTA requires functional adhesion molecules, so platelet adhesion and aggregation tests are expected to be in normal range. We aimed to investigate the capacity of simple platelet function analizers for making the distinction between pseudo TCP and real thrombocytopenia.
Methods: Platelet functions were measured as collagen-ADP and collagen-epinephrine closure times (ColADP and ColEPI) by Platelet Function Analyzer (PFA-200™) for all patients who are referred to our clinic as thrombocytopenia (TCP, plt <150 x10^3/µL) and value of this new method for determining peseudoTCP is compared with PBS which is accepted as the gold standard by using Receiver Operating Characteristic (ROC) curve analysis. PFA-200 system closure time is expected to be longer in true thrombocytopenia and normal in pseudoTCP, but there is no study investigated this system for this purpose. Descriptive analyses were presented using means ±standard deviations for normally distributed variables or median and interquartile range (IQR) for nonparametric continuous variables. An overall p-value of less than 0.05 was considered to show a statistically significant result. This study is supported by Duzce University with project number of 2015.04.03.370 and these are preliminary results.
Results: We included 59 patients who were referred to our clinic with thrombocytopenia (TCP, Plt<150 x10^3/µL) and 11 healthy controls (Plt>150 x103/µL). Median age was 54 (IQR:37-68) for thrombocytopenic subjects and 37 (%63) of them were female. Median Plt count was 61 x103/µL (IQR:30-90) in TCP group but WBC and Hb were not different from control subjects. Subjects referred with TCP were grouped with PBS as pseudoTCP and real-TCP. There was no difference in terms of Plt, MPV, PCT, WBC or Hb between these groups but age was younger (median age 46 *vs* 62, p<0.05) and PDW was higher in pseudoTCP group (med 17.6 *vs* 16.8, p<0.01). ColEPI and ColADP measures were significantly lower (med 125 *vs* 287 for ColEPI, med 84 *vs* 224 for ColADP, p<0.001 for both) at pseudoTCP group. The capacity of ColEPI and ColADP values in predicting pseudoTCP were analyzed using ROC curve analysis. We found that, when the manufacturer's recommended cut-off value (150 s) was used, the sensitivity and specificity were 74.4% and 95%, with overall accuracy of 81.4% for ColEPI (AUC 0.813, SD:0.061, p<0.001,%95CI: 0.694-0.933). Similarly sensitivity and specificity were 79.5%, and 95%, with overall accuracy of 84.7% for ColADP using manufacturer's cut-off value of 100 s (AUC 0.878, SD:0.055, p<0.001,%95CI: 0.770-0.986).
Summary/Conclusions: We concluded that, running PFA tests for everybody with thrombocytopenic counts, could be used for differentiate pseudoTCP and realTCP in centers which does not have conditions for proper BS. Especially long closure times excludes pseudoTCP with a high specificity and could make clinicians quick decisions for further investigations.

PB2124

MANAGEMENT OF ADULT CHRONIC IMMUNE THROMBOCYTOPENIA. SINGLE CENTER EXPERIENCE

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Background: The investigation and management of patients with Chronic immune thrombocytopenic purpura (ITP) varies widely. Although many treatments have been recommended for ITP, there are no evidence-bassed recommendations for when different treatments should be used, or when any treatment should be used rather than managing a patient by observation alone.
Aims: To evaluate the treatment of ITP patients in Departament of Hematology, County Hospital, Timişoara.
Methods: A retrospective study for 350 ITP patients was performed. Patients demographics, medical history, current treatments and side effects, were abstracted from the patient's medical charts for the 15 months prior to their most recent visit.
Results: The mean age was 45.6 years with 58% women and 42% men. Median time from the diagnosis of ITP to the start of the observational period was 23 months. Regardless of the presence of bleeding symptoms, for majority of patients we started treatment based on plateled count. Treatment was considered when platelet counts are less than $20x10^9$/L in patients without bleeding, and less than $30x10^9$/L in patients with bleeding. Prior to the observational period, 36% of patients had been splenectomized and the most reported treatment was corticosteroids. During the observational period, 72% of all patients were treated. The most frequent reasons given for treatment were platelet count (58%), followed by bleeding symptoms (42%). Corticosteroids represented 52% of treatments, followed by IVIg (20%), azathioprine (12%) rituximab and 8% Nplate. Splenectomies (8% of patients) and platelet transfusions (27% of patients) were performed during the observational period. In the patient survey, 52% of participants were 60 years of age or older and the duration of disease was more than 10 years in 43% of patients. The minimum platelet counts were less than $10x10^9$/L in 49% of patients. The most common symptoms of ITP was fatigue (45%). Approximately 60% of patients reported at least one side effect associated with ITP treatment. The side effects were most frequently associated with corticosteroid use (43%). Overall, 40% of patients required hospitalization. Mean duration of hospitalization was 13,5 days.
Summary/Conclusions: The retrospective study of 350 patients provides the results of treatment practices in our country. It showed that bleeding symptoms remained quite frequent among patients with chronic ITP. Corticosteroids were the most widely used treatment.

PB2125

IMMUNOLOGICAL THROMBOCYTOPENIC PURPURA AND PREGNANCY: A RETROSPECTIVE STUDY OF 89 PREGNANCIES IN 59 PATIENTS

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Background: Immunological thrombocytopenic purpura (ITP) occurs for about 1 case for 1000 pregnancies. The risk of onset, aggravation or relapse of ITP during pregnancy is not clearly established.
Aims: The aim is to describe the prevailing ITP progression profile in pregnant women and to evaluate the risk of neonatal thrombocytopenia in two situations, when ITP was known before pregnancy and when ITP was discovered for first time during pregnancy.
Methods: It is a retrospective study carried out in the hematology department of CAC Blida, Algeria, between 1993 and 2016. All patients (pts) who had a pre-pregnancy ITP or thrombocytopenia during pregnancy attached to an ITP were included.
Results: The development of 89 pregnancies (PG), including two twins, occurred in 59 women was analyzed. There were one PG in 40 pts, 2 PG: 13 pts, 3 PG : 5 cases, 4 PG : 1 case and 5 PG : 1 case. Of the 59 pts: in 42 cases it was a history of ITP before pregnancy (group 1: G1) with a history of splenectomy in 9 patients, and in 17 cases it was ITP discovered on the occasion of Pregnancy (group 2: G2). The average age at diagnosis=26.7 years (7-44) and that at delivery=30.4 years (19-44). The mean platelet count at diagnosis: G1: 34000 / µL , G2: 47000 / µL. In the first group (G1): At the beginning of pregnancy the ITP was chronic in 30 cases, newly diagnosed in 1 case, persistent in 2 cases and transient cured in 7 cases; treatments previously received were: corticosteroid therapy (n=34), splenectomy (n=9), Danazol (n=1), cyclosporine in 1 case and cyclophosphamide in 1 case, abstention in 7 pts, 2 of whom required corticosteroids during pregnancy. The status of the ITP at the beginning of each pregnancy was: out of treatment (n=8), corticosteroid dependence (n=5), non-response (n=7), PR (n=11), CR (n=24).In the second group (G2): the discovery of thrombocytopenia was in the first trimester (T) in 4 cases, in the second T in 6 cases and in the third T in 7 cases; 17 pts had platelet counts <80000 / µL and were included due to the persistence or even worsening and / or necessity to resort to treatment of thrombocytopenia after delivery. In both groups: in 26 pts (G1:16; G2: 10)variable dose and duration treatment were required during pregnancy; at delivery, 19 patients needed a treatment, out of them, a bolus of corticosteroids (n=11)+transfusion of platelets (n=4), immunoglobulins in 4 cases and transfusion of platelets alone in 4 cases. At birth, thrombocytopenia was observed in 40 pregnancies (50.6%): platelets <30000 / µL (n=7), between 31000 and 50000 /µL (n=13), between 51000 and 100000/µ (n=20), between 100000 and 150000/µl in 2 cases. All pregnancies were completed: 14 by caesarean section, one for thrombocytopenia, with an average platelet count=95000 /µL and 75 by natural delivery with a mean platelet count=100000 / µL with 4 deaths born, one anencephaly and 88 newborns No hemorrhagic syndrome was observed in pregnancy; two postpartum hemorrhages were seen in G2 group. Eleven newborns (5 in G1 and 6 in G2) were thrombocytopenic with platelet count <20000/µL in 4 cases; between 20000 and 50000/µL in 7 cases; neonatal thrombocytopenia occurred during the first 7 days. Only 4 newborns were treated, one by corticosteroid and 3 by immunoglobulins, with a good progression and only one of the untreated is always followed for thrombocytopenia.
Summary/Conclusions: The de novo ITP appearing during pregnancy is an etiological eventuality to be evoked in front of a thrombocytopenia of the pregnant woman after elimination of the other causes related to the pregnancy and in front of the non-resolution after the delivery. The pre-existing ITP does not necessarily.

Quality of life, palliative care, ethics and health economics

PB2126

QUALITY OF LIFE AND SYMPTOM BURDEN IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Multiple myeloma (MM), the second most common hematological cancer, remains incurable. Its incidence is rising due to population ageing. Despite the impact of the disease and its treatment, not much is known about health-related quality of life (QoL) of patients with MM.
Aims: This study aimed to (1) Determine symptom prevalence in patients with MM on disease-modifying treatment, and identify the range and nature of these symptoms within the dimensions of physical, psychological, social well-being. (2) Measure the QoL of patients. (3) Compare the above-mentioned parameters to the general population.
Methods: Adults with multiple myeloma attending the hematology day unit in hematology department from November 2016 to January 2017 were eligible for inclusion in a cross-sectional. Consenting patients completed 2 validated questionnaires, the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 (EORTC QLQ-C30) supplemented by the myeloma-specific module (EORTC QLQ-MY20).
Results: Forty-seven patients were included for analysis: 51, 1% were male and 48,9% were female. Mean age was 64,7 years (range 42-82, standard deviation 11.50). The QoL scores were significantly lower than the general population (54,7 *vs* 71,2). The most commonly reported physical symptoms were pain (72%), fatigue (70%) and insomnia (66%). About 61% of the patients were burdened by financial worries. On multivariate analysis, a good performans status (PS≤1) and a response of the disease to therapy (at least a partial response) were associated with high scores of QoL (P=0,01, P=0,03 respectively).
Summary/Conclusions: Patients with MM have a lower QoL than the general population and are symptomatic across physical, psychological and financial domains. They represent a polysymptomatic patient cohort with a complexity of need that merits a holistic multidisciplinary approach, and consideration of specialist symptomatic or palliative care review.

PB2127

QUALITY OF LIFE IN ANEMIC PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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Background: Anemia is a common complication of patients with hematological malignancies (HM), which may progress undergoing antitumor treatment significantly decreasing hemoglobin concentration and occur symptoms as fatigue, dizziness, palpitations, dyspnea markedly reduce patient activity, resulting in impaired Quality of Life (QoL).
Aims: To compare of QoL in HM's patients with different grades of anemia.
Methods: In this study were included following patients (n=326) in the age of 19-82 (Me=65) years: myelodysplastic syndrome (n=37), acute myeloid leukemia (n=20), acute lymphoid leukemia (n=7), primary myelofibrosis (n=23), chronic myeloid leukemia in blast crisis (n=6), multiple myeloma in II and III st. (n=126), Non-Hodgkin's lymphoma in III-IV st. (n=40) and chronic lymphocytic leukemia in B or C st. (n=67). Patients were examined: 1) clinical blood test (hemoglobin concentration) to assess anemia's grade; 2) the Functional Assessment of Cancer Therapy-Anemia (FACT-An) scale to measure of QoL. The FACT-An questionnaire consists of a general questionnaire (FACT-G), measuring domains of physical well-being (PW), social/family well-being (S/FW), emotional well-being (EW), functional well-being (FW), an anemia-specific questionnaire – Anemia subscale (AnS), measuring fatigue-associated items – Fatigue subscale (FS) and non-fatigue-associated items – Non-Fatigue subscale (NFS). Patients were divided into six groups according to the Hb concentration: 1) the first group – Hb was 4.0-6.4 g/dl (Me=5.7 g/dl); 2) the second – Hb 6.5-7.9 g/dl (Me=7.2 g/dl); the third – Hb 8.0-9.4 g/dl (Me=8.6 g/dl); the forth – Hb 9.5-10.9 g/dl (Me=10.8 g/dl); the fifth – Hb 11.0-11.9 g/dl (Me=11.4 g/dl); the sixth – Hb 12.0-14.4 g/dl (Me=13.0 g/dl). The sixth group was control.
Results: In the first group of patients (n=34) with severe anemia grade 4 QoL was revealed too poor; number of points in the subscale of PW was 14.0±0.9, in S/FW – 14.2±0.7; EW – 10.3±0.9, FW – 18.5±0.8, AnS – 41.2±1.6, FS – 27.8±1.3, NFS – 13.4±0.6. In the second group of patients (n=53) with anemia grade 3 QoL was poor too; in PW was 13.3±0.8, in S/FW – 14.4±0.6, EW – 9.9±0.7, FW – 18.2±0.6, AnS – 38.5±2.3, FS – 26.8±1.7, NFS – 12.0±0.7. In the third group of patients (n=72) with anemia grade 2 QoL in the subscale of PW was 11.5±0.7, in S/FW – 14.0±0.5, EW – 8.6±0.6, FW – 16.9±0.5, AnS – 36.1±1.9, FS – 25.5±1.4, NFS – 11.6±0.6. In the forth group of patients (n=70) with anemia grade 1 QoL in PW was 11.3±0.7, in S/FW – 14.3±0.6, EW – 8.4±0.8, FW – 16.9±0.7, AnS – 34.7±1.6, FS – 23.0±1.2, NFS – 11.7±0.6. In the fifth group of patients (n=41) with anemia grade 0 QoL in PW was 11.1±0.9, in S/FW – 14.9±0.8, EW – 7.6±0.6, FW – 16.4±0.5, AnS – 34.6±2.2, FS – 23.7±1.6, NFS – 10.9±0.7. In the sixth group of patients (n=56) without anemia QoL in the subscale of PW was 7.5±0.7, in S/FW – 13.6±0.6, EW – 6.4±0.5, FW – 14.8±0.7, AnS – 23.4±1.5, FS – 14.9±1.0, NFS – 8.4±0.6.
Summary/Conclusions: QoL was found too poor in patients with Hb <8.0 g/dl. QoL wasn't satisfactory in patients with Hb 8.0-11.0 g/dl. But the QoL improvement were greater in patients with Hb levels >11.0-12.0 g/dl (p<0.05). These data suggest that early correct anemia with red blood sells transfusions and erythropoiesis-stimulating agents can improve QoL in a clinically meaningful way.

PB2128

AN ANLYSIS OF THE IMPACT OF LOCAL COSTS OF MEDICINES ON COST EFFECTIVENESS OF THE TREATMENT OF CANCER ASSOCIATED THROMBOSIS.

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Background: New research has surfaced in relation to health care resource utilization and costs in Cancer Associated Thrombosis (CAT). The studies originate from the US and are difficult to transfer directly to other countries. A few studies in Europe focusing on the total cost of CAT seem to indicate that the cost data in the field of CAT varies greatly between regions. To examine the importance of region specific cost elements in relation to research related to CAT, we studied the cost driver in the newest and most relevant health economic research and compared it with the costs from 6 European countries as well as Canada.
Aims: To highlight the importance of localized or regionalized cost inputs as cost drivers when considering cost effectiveness in relation to CAT.
Methods: The cost driver is the medication in a recent analysis by Connell 2016 and thus the focus of our analysis. The American paper incorporates outcomes from 6 RCTs for treatment with LMWH in patients with CAT. The annual medication costs of LMWH for daily treatment in 365 days were 32,120 USD in wholesaler acquisition cost (WAC). For VKA the annual medication cost for 365 days was 44 USD. LMWH is the cost driver but is not cost effective due to the cost of it. The study finds that " *The one-way sensitivity analysis shows that LMWH would become the preferred strategy once its annual cost was less than $7177*" . In the present analysis, the daily cost acquisition cost Wholesaler Purchasing Price (WPP) (which corresponds to the American WAC) for a LMWH (prefilled treatment syringes with Tinzaparin) was gathered in 7 large markets using a data retrieval from IHS global insights systems (Jan 2016). In addition to this, the role of the cost driver was also compared to other publications.
Results: Simply by applying the local unit cost for the treatment with LMWH for these countries, the conclusion becomes notably different. LMWH becomes the cost effective alternative in the European countries as well as in Canada with annual costs below 7177 USD. The price for VKA is comparable to that in the US, and does not change the cost effectiveness ratio.
The data from the retrospective cost of CAT study that the cost of the hospitalization of was 19% of the total cost of CAT and the CAT medication 11% of the total cost of CAT. This outlines hospitalization is a cost driver as well and not only the medication. Similar conclusions were reached in other studies. In summary, the role of the cost driver can change as a consequence of the localization of the costs. This outlines the great variation in costs in terms of CAT, and the caution it must be used with (Table 1).

Table 1.

Average Wholesaler Purchasing Price (WPP) in USD for Tinzaparin prefilled treatment syringes	Annual WPP cost (365 days of treatment)	Tinzaparin below threshold for Cost Effectiveness outlined by Connell, 2016; 7177 USD pr year
UK	USD 2.847	*Yes*
Germany	USD 3.358	*Yes*
Spain	USD 3.176	*Yes*
France	USD 3.869	*Yes*
Sweden	USD 2.811	*Yes*
Greece	USD 2.993	*Yes*
Canada	USD 6.879	*Yes*

Summary/Conclusions: The exercise shows that using local input changes the conclusion and could potentially influence local evaluations related to the access for LMWH treatment for CAT. Tinzaparin was found to be a cost effective LMWH over VKA in 6 European Countries as well as in Canada, when local medication costs were used. This was in contrast to the conclusion in the US. Not using localized or regionalized cost inputs could potentially lead misinterpretations about cost effectiveness of CAT treatments.

PB2129

MINIMIZING THE RISK OF MUCOSITIS IN HEMATOLOGIC PATIENTS WITH TOPICAL PRODUCTS

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Background: Mucositis is a frequent severe complication associated to aggressive therapies of hematological malignancies with chemo and/or radiation (therapy), conditioning therapy in stem cell transplants. Regularly occurs at 3 to 10 days after chemotherapy and about 6 to 8 weeks after radiotherapy. It is self-limited within 2-4 weeks, but in this period the patient is vulnerable to systemic infections (bacterial and fungal). It could also compromise the optimal timing and dosage of the chemotherapy schedule, induce psychosocial distress, prolonged hospitalization and finally, higher costs.
Aims: Evaluating the efficacy of Gel X® in chemotherapy induced mucositis. GelX® is a topical product that contains Zinc gluconate+taurine, with bacteriostatic and anti-inflammatory effect, easy to use for the patient, in order to prevent and reduce pain and severity of oral ulcers, making a barrier for mucosae
Methods: A retrospective analysis of 77 adult patients: 17 with hematological treatments and 60 with allogeneic stem cell transplantation. 17 were diagnosed and treated between January 2015 and December 2016 with various hematologic malignances (5 AML, 2 ALL – 1 Ph positive, 2 blastic phases of CML, 3 AILT (CHOP/DA-EPOCH), 2 DLBCL (RCHOP), 1 FL (RCVP), 1 MM (radiotherapy), 1Hodgkin disease (ABVD). Treatment regimens used for acute leukemias/blastic phases of CML were: "3+7" (3 cases), MEC (1 case), high doses ARA-C (1), GMALL protocol (1), HyperCVAD (1), Idarubicine and ARA-C(1) HD-MTX(1) . GelX® was indicated as prophylactic treatment for eight patients, because the risk of mucositis was high (aggressive chemotherapy, bad oral condition, risk of prolonged neutropenia). Curative treatment of grade 3-4 mucositis was indicated for 10 patients (one was initially treated with curative intention and after that with prophylaxis). In 60 patients allografted for various hematological conditions (35 unrelated, 4 haplo and 21 sibling) GelX® was prescribed for treating grade 3-4 mucositis. For the 35 cases with unrelated allotransplant (21 AML, 4 ALL, 2 SA, 2ATLL, 2 MMM, 2 CML, 1 MDS, 1 BH), 16 cases of grade 3-4 mucositis has appeared .The conditioning regimen was mieloablative(14 cases) and reduced intensity(21 cases) .There were 21 cases of sibling allotransplants (6AML, 3 ALL, 1 ATLL, 5 LMNH, 1CLL, 2 SAA, 2 CML, 1 mycosis)with 10 cases of mucositis grade 3-4. The regimens used were 6 mieloablative and 15 nonmieloablative. 3 from 4 cases of haplotransplant with nonmieloablative conditioning (2MDS, 1 AML and 1 SAA) had grade 3 mucositis.
Results: Prophylactic treatment induced a reduction in the grading of mucositis (grad 1-2) and a shorter period of evolution (5 days) *versus* grade 3-4 mucositis and prolonged duration of oral lesions for those with curative treatment. From 60 patients allotransplanted, 30 patients experienced grade 3 and 4 mucositis with a medium duration of five days. All of them received GelX® as prophylactic treatment.
Summary/Conclusions: Prophylaxis is the key of successful evolution in mucositis (time to heal shorter than 10 days). Identifying candidates for mucositis is mandatory and the product should be applied starting with the chemotherapy (or in the first 24 hours on the onset of chemotherapy) in order to minimize the risk of mucositis appearance.

PB2130

EUROBLOODNET: THE EUROPEAN REFERENCE NETWORK IN RARE HEMATOLOGICAL DISEASES

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Background: Almost all hematological disorders are rare diseases, affecting less than 1 in 2000 individuals, justifying their inclusion in a European Reference Network (ERN). ERN are networks created following the Directive 2011/24/EU on cross border health which include nationally recognized Centres of Expertise aiming to ensure the same level of access to health services of European citizens affected by a rare disease. EuroBloodNet, the ERN in Rare Hematological Diseases (RHD), results from a joint effort of the European Network on Rare and Congenital Anaemias (ENERCA), the European Hematology Association (EHA), and European hematology patient organisations represented in both the EURORDIS European Patient Advocacy Groups (ePAGS) and the EHA Patient Organisations Workgroup. EuroBloodNet gathers 66 highly skilled and multidisciplinary healthcare teams in 15 Member States, and advanced specialized medical equipment and infrastructures which will facilitate concentration of resources for the design, validation and implementation of high-quality and cost-effective services aimed at facing the challenges of RHD.
Aims: EuroBloodNet's main goal is to improve the healthcare and overall quality of life of patients with a RHD by 1) Improving equal access to highly specialized healthcare delivery for RHD across Europe 2) Promoting best practices in prevention, diagnosis and safe clinical care across Europe 3) Disseminating cutting-edge knowledge and facilitating continuous medical education in the field of RHD 4) Providing inter-professional consultation by sharing of expertise and safe exchange of clinical information 5) Fostering European cooperation in highly specialized procedures for diagnosis, promotion of clinical trials and innovative treatments and research.
Methods: RHD are covered in two main thematic groups: non-malignant diseases and malignant diseases, and six sub-thematic areas. Non-malignant diseases include 4 sub-thematic areas: 1) Rare red blood cell defects 2) Bone marrow failure (BMF) and hematopoietic disorders 3) Rare Bleeding-Coagulation disorders and related diseases and 4) Haemochromatosis and hereditary iron metabolism disorders. Malignant diseases include 2 sub-thematic areas: 1) Myeloid malignancies and 2) Lymphoid malignancies. Methods and tasks aiming to achieve EuroBloodNet specific objectives have been split into five categories of Transversal Field of action (TFA): 1) Cross border health 2) Best practices 3) Continuing medical education 4) Telemedicine 5)Clinical trials and research.
Results: Expected outcomes include reduction of healthcare inequalities for RHD in the EU by a) establishing a cross-border referral system allowing safe information, samples and patient mobility, b) provision of equal access to highly specialised procedures and innovative therapies resulting from best practice sharing, continuous medical education and virtual inter professional consultation for complex RHD cases, and c) facilitation of a timely and efficient translation of research results into patient oriented strategy at the clinical and the public health level.
Summary/Conclusions: EuroBloodNet, with the experience gained thanks to the EU-funded ENERCA and EHA, will seek to improve access to healthcare for RHD patients, to promote guidelines and best practice, to improve training and knowledge sharing, to offer clinical advice where national expertise is scarce, and to increase the number of clinical trials in the field.

PB2131

2016 REVISION OF WHO CLASSIFICATION OF TUMOURS OF HAEMATOPOETIC AND LYMPHOID TISSUES: IMPACT ON INVESTIGATING PATIENTS WITH ISCHAEMIC STROKE

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Background: Under diagnosis related to the earlier hemoglobin (Hb) or hematocrit (Hct) diagnostic criterion is one reason to the 2016 revision of the diagnosis of PV in the World Health Organization (WHO) classification of Tumours of Haematopoetic and Lymphoid tissues. Bone Marrow Biopsy (BM) and molecular markers (JAK2) are recommended to establish the diagnosis in those with the lower threshold(Arber DA et al,2016). This potentially could result in increased numbers and costs of investigations. The lower thresholds are aimed to identify those previously referred to as masked PV (mPV) who have been recognized to have an increased incidence of thrombosis (Barbui T et al, 2014 & 2015).We hypothesized that the revision would increase the incidence of patients with Ischaemic stroke and potential PV who would then require additional investigations.
Aims: To determine number of patients with young strokes with potential PV on application of the 2016 revised WHO criteria for PV.
Methods: We undertook an analysis of records of patients with ischemic stroke prospectively maintained in the The Indo-US Stroke Registry and Infrastructure Development Project. This registry enrolled adult patients admitted with imaging-confirmed ischemic stroke <2 weeks after symptom onset. The Indo-US Stroke Registry and Infrastructure Development Project, includes 5 geographically diverse centers in India and one in USA. The registry data was entered into a central web-based electronic database. From January, 2012 to March, 2014, 2076 patients with new onset ischemic stroke were evaluable in the Indian arm of the Indo-US Stroke registry. We compared the incidence of polycythemia as per the 2016 revision against the earlier (2008) Hb diagnostic criterion.
Results: There were 24 (1.2%) patients with potential PV which was revised to 107 (5.2%) on applying the 2016 Hb criterion. The exact McNemar's test determined that there was a statistically significant difference in the proportion of polycythemics, p=.000. Considering the potential of comorbidities in the elderly to confound the association of polycythemia with Ischaemic stroke, we

separately analyzed only those with young stroke (Age <45). In this cohort there were 420 patients. A total of 6 (1.4%) patients had potential PV based on the 2008 Hb criteria. On applying the 2016 revision; 37 (8.8%) patients fulfilled the Hb criteria. An exact McNemar's test determined that there was a statistically significant difference in the proportion of polycythemics, p=.000. Separate analyses by gender was not significant in females, P=0.5; but significant in males, p=.000. There were an additional 29 males with the revised criteria for polycythemia. The impact of cost in influencing treatment decision from resource limited countries with predominant out of pocket health expenditure has been earlier reported (Philip C et al, 2015). This revision promotes the routine use of BM and JAK-2. In our analysis we estimate this new criterion would add to the costs to each patient (7000 per our centre estimate).
Summary/Conclusions: The present data shows that there exists a significant difference in the incidence of polycythemia in thrombosis (Ischaemic Stroke) on applying the revised criteria. The requirement to additionally investigate them with BM and molecular markers for PV has potential economic implications.

PB2132

PATHOPHYSIOLOGICAL MECHANISMS INVOLVED IN THE DEVELOPMENT OF ANEMIA IN PATIENTS WITH NON-HODGKIN'S LYMPHOMA

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Background: Non-Hodkin's lymphomas (NHL) are a group of heterogenous malignant lymphoid disorders that associate anemia either from diagnosis or during the evolution of the disease. The anemic syndrome can be present at the moment of diagnosis or can develop during the evolution of non-Hodgkin's lymphomas, with negative effects on the therapeutic regimen due to reduction of intensity and density of drug doses, overall survival and quality of life of these patients. Various pathophysiological mechanisms responsible for the development of anemia are depicted in literature: pro-inflammatory cytokines and hepcidin action on iron metabolism and erythropoiesis, bone marrow failure caused by infiltration of malignant lymphomatous cells, cytopenias secondary to chemotherapy, immune peripheral destruction of red blood cells, iron and folate deficiency due to chronic bleeding.
Aims: To evaluate the prevalence of anemic syndrome in patients with non-Hodgkin's lymphomas and the pathophysiological mechanisms involved in the development of anemia.
Methods: A retrospective study was conducted on 85 patients (informed consent obtained) with non-Hodgkin's lymphoma, who were admitted to the Clinic of Hematology, Filantropia City Hospital, Craiova, Romania, in between 2013 and 2015, in order to evaluate the prevalence and pathophysiological mechanisms involved in the development of anemia in this study group.
Results: In our study group, the median age at diagnosis of non-Hodgkin's lymphoma was 64 years, sex distribution was males:females=1,3, and the rural to urban area index=1,2. 85,88% of patients had B type NHL and 14,12% T type NHL. 20% of NHL were indolent lymphomas, aggressive lymphomas in 54% cases and very aggressive lymphomas in 26%. NHL repartition on stage of disease revealed: type I – 2.35%, type II – 18.81%, type III – 57.64%, and type IV – 21.16%. In our study group, 84% of patients enrolled had anemia, with the anemic syndrome affecting the 50-59 years and 70-79 years age groups. 59.73% of patients had anemia at diagnosis and 40.27% of patients developed anemia during the evolution of NHL. The pathophysiological mechanisms involved in the development of anemia were: perturbations of iron metabolism and erythropoiesis under pro-inflammatory cytokines and hepcidin actions (47.25%), bone marrow failure induced by lymphomatous infiltration (25%), anemia induced by chemotherapy (18.05%), and autoimmune hemolysis (9.7%). Five patients with anemia induced by chemotherapy and three patients with lymphomatous infiltration of the bone marrow also associated iron and/or folate deficiency.
Summary/Conclusions: In our study, anemia was present in 84% of NHL cases, more frequently found in patients that associated comorbidities and belonged to the 50-60 years and 70-80 years age groups. In half of the cases, anemia was moderately severe. 47.25% of patients had simple chronic anemia due to perturbations of the iron metabolism and of erythropoiesis, and 25% of patients presented anemia due to bone marrow failure. Chemotherapy lead to an anemic syndrome in 18.05% of cases, whereas hemolysis of autoimmune cause was responsible for 9.7% of cases of anemia diagnosed. The management of anemia is extremely important in patients with NHL because it influences the administration of chemotherapy (dose intensity and density), prognosis and quality of life.

PB2133

SAFETY OF RITUXIMAB BIOSIMILAR (NOVEX®) IN THE ROUTINE USE TREATMENT IN ARGENTINA.

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Background: Novex® is a biosimilar by design of the reference product Mabthera®/Rituxan®. Novex® was approved in Argentina following ANMAT's Biosimilar guidelines, having the same indications as the reference product, and is commercialized by Laboratorio Elea. As part of its Risk Management Plan (RMP), Laboratorio Elea implements an active pharmacovigilance program as defined in Argentina regulation. Periodically reports ANMAT RMP status and results.
Aims: To describe frequency and pattern of adverse events during the use of NOVEX® in treatments registered along an active pharmacovigilance program in order to oversee the safety profile of NOVEX® in the real clinical practice and maintain the benefit-risk evaluation.
Methods: A treatment Registry for NOVEX® was implemented from the beginning of NOVEX® commercialization as part of the RMP. The Data Lock Point for this report is Jan 31st, 2017. Physicians prescribing NOVEX® were requested to fill a form indicating age and gender, treatment start date, treated pathology, dosing and dose frequency. Such data was recorded in a database. After a preset time, physicians were contacted by Laboratorio Elea to ask them about the treatment outcome and Adverse Event occurrences. If adverse events were detected they reported each occurrence as Individual Case Safety Report (ICSR), they were registered using the MedDRA dictionary (version 19.1) for its codification.
Results: The total number of participating physicians was 151. During this period, they reported 638 treatment initiations, 389 of which had at least 1 follow up point and were included in further analysis. 53% male. Mean age 64.1 years. Hematological indications were more than 90%. More than 90% of indications were approved indications. Nevertheless, we detected off-label use**.** Total cycles received for any approved indication had a mean number of 5.7. Total received Individual Case Safety Reports were 17, indicating a relative frequency of 4.4% of Individual Case Safety Report. Occurrence rates were 1.2 Individual Case Safety Report per 100 administered cycles, and 0.020 per 100 treatment days. Eleven Individual Case Safety Reports were classified as serious (SAE) because they had at least one manifestation that prolonged hospitalization, endangered life or was death-associated. The most frequent AE reported was acute reaction related to infusion (9 cases), followed by cardiovascular manifestations (2 arrhythmia, 1 cardiac failure and 1 ischemic stroke), infections (1 pneumonia, 1 progressive multifocal leukoencephalopathy), neurologic (1 paresthesia), cytopenias (1 pancytopenia) and cutaneous (1 bullous dermatitis).
Summary/Conclusions: The activities developed under this active pharmacovigilance program showed great value allowing us not only to monitor the adverse event pattern but also to detect off-label use as part of real life treatments. This report showed a similar safety profile to that of the reference product concluding that NOVEX®, in terms of tolerability, is similar to the reference product. Pharmacovigilance is cornerstone in the development of biologicals, especially biosimilars, as a tool to assist in the knowledge about their safety profile.

PB2134

DEPRESSION AS THE PRESENTING SYMPTOM OF CENTRAL NERVOUS SYSTEM LYMPHOMAS IN NORTHWESTERN TURKEY

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Background: PCNSL represents approximately 4 percent of newly diagnosed primary central nervous system (CNS) tumors, with an age-adjusted incidence rate of four cases per million persons per year. Most cases of non-AIDS related PCNSL are diagnosed in patients between 45 and 65 years of age, with a median age at diagnosis in the fifth decade. The most notable risk factor for the development of PCNSL is immunodeficiency including HIV infection, iatrogenic immune suppression, and congenital immune deficiencies. Antecedent flu-like or gastrointestinal illnesses or a history of autoimmune diseases were reported. Presenting symptoms may include focal neurologic deficits, neurophyschiatric symptoms, signs of increased intracranial pressure, seizures or ocular symptoms. Neurophyschiatric symptoms like depression, apathy, psychosis, confusion, memory impairment, slowness of thought are generally undernoticed or underestimated due to the increased rates of depression and tendancy towards antidepressant use. Diagnosis is based on imaging of the central nervous system (CNS), ideally with contrast-enhanced magnetic resonance imaging (MRI), cerebrospinal fluid (CSF) analysis, unless contraindicated due to elevated intracranial pressure The radiographic lesion tends to be a solitary non-hemorrhagic mass, situated in the deep white matter adjacent to the ventricular surface.
Aims: We aimed to evaluate the presence of depression and antidepressant use before the diagnosis of CNS lymphoma and emphasize the duration between the diagnosis of depression and lymphoma.
Methods: Data of 40 patients with CNS lymphoma were evaluated in a retrospective manner. From their national health records, prescription for antidepressant and anxiolytic drugs with their psychiatric diagnosis, time before the diagnosis of CNS lymphoma, the branch of the prescribing physician, presenting symptoms

from their medical files, type and treatment of lymphoma and survival were recorded. OECD international statistics as well as Turkish Statistical Institute data for national antidepressant use were collected and interpreted.
Results: Of the 40 patients, 14 were male (35%) while 26 were male(65%). Mean age was 60,5 years (38-78). 7 patients were alive (17.5%). Method for diagnosis was radiological imaging (magnetic resonance imaging) in 27 patients (67.5%) while in 13 patients, diagnosis was supported with histopathological confirmation (32,5%). Mean survival was 8,6 months (2-24 months). As the complaint for medical help seeking, 4 patients presented with neurophyschiatric symptoms while 16 patients presented with headache (40%) and 20 patients (50%) presented with neurological defects. On the other hand, prior to lymphoma diagnosis, 7 patients were diagnosed as anxiety disorder and 13 as depression (total, 19 patients, 47,5%) and were prescribed antidepressant and anxiolytic medications. The mean duration between prescription of antidepressants and diagnosis of lymphoma was 2,6 months (0-10 months). Within the patients who were on antidepressants, 6 were female and 14 were male.
Summary/Conclusions: OECD Health at a Glance data revealed that in 2013, the defined dose per 1000 per day is 35, range of Europe is 21-88. According to our data of Ministry of Health, use of antidepressants in the general population is 10,52%, mostly in women. Within these patients, 42,37% were anxiety disorders and 22,99% were depression. In the last five years' statistics, 30% of our population was prescribed for an antidepressant. The major group of physicians presciribing these medications was family and general physicians (>45%). The most striking finding of our study was the majority of male patients receiving antidepressants before the diagnosis of CNS lymphoma with a mean delay of diagnosis as 2,6 months (0-10 months). Depression and anxiety disorders are the leading diseases of disability and the importance of organic and underlying conditions should not be underestimated relying on the increasing need of antidepressants.

PB2135

IMPACT OF U.S. FDA APPROVAL OF LENALIDOMIDE MAINTENANCE THERAPY IN THE FIRST-LINE TREATMENT OF MULTIPLE MYELOMA AFTER AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANT ON TOTAL HEALTHCARE COSTS

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Background: Lenalidomide maintenance therapy after autologous hematopoietic stem cell transplant (auto-HSCT) in the first-line treatment has been shown to improve progression-free survival (PFS) and overall survival (OS) in multiple myeloma (MM) patients.
Aims: This study assessed the budget impact of the United States (U.S.) Food and Drug Administration (FDA) approval of lenalidomide maintenance therapy on total healthcare costs of a U.S. health plan.
Methods: An economic model was developed to estimate the incremental (additional) total plan costs (in 2016 USD) of maintenance therapy in each year for the first 3 years after lenalidomide monotherapy (R) maintenance therapy approval. The number of post auto-HSCT adult MM pts eligible for initiating maintenance therapy was estimated from published epidemiological data and an analysis of Connect® MM Registry data. Clinical endpoints for R-maintenance, including time on treatment, PFS and OS, were obtained from a meta-analysis of published clinical trials (CALGB, IFM, and GIMEMA). The use of common off-label maintenance therapies was considered. Types of costs included in the model were drug, drug administration, adverse events (AE), AE monitoring, one-time progression and terminal care costs.
Results: In a hypothetical U.S. health plan with 1 million members, the number of adult MM pts eligible to initiate post-ASCT maintenance therapy was estimated to be 28. Among them, 14.8 pts initiated R-maintenance in Year 1, 15.2 in Year 2, and 15.3 in Year 3, representing an incremental increase of 2.9%, 4.2% and 4.4% after R-maintenance therapy approval, respectively. After considering additional costs of maintenance, as well as potential offsets resulting from delayed progression the incremental total healthcare costs by year are listed in the Table 1. Results were consistent across all total plan, per patient per year, and per member per month costs. Deterministic sensitivity analysis showed that the model results were robust to the variations of key model inputs.

Table 1.

	Healthcare Costs		
	Year 1	Year 2	Year 3
Incremental Plan total	$67,617	$176,262	$271,929
Incremental Per patient per year	$2,405	$3,222	$3,402
Incremental Per member per month	$0.0056	$0.0147	$0.0227

Summary/Conclusions: Approval of lenalidomide monotherapy for maintenance after auto-HSCT in the first-line treatment of MM has minimal impact on total plan costs, primarily due to the small incident population and the already common use of lenalidomide in post auto-HSCT maintenance.

PB2136

LAPAROSCOPIC APPROACH CAN EXTEND THE INDICATIONS OF SPLENECTOMY: ANALYSIS OF 31 CONSECUTIVE PATIENTS WITH MALIGNANT HEMOPATHIES

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Background: Surgical resection of large spleens may eliminate a significant amount of tumor, allow definite diagnosis of malignant disorder, ameliorate abdominal symptoms and resolve cytopenia. However, because of short term perioperative events (25%) and long term immunosuppression (increased risk of infections caused by encapsulated bacteria) physicians can be reluctant to choose splenectomy, especially in older patients or patients with comorbidities. The role of laparoscopic splenectomy (LS) in patients with hematological malignancies is still unclear. Nevertheless, the ageing of the world's population and the increased incidence of Non-Hodgkin's Lymphoma are increasing the indications for splenectomy, requiring a well-tolerated and less invasive procedure.
Aims: The aim of this review is to analyze our single-center experience of LS performed for malignant Hemopathies. Results are compared with LS for benign splenomegaly and the risk of locoregional dissemination or inadequacy of fragmented histological sample were analyzed.
Methods: We retrospectively analyzed 50 patients who underwent LS between 2005 and 2016 at Saint-Pierre Hospital. Anterior approach was used in 12 patients whereas in the remaining 38 cases, a semi-lateral position was chosen. All the patients received the triple vaccination (Streptococcus pneumoniae, type B Haemophilus influenzae, and Neisseria meningitidis). Patients characteristics, safety data such as early (<30 days) and late (>30 days) morbidities and mortality and efficacy (hematological recovery, accuracy of histological diagnosis) were analyzed.
Results: 19 patients underwent splenectomy for benign hemopathies (SBH) and 31 patients for malignant hemopathies (SMH). Non-Hodgkin's lymphomas (12) and idiopathic myelofibrosis (10) were the most common causes of splenectomy followed by chronic lymphocytic leukemia (7), hairy cell leukemia (1) and hodgkin's lymphoma (1). Patients' age (67 +/- 12 years, ranging from 36 to 87 in SHM, and from 11 to 71 in SBH), prior abdominal surgery (18/31) and spleen volume (1515 +/- 662 mL, ranging from 220 to 3000ml in SMH, and from 90 to 1500ml in SBH) were significantly higher in the SMH group (p <0.05). There was no significant difference in surgical time (150 *vs* 146 min, p=0.8), blood losses (243 *vs* 402 mL, p=0.26) and duration of hospitalization (5.4 *vs* 7.5 days, p=0.19) between SMH and SBH. No case of locoregional dissemination was experienced. The early morbidity of the SBH group was 10% and 13% for the SMH group (p=1). Late morbidity was 0% in the SBH group and 13% in the SMH group (p=0.28). This could be explained by a combination of underlying disease and immunosuppression (2 sepsis and 2 deep vein thrombosis). There was one conversion to open surgery and perioperative mortality in each group (p=1). There was no significant difference in efficacy of splenectomy, with respectively 83% and 79% (p=0.91) or quality of histological sample for pathological report between SBH and SMH. In the SMH group, 4 out of 31 patients received a pre-surgical corticosteroid treatment, with a pre-surgical platelets level of 156 +/- 108 x 10^3/mL, white blood cell level of 15696 +/- 18950/mL and Hemoglobin level of 10.1 +/- 1.6 g/dL. Regarding the efficacy of LS in correcting hypersplenism in the SMH, a significant difference in term of platelets recovery after 1 month from the surgery was shown in patients efficiently *Vs* inefficiently operated (respectively 387 +/- 125 *Vs* 138+/- 90 x 10^3/ml, p <0,05). The median follow up is 39 +/- 37 months and 80% achieved a hematological recovery.
Summary/Conclusions: LS is a safe and less-invasive procedure in patients affected by Malignant Hemopathies. This approach is also well tolerated in older patients (median 67yrs) and in patients with large spleen (1515+/-660 ml), extending the indication for laparoscopic SHM even in older patient and in patients with high volume spleen. Compared to historical data, LSy for Malignant Hemopathies shows better early and late morbidities. Our data shows however a trend for higher late morbidity in the SMH group, warranting a careful long term follow-up in this subset of patients.

PB2137

ARE WE AWARE OF ANXIETY AND DEPRESSION IN PATIENTS WITH NEWLY DIAGNOSED ACUTE LEUKEMIA?

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Background: Acute leukemia poses a high risk of stress for the patient during the process of diagnosis. The process after the diagnosis is challenging for the

patient due to urgent admission, long duration of stay in hospital, chemotherapeutic agents used in the treatment and the disease itself. Evaluating this group of patients for anxiety and depression, providing necessary professional support and revising medical treatment is therefore substantial.
Aims: In our study, we aimed to assess the risks of anxiety and depression in newly diagnosed acute leukemia patients who were admitted to hematology clinic to receive chemotherapy and provide necessary professional support along with treatment revisions and follow-up according to our findings.
Methods: Our study was performed with newly diagnosed acute leukemia patients, who were admitted to our hospital hematology clinic in a six-month period to receive chemotherapy. Demographic characteristics were noted and Hospital Anxiety and Depression Scale (HADs) was used to assess depression. Hospital Anxiety and Depression Scale (HADs) is an assessment scale developed by Zigmond and Snaith to determine the risks and assess the severity of anxiety and depression (8). The validation and reliability studies of the scale in Turkey were carried out by Aydemir et al (9). The questionnaire has a total of 14 items; seven of which measure anxiety (odd numbers) and the remaining seven (even numbers) measure depression. Each item is scored from 0 to 3. The scoring order of each item in the questionnaire is different. Items numbered 1, 3, 5, 6, 8, 10, 11 and 13 indicate decreasing severity and are scored as 3-2-1-0. On the other hand; items numbered 2, 4, 7, 9, 12 and 14 indicate increasing severity and are scored as 0-1-2-3. The cut-off value for the total score of the odd-numbered questions assessing anxiety is 10; while it is 7 for the even-numbered questions assessing depression.
Results: 21 patients were included in the study. 13 of these patients (61.9%) were diagnosed with acute myeloid leukemia (AML) and 8 (38.1%) were diagnosed with acute lymphoblastic leukemia (ALL). Median age of the patients was 45 (range: 21-69). 11 patients (52.4%) were female and 10 (47.6%) were male. 5 patients (23.8%) had comorbidities while 16 (76.2%) had none.
Anxiety evaluation revealed that 38.1% of all patients in the study experienced anxiety. The rate of anxiety was 38.5% in AML patients and similarly 37.5% in ALL patients. 45.5% of the female patients had anxiety while the rate was only 30% in male patients. The difference was not statistically significant ($p > 0.05$).
Depression evaluation revealed that 81% of all patients in the study. The rate of depression was 84.6% in AML patients and 75% in ALL patients. 81.8% of the female patients had depression while it was 80% in male patients. Neither anxiety nor depression had a significant correlation with comorbidities or gender ($p > 0.05$). Correlation analysis revealed a positive correlation between anxiety and depression ($r=0.846$; $p < 0.01$).
Summary/Conclusions: In conclusion, assessing anxiety and depression in patients with acute leukemia at the time of hospital admission is substantial for the course of and adherence to treatment. In our study, depression was distinctively more common than anxiety and there was a positive correlation between depression and anxiety. We think that including a professional for psychological support in the medical team is important for the treatment of these patients.

PB2138

GENDER DIFFERENCE IN ANXIETY FOR THE FIRST BLOOD TRANSFUION

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Background: Blood transfusion has several risks including allergic reaction, acute hemolysis, infectious disease and so on. Both physicians and patients are always cautious to decide on blood transfusion.
Aims: The purpose of this study was to explore whether there are gender difference in anxiety for the first blood transfusion in patients with different diseases.
Methods: 315 patients (153 men and 162 women) were enrolled in this prospective, comparative study and median age was 38 years(range 17-72). The disease consisted of 85 chronic hepatitis B, 73 leukemia, 69 gastric ulcer, 48 chronic renal failure and 40 gynecological oncology. Various blood products including plasma, red blood cells suspension and platelet were infused. Anxiety was evaluated according to the HAMA self-rating anxiety scale (SAS) during the first blood transfusion. Patients got 50 points below were divided into no anxiety group, 50 to 59 points were divided into mild anxiety group, 60-69 points were divided into moderate anxiety group and 70 points or more were divided into severe anxiety group.
Results: For patients with the same disease, more female patients were divided into moderate to severe anxiety group than male ones. The number of patients with mild anxiety was similar in female and male, and no one was divided into no anxiety group.
Summary/Conclusions: Women were more anxious than men during the first blood transfusion, which is independent of age, race, education level and kinds of blood product.

PB2139

A ROOM OF MY OWN

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Background: Three years ago, a unit for autologous bone marrow transplant for hematological patients has been established in Shaare Zedek medical center. The patients meet with the doctors for the treatment plan usually following the diagnosis. From the point of view of a part of the patients, the process appears simple, short term, and promises cure. In reality, the process is long term, including aggressive chemotherapy prior to the transplant. The treatment is highly aggressive and toxic with many physical and mental side effects for the patient and his/her family. The transplant process requires hospital admission for about a month in an isolation room. No one is allowed in the room except for close relatives and the medical staff. The social worker, part of the caring staff, accompanies patients and families from the initial diagnosis through this taxing and stressful process. Most patients are young, average 45 years, in the middle of their careers, from a broad spectrum of occupations, education as well as social status, representing Israeli society.
Aims: 1. To accompany and empower patients by means of giving them tools to cope with the transplantation process which is a crisis situation in the midst of their lives. 2. To teach patients self-awareness. 3. Promote quality of life for the patients especially during the stay in the isolation room by way of creating a safe domain.
Methods: The following tools had been utilized: 1. The "Empowerment method". An advanced view of the powers and experiences of patients that constitute resources in addressing crisis. 2. Work of hope- finding unique meaning in life crisis.
Results: This work is based on therapeutic conversations that took place inside the isolation room with about 30 patients, mostly men, average age was 50, during the past three years. With the understanding that a patient goes from the public sphere to a private one -the isolation room- my entrance into the room was based on the ability and willingness of the patients to go into a treatment dialogue at that point and time. From the narratives of the patients, a few themes were extracted that were repeatedly discussed by most patients. 1. Fear of death. 2. Post-traumatic issues. 3. Fear of isolation. 4. The issue of relationships. 5. Mind and body. 6. Children. 7. Faith. 8. Closure
As cited by S.A, a 49 year old man "I'm afraid to give in and die, help me to stay alive. And if I die, I want to know that I have left no unfinished business."
Summary/Conclusions: From the therapy sessions it appears that the central issues that bother the patients belong to the private space and the coping with it. The process of treatment helps patients to go from the private sphere back to the public one.
Recommendations: It seems essential for the patients in the isolation room, undergoing autologous bone marrow transplant, to have therapy sessions with a qualified social worker as part of the holistic care. 'Having a room of his own' in the process enables an opportunity to examine the inner self esteem and strengths of the patients thereby patients learn to contribute to themselves from themselves.

Sickle cell disease

PB2140

HYDROXYUREA INHIBITS MYELOID DIFFERENTIATION VIA NITRIC OXIDE SYNTHASE

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Background: Hydroxyurea and nitric oxide (NO) inhibit erythroid differentiation, while hydroxyurea is NO-releasing agent used in therapy of sickle cell diseases.
Aims: To study the mechanism of hydroxyurea inhibition of erythroid differentiation by exploring NO synthase (NOS) dependence.
Methods: The erythroid differentiation is studied by methylcellulose colony assay in mice, whereas presence and activation of endothelial NOS (eNOS) by immunocytochemistry and immunoblotting, respectively in K562 erythroleukemic cell line.
Results: In *ex vivo* experiments, mice exposed 7 days to hydroxyurea demonstrated significant decrease in the number of nucleated cells per femur, partially reversed by NOS inhibitor N-nitro L-arginine methyl ester hydrochloride (L-NAME). The same, but less prominent reduction has been observed with NO metabolites nitrite (NO2) and nitrate (NO3). Moreover, hydroxyurea demonstrated a large diminution in the number of bone marrow derived myeloid colony-forming unit -granulocyte/macrophage (CFU-GM), burst-forming-units-erythroid (BFU-E) and colony-forming unit-erythroid (CFU-E) colonies in methylcellulose cultures. L-NAME attenuated hydroxyurea reduction of myeloid and erythroid colonies, while by itself increased CFU-E and CFU-GM colonies and slightly BFU-E colonies. NO metabolites NO2 and NO3 generally inhibited myeloid and erythroid colonies, but the reduction was more prominent by NO2 compound. Moreover, the hematological parameters and weight (before and after treatment) of mice did not show any significant difference among studied groups. Hydroxyurea increased NO production and the number of eNOS positive K562 erythroleukemic cells, while phosphorylation of eNOS and activation of AKT/mTOR signaling was not blocked by phosphatidylinositol 3-kinase inhibition.
Summary/Conclusions: NO prodrug hydroxyurea demonstrated NOS dependence in inhibition of myeloid / erythroid differentiation, not influencing the hematological parameters.

PB2141

SLEEP DISORDERED BREATHING IN CHILDREN AND ADOLESCENT WITH SICKLE CELL DISEASE: IMPACT ON EXECUTIVE FUNCTION AND PROCESSING SPEED INDEX

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Background: Studies in non-syndromic children have shown that sleep-disordered breathing (SDB) increases the risk of neuropsychological deficits and neuronal brain injury. Few authors have investigated the role in cognitive deficits of SDB and the associated hypoxia in children with sickle cell disease (SCD). Snoring and SDB is very common in children with SCD and may affect cognitive function in very young children. Previous data suggested that executive function was worse in older children with SCD and low mean overnight oxygen saturation.
Aims: We aim to investigate if SDB could be a potential factor contributing to developmental problems in cognition in children and adolescent with SCD.
Methods: We have followed up children and adolescents in the Sleep Asthma cohort who underwent Polysomnography at two different time points (1) 2006-2009 and (2) 2011-2014 and compared the sleep data with subsequent neuropsychological assessment.
Results: Worse performance was found for processing speed: PSI (p<0.01) and general intelligence (p<0.05) compared to control siblings. SDB, measured as apnea and hypoxia index (*i.e.* AHI >3%: Apnoeas and hypopnoeas with more than ≥3% desaturation), was found to impact executive function, as measured with the Tower test, (p<0.05) and PSI (p<0.05). Mean oxygen saturation during total sleep time was significantly associated with lower PSI (p<0.05). Additionally, participants who showed a worsening of their SDB symptoms in their second sleep study had lower cognitive scores (*i.e.*, executive function, p<0.05 and PSI, p<0.05) (Figure 1).
Summary/Conclusions: SDB symptoms seem to worsen into adolescence and therefore, might have a neurodevelopmental impact if left untreated; appropriate intervention might improve cognition and quality of life.

PB2142

LUNG FUNCTION IN CHILDREN AND ADOLESCENTS WITH SICKLE CELL ANEMIA: A COMPARISON BETWEEN UK AND ITALY

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Background: Acute and chronic respiratory complications are common in sickle cell anemia (SCA). Subjects with SCA often have a progressive decline of lung function with age that could be influenced by the quality of healthcare and by environmental factors, such as the level of exposure to air pollution.
Aims: To compare lung function, evaluated cross-sectionally through spirometry, in children and adolescents attending sickle cell centers in UK and Italy.
Methods: Anthropometry and spirometry were recorded in patients with SCA (SS,Sb^0) aged 6-17 years of African ancestry followed at the Evelina Children's Hospital, London, UK, and at the University Hospitals of Padova and Udine, northeast of Italy. Subjects from the British cohort lived in an urban area while those from Italy came from urban and non-urban areas. Exclusion criteria were the presence of SCA-related morbidity within the last two weeks and the inability to perform a spirometry meeting the *European Respiratory Society* acceptability and repeatability criteria (Miller, Eur Respir J 2005;26:319–338), modified for children (Kirkby, Pediatr Pulmonol 2008;43:1233–1241). Portable spirometers (Pony FX, Cosmed-IT, Easy-on PC, NDD-CH) were used. Z-scores of anthropometric and spirometric data were derived, respectively, from CDC2000 and from the *Global Lung Initiative 2012* predictive equations for African Americans (Quanjer, Eur Respir J 2012; 40:1324–1343). Spirometry patterns were classified as normal, obstructive ($zFEV_1/FVC$<-1.64) or restrictive ($zFVC$<-1.64+$zFEV_1/FVC$ ≥ -1.64). Differences between groups were assessed by t-tests and considered statistically significant for p values <0.05.
Results: A total of 101 children and adolescents were included (n. 62 in UK; n. 39 in Italy; 42% girls; age-range: 6.2-17.9 years). We didn't find significant differences in mean spirometry indices between the SCA cohort from London and northeast Italy (Table 1). Nevertheless while an obstructive spirometry pattern was more common in the British cohort compared to the Italian one (respectively 22.5% *vs* 7.7%), the picture was the opposite for the restrictive pattern (respectively 11.2% and 20.5%) (Table 1). In the whole sample age was negatively correlated with both zFEV1 (Spearman's rho-0.20) and zFVC (Spearman'srho -0.24).

Table 1.

Index	Sickle cell UK	Sickle cell ITA	Diff between means (95% CI)
n (%male)	62 (54%)	39 (61%)	
Age (years)	11.9 (2.7)	11.3 (3.5)	0.6 (-0.6 to 1.9)
Height z-score	-0.11 (1.23)	-0.08 (1.09)	-0.03 (-0.53 to 0.47)
BMI z-score	-0.11 (1.71)	-0.55 (1.17)	0.44 (-0.03 to 0.91)
FEV1 z-score	-1.10 (1.04)	-0.80 (0.97)	-0.30 (-0.72 to 0.10)
FVC z-score	-0.71 (1.03)	-0.71 (0.83)	0.00(-0.43 to 0.39)
FEV1/FVC z-score	-0.43 (1.09)	-0.27(0.93)	-0.16 (-0.58 to 0.25)
Spirometry pattern			
Obstructive (% of total)	14 (22.5%)	3 (7.7%)	
Restrictive (% of total)	7 (11.2%)	8 (20.5%)	

Summary/Conclusions: Lung function of pediatric subjects with SCA living in London and in the northeast of Italy is overall comparable. Obstructive lung disease is more common among subjects with SCA living in London than in urban and non-urban areas in Italy. Differences in the level of exposure to ambient air pollution and in the prevalence of allergies between the rural and urban environment might have contributed to this finding and need to be further investigated.

PB2143

SICKLE CELL DISEASE: A NEW DISEASE IN MADRID

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Background: Sickle cell disease (SCD) was scarcely diagnosed 2 decades ago in Spain, and the Community of Madrid is a paradigm of the adjustments that had to be implemented to attend an increase of cases due to immigration.
Aims: The aim of our study was to find out the prevalence of SCD in the referral center for sickle newborn screening in the Community of Madrid, in addition to the demographic characteristics of these patients. The secondary objectives were to obtain the frequency of specific treatments or prophylaxis accomplished by these patients, and the reasons for loss to follow-up.

Methods: The study is observational, unicentric, descriptive and retrospective, carried out in February 2017 in a tertiary hospital in the Community of Madrid, Spain. All patients diagnosed with SCD and who had attended at least once to the hematology clinic for this reason were included. Demographic characteristics (date of birth, gender, country of birth) and clinical characteristics (genotype, therapy and update in follow up, like alive, deceased or lost patient) were collected. Written informed consent was signed by patients or legal guardians in accordance with the Declaration of Helsinki. The study was approved by the hospital Ethical Committee. Statistical analyses were performed using SPSS version 18.0. Quantitative variables were reported as median or mean value and range, while categorical variables were expressed as absolute value and percentage.
Results: The total number of SCD patients included was 209. Ratio boy/girl is 1.3. Most of patients were born in Spain (85%), although 8% and 5.26% were born in Africa or America respectively. Seventy three percent of the progenitors came from Africa and 24% from America. Ninety two percent of those SCD patients born in Spain were detected in their first days of life due to universal screening detection implemented in Community of Madrid since 2003. Median age at first diagnosis was 1.42 years (0-21.4). Median age at the end of inclusion was 9.91 years (range 0.13 to 35.14). SS or S/Betathal^0 was reported in 86%. In addition, 2.39% associated alfa gen deletion, and 1 (0.48%) glucose 6 phosphate dehydrogenase deficiency. No patient had congenital thrombotic diathesis. Eighteen patients (8.65%) had human leucocyte antigen (HLA) identical siblings. Hydroxyurea was added to standard treatment in 65 patients (31%) of which 47 continue to be treated to date. Penicillin prophylaxis was communicated in 165 patients (79%). Vitamin-D prophylaxis was initiated in 128 patients (61%). Chronic exchange or simple transfusions existed in 26 cases (12%) and 9 children (4%) underwent splenectomy. None of these patients had sepsis or meningitis. Cholecystectomy was performed in 9 cases (4%). There were 18 progenitor stem cell transplantations (8.61%) performed between 2.09 to 13.97 years of age (median 6.77 years). Ten patients remained on complete chimerism, 6 on mixed chimerism and 1 attained a marrow rejection. One patient died of graft-*versus*-host disease. Patients lost in follow-up summed up 128: 23 for emigrating to other countries, 65 for continuing the monitor of their diseases in other centers or in adults units and 31 for unknown reasons. There were 9 deaths (2.87%).
Summary/Conclusions: Early diagnosis like universal neonatal screening allows an effective health education, and antibiotic and osteopenia prophylaxis with vitamin D and general and specific vaccination can be started.

PB2144

COMPLEMENT ACTIVATION IN PATIENTS WITH SICKLE CELL DISEASE IS ASSOCIATED WITH HIGHER HBS LEVELS

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Background: Older studies have suggested activation of the alternative pathway of complement (APC) in sickle cell disease (SCD). Despite the renewed interest in SCD therapeutics, little is known about APC activation in the clinical setting of SCD, possibly due to the complexity of complement diagnostics.
Aims: We investigated firstly, whether complement activation can be detected in the sera of asymptomatic SCD patients using a simple functional assay, secondly whether it is associated with clinical parameters and thirdly whether it can be blocked *in vitro* by the complement inhibitor eculizumab.
Methods: Consecutive asymptomatic SCD patients were enrolled prospectively from November 2016 to January 2017. Patient history, clinical and laboratory data were recorded. Complement activation was detected in patient sera using the modified Ham test, a cell proliferation assay based on the susceptibility of a PNH (paroxysmal nocturnal hemoglobinuria)-like cell line to complement activated serum. Normal human serum (NHS) was used as a negative control and lipopolysaccharides (LPS)-incubated normal serum as a positive control. All samples were tested in triplicates and twice. Eculizumab containing serum (ECU) was collected from a PNH patient within 60 minutes after the infusion and used to test complement blockade by eculizumab in the modified Ham test.
Results: We studied 26 SCD patients (36±11 years of age, 12 male:14 female, 8 HbS/S: 18 HbS/β-Thal). Among them, only 6 had a history of painful crisis and 15 a splenectomy, 15 were on hydroxyurea and 12 on antithrombotic treatment. Based on previous studies and present controls, percentage of non-viable cells higher than 20% was considered a positive modified Ham test, indicating increased APC activation in 4 SCD patients (Figure 1). Positive patients had significantly increased HbS levels (80.5±11 *vs* 61.8±16%, p=0.037), while 2/4 were homozygous (HbS/S) and 2/4 heterozygous (HbS/β-Thal). The majority (3/4) were not on hydroxyurea. No significant difference was found regarding age, gender, platelets, white blood cells, Hb, HbF, LDH and bilirubin levels between patients with and without complement activation. Then, we evaluated *in vitro* the efficacy of complement inhibition by eculizumab in the modified Ham test. Mixing eculizumab serum (ECU) with complement activated sera demonstrated a dose-killing relationship that was consistent across the 4 patients.

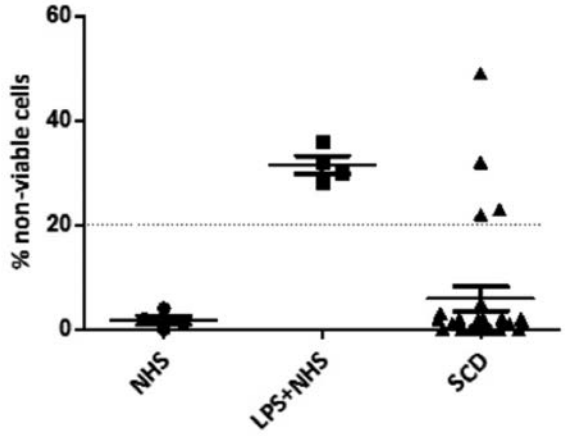

Figure 1.

Summary/Conclusions: Our results suggest that complement dysregulation is evident in asymptomatic SCD patients with increased HbS levels, an important tool in everyday clinical practice. APC activation during a painful crisis and the role of hydroxyurea need to be further investigated in larger series validating the role of different functional assays. Effective inhibition of complement activation *in vitro* is promising for future studies in selected patients.

PB2145

THE ROLE OF EXECUTIVE DYSFUNCTIONS IN THE VERBAL LANGUAGE DEFICITS OF CHILDREN WITH SICKLE CELL DISEASE

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Background: Children with Sickle Cell Disease (SCD) frequently present impairment of general and specific neurocognitive functions, even in the absence of clear neurological damage at brain neuroimaging. Verbal language deficits are also common, but the etiology of poor performance in the verbal domain is still not clear. The ability to speak and communicate verbally relies on a complex interaction of cognitive and linguistic functions as well as on environmental factors, like bilingualism or second language (L2) learning. The majority of children with SCD in Italy are of immigrant families whose first language is not Italian. These children thus perform poorly in tests assessing the verbal domain with adverse impact on school performances.
Aims: To evaluate if verbal language deficits in bilingual children with SCD are mainly due to linguistic and environmental issues or to impairment of executive functions.
Methods: In this study a cohort of bilingual children with SCD and social-demographically matched healthy controls recruited from elementary schools of the same city, performed an extensive battery of tests to assess naming skills, phonological and semantic fluency, attention and Executive Functions (inhibition and planning skills) and visuo-spatial skills (Boston naming test, phonological and semantic fluency tests, Flanker task, Elithorn test, PMA spatial relations subscale). All tests were administered in Italian. A composite index considering parental education and employment was used to match socially, demographically and economically children at best. Analyses of variance (ANOVAs) were performed to test differences between the two groups in verbal language, attention and executive functions. Hierarchical regressions explored the contribution of linguistic knowledge and executive functions (*i.e.* inhibition) to the verbal language deficit of children with SCD.
Results: Thirty-two children with HbSS SCD aged 6 to 12 years (mean age= 9.03) and 35 controls (mean age= 9.14) were enrolled. Patients and controls were matched for gender (F 53 *vs* 61%), ethnicity (African 30 *vs* 29),% of children born in Italy (81 *vs* 80%), number of years lived in Italy (8.09 *vs* 8.31) and Socio-Demographic Index (5.15 *vs* 4.59). Children's Performance (in Z scores) at Visuo-Spatial, Boston Naming, Phonological Fluency and Semantic Fluency Tests are shown in Figure 1. The results overall showed significant differences between patients and controls in inhibition and planning (p= 0.001 and 0.001 respectively) and in verbal tasks that involved Executive Functions more (*i.e.* phonological fluency) (p=.004). The poorer verbal performance of children with SCD was not associated to visible lesions to Broca's area; in fact only 9 patients presented Silent Infarcts that were all in the white matter, in watershed areas. Regression analyses showed that in children with SCD inhibition skills explained unique variance in phonological fluency, suggesting that poor executive control

was a factor of the lower performance in this task. Figure 1. Children's Performance (in Z scores) at Visuo-Spatial, Boston Naming, Phonological Fluency and Semantic Fluency Tests. P-values: Visuo-spatial intelligence: not significant(ns); Boston naming: ns; Phonol-Fluency: 0.004; Semantic fluency: ns.

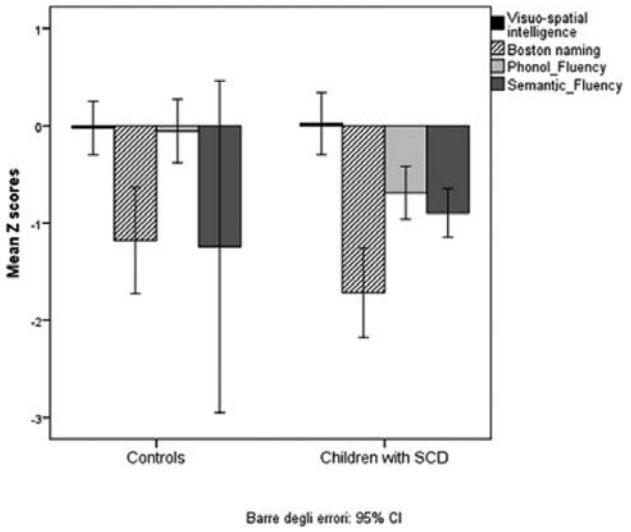

Figure 1.

Summary/Conclusions: Selective language problems may occur in children with SCD in the absence of clear neurological damage to language areas. These problems are explained by the executive dysfunction of patients with SCD and not by environmental factors like bilingualism. Cognitive rehabilitation or extra tuition may aid in overcoming these difficulties.

PB2146

UNDERSTANDING MEDICAL HISTORY, LIFESTYLE AND NEEDS FOR FUTURE THERAPIES FOR PEOPLE LIVING WITH SICKLE CELL DISEASE - IMPLICATIONS FROM A PATIENT SURVEY

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Background: Sickle Cell Disease (SCD) is an inherited blood disorder affecting millions of people. Sevuparin/DF02 is being developed to treat people suffering from SCD and is currently in clinical phase 2 for the treatment of the acute painful crisis in hospitalized SCD patients with intravenous infusion. This is called the Resolve program. In a second program called EASE, sevuparin/DF02 will be investigated as an on-demand treatment of early symptoms of painful sickle cell crisis in an at-home setting via a subcutaneous injection.Searching in the literature and discussing with health care providers, it becomes clear that little is known about how the SCD patients sense these early symptoms of a painful crisis. In order to gain increased understanding of how people living with SCD experience daily life, coping with disease, support by health care providers and the demand for new therapies, a patient survey addressing these areas was conducted.
Aims: The aim with this survey was to gain deeper understanding of different aspects of life with SCD by providing a channel for patients to air their own views. The outcome will provide important information and, in combination with future feasibility studies, will guide the design of the first clinical study aimed at treating the early symptoms of pain crises in SCD patients.
Methods: A 29-question survey was created to gather input on a wide variety of topics related to the lives of people living with SCD. This questionnaire was developed by Modus Therapeutics AB, Sweden, in conjunction with Micromattie Consulting Inc., USA. Experts and leaders of community-based organizations participated in two focus group sessions to ensure that the text and structure were ethical and appropriate for the intended purpose. The survey was hosted at www.modustxpatientsurvey.com. Patients answered the survey directly, or had their views entered in by a caregiver. The answers are anonymous. During the initial period, survey promotion occurred within the Sickle Cell Warriors online community and later, additional connections within the network of community-based organizations were leveraged. The survey was open for access during the period of January 10, 2017 through March 1, 2017.
Results: An interim analysis was conducted on January 31, 2017. Basic demographic data is presented in Table 1. Responders were located mainly in the US. Medical history related questions indicate that fatigue (40%), aches/pain (37%), irritability (27%) and appetite (20%) are early symptoms and increase just before the onset of a pain crises. However, 7% reported infrequent signs and 19% never experienced an indicator of pain crisis. Patients take initiative at home to manage the onset of an acute crisis and the top 5 home strategies reported were: prescription pain medication (15%), sleep/rest (15%), apply heat using heating pad/blanket/bath/shower (13%), increase fluid intake (12%), and finally avoid stress (9%). Further it is clear, that people living with SCD are motivated to try a new therapy that could provide "significant relief" and "prevent symptoms from happening" due to their SCD.

Table 1.

Demographic data from interim analysis January 31, 2017.

Demographic data	
Number of responders	70
Age	4-74 years
Female	80%
Male	20%
SCD diagnosis	100%
Ethnicity	
• African American/African Descent	97%
• Other	3%

Summary/Conclusions: The survey collected feedback about topics for which the patient is the best source of information. It is obvious that people with SCD are willing to self-medicate by subcutaneous injections and that there is a need for new tools and medications. With support from the answers from the survey, specific aspects will be considered while designing a first clinical study for subcutaneous sevuparin/DF02 administration to treat early symptoms of painful crisis in an at-home setting.

PB2147

LONG-TERM USE OF HYDROXYUREA IN CHILDREN AND ADOLESCENTS WITH SICKLE /BETA THALASSEMIA

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Background: Hydroxyurea (HU) has lately been used in the treatment of patients with severe sickle cell disease (SCD). Despite documented benefits on laboratory and clinical parameters in SCD patients, there are few reports about drug's long-term safety and efficacy in pediatric patients with SCD – even more so in the rare patient subgroup of sickle/beta thalassemia.
Aims: A prospective, long term evaluation of HU efficacy and safety in children and adolescents with sickle/beta thalassemia (S/b thal).
Methods: Ten patients with S/b thal aged 3.5-18 years were followed for a 6 year period (Jan 2011- Dec 2016). HU was given at a daily dose that ranged from 10 to 20 mg/kg, with a mean of 14.1 mg/kg. Laboratory follow-up consisted of WBC, Hb, Ht, RBC, reticulocyte count and PLT count measured every 2 weeks until dose escalation to a stable dose, biochemistry assessed every 2 months and Hb F measured every 2-3 months. Patients were clinically evaluated prior to HU treatment and every 12 weeks during the study period. Evaluated data on clinical course included frequency of vaso-occlusive crises, hospitalizations and transfusions, as well as presence of severe clinical events. Hematologic toxicity of hydroxyurea was defined as a more than 20% decline from baseline in Hb, as an absolute neutrophil count of less than 1,000/μl and/or a PLT count of less than 80,000/μl. Moreover, presence of alopecia, rash, skin hyperpigmentation or headache was reported as drug-related toxicity.
Results: A significant reduction in vaso-occlusive crises as compared to prior to HU treatment was noted (median: 1 episode per year before HU, range: 0-2.5 *vs* median: 0.24 episodes per study year after HU, range: 0-1.33, p=0.011). A significant reduction in hospitaliizations was also reported (median: 1 per year before HU, range: 0.3-2 *vs* median: 0.16 per study year after HU, 0-0.83, p=0.005). None of the patients presented with severe clinical events such as acute chest syndrome, avascular bone necrosis, stroke or splenic sequestration during the study period. With regards to hematological parameters, a significant increase in HbF (10.2±6.5% *vs* 16.6±7.1% p=0.02), MCV (66.1±3.9fl *vs* 79.3±8.4fl, p<0.001) and MCH (20.9±1.2pg *vs* 25.3±2.2pg, p<0.001), as well as a decrease in reticulocyte count (7.7±3.3% *vs* 5.0±1.9%, p=0.039), WBC count (9,566±3,674/μl, *vs* 7,466±3,460/μl, p=0.009) and PLT count (333,778/μl,±170,227 *vs* 272,111±160,304/μl, p=0.007) was noted. Concerning adverse events, one patient presented with mild transaminasemia, one with mild elevation of serum creatinine levels and one with pancytopenia. Due to persistent pancytopenia HU treatment was discontinued in the last mentioned patient, but was restarted a year later due to frequent vaso-occlusive events - despite the patient being put on transfusions after initial HU discontinuation. Besides the pancytopenia episode, the rest of the mentioned toxicities were short-term and dose-dependant.
Summary/Conclusions: The study indicates that HU has an overall safe profile and results in a marked improvement of clinical course in pediatric S/b thal patients.

PB2148

IN VITRO AND *IN VIVO* EVIDENCES OF SICKLING REVERSAL INDUCED BY REHYDRATION WITH HIGH K+-ISOTONIC SOLUTION

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Background: Erythrocyte sickling and adhesion are favoured by cellular dehydration, which increases the rate of hemoglobin polymerization and cell sickling. Potassium chloride co-transport and calcium-activated potassium channel (Gardos channel) mediate erythrocyte dehydration in sickle cell disease and β-thalassemia. We investigated the in-vitro and in-vivo effects of various concentration of K^+ ions in physiological solutions (PSS) as well as in *cocos nucifera* water (CNw) which is known for its natural high potassium content and isotonicity.
Aims: This study was aimed at ascertain the efficacy of high potassium isotonic solutions in rehydrating sickle cell and possibly reversing the sickling phenomenon at *in vivo* and *in vitro* situations
Methods: : Erythrocytes from twenty sickle cell anaemia (SCA) as well as 46 healthy subjects were studied. One part was treated with sodium metabisulphite ($Na_2S_5O_7$) solution to induce maximum sickling as controls while the other was subjected to different high concentrations of K^+ in PSS as well as *Cocos nucifera* water (40mM, 80mM and CNw - 65mmol/L) respectively. The procedure was repeated for the normal HB AA subjects. Also, both groups of subjects were given 10ml/kg body weight of coconut water to drink as a single dose for the in-vivo experiment. Blood samples were collected longitudinally before and after the oral ingestion, at 1hr and at 24hrs for analysis of red cell indices as well as stained blood films used to ascertain the percentage sickled erythrocytes count before and after the treatment in both cases.
Results: Maximum percentage counts of sickled cells after the addition of $Na_2S_5O_7$ (45%) were observed which decreased significantly ($P<0.05$, respectively) to about 2% with Cocos nucifera and 10% with 80mM K^{+PSS}. The count in 40mM K^{+-PSS} was not statistically significant. In both Hb AA and SS subjects, MCH and MCV remained relatively stable when compared with the pre-ingestion sample ($P>0.05$, respectively) while MCHC increased significantly in both groups as early as 1hr and sustained till the 24th hour. MCHC was equally raised in the in-vitro samples ($P<0.05$, respectively). The morphology of red cells also indicated a lesser count of sickled red cells after the oral ingestion
Summary/Conclusions: *Cocos nucifera* water and other high potassium ion solutions can activate the rehydration of sickled erythrocytes by probably de-activating the Gardos channel to increase the mean corpuscular haemoglobin concentration(MCHC) and thereby restoring the normal red cell shape. We suggest a probable pharmacological value of the *cocos nucifera water* as well as other formulated high potassium but isotonic fluids in SCA management.

PB2149

VITAMIN D IN SPANISH CHILDREN WITH HEMOGLOBINOPATHIES.

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Background: Although vitamin D deficiency has been documented as a frequent problem in studies of children, there are limited data on the prevalence of this nutritional deficiency among children who suffer from sickle cells disease (SCD) or thalassemia. Vitamin D homeostasis is important to prevent osteopenia. Furthermore vitamin D deficiency has been associated with increased risk of common cancers, autoimmune diseases, hypertension, and infectious diseases. Vitamin D deficiency is now recognized as a pandemic. The major cause of vitamin D deficiency is the lack of sun. Although Spain has a high rate of sunny hours, we have found low levels of vitamin D in our patients with SCD or thalassemia.
Aims: The purpose of this work is to assess the status of vitamin D in children with SCD and thalassemia in our setting.
Methods: We have recruited children diagnosed with SCD and thalassemia between 1998 and 2016 and we have reviewed their vitamin D levels. We have chosen the first vitamin value we obtained and the last one till today. Vitamin D was measured by quantitative determination of 25(OH) D. Deficit of vitamin D was defined by<30 ng/ml. The study enrolled 114 children. Most of them, with SCD diagnosis (94%). The type of anaemia was Hb SS (94 patients), Hb SC (8 patients), Hb Sß0 (3 patients) and HbSß+ (2 patients). The remaining 6% were diagnosed with Thalassemia Maior. Mostly of the children were African or Central-South American. In our centre, vitamin D prophylaxis is made since the first year of life.
Results: 60% of the children had vitamin D deficiency. We have divided children into 4 groups depending on the age. When considering vitamin D first determination: mean vitamin D levels in children below 2 years old were 39.5±13.3 ng/dl. The group between two and five years old had a mean serum vitamin D of 35.5±14.8 ng/dl. Children aged between five and ten had 26.1±13.5 ng/dl of mean 25(OH)D. Finally in the group older than 10, we observed mean of 7.4±14 ng/dl. When having these low levels of vitamin D, we strongly recommend to start treatment with Cholecalciferol 25000U/month. Regarding second levels of vitamin D, we have divided patients into those who presumably have the treatment against children who do not. We present the results in the following Table 1.

Table 1.

	<2 years old	2-5 years old	5-10 years old	>10 years old
Vit D treatment	33.7±9.02ng/dl	35.5±12.3ng/dl	34.3±7.3 ng/dl	26.4±11.2ng/dl
No Vit D treatment	-	33.8±1.5 ng/dl	27.3±4.9 ng/dl	6.6±2 ng/dl

Summary/Conclusions: The study found a high prevalence of vitamin D deficiency in children older than five years old(in the first determination) with SCD or Thalassemia Maior and significant decrease of levels in those not having vitamin D therapy. It is not well known the physiopathology of this factor deficiency, although it is supposed to be multifactorial. However we confirm that living in a sunny geographical situation with a healthy diet is not enough to maintain an adequate 25(OH)D levels. Although it is difficult to reach correct levels of vitamin with oral treatment, vitamin D levels increase when having correct doses. We have also checked that older children have lower levels of vitamin D than younger boys. This could be explained by the fact that preteenagers spend lot of time at home instead of going out. If prophylaxis is made not only the vitamin levels will increase but bone growth also.

PB2150

KNOWLEDGE OF SICKLE-CELL DISEASE IN HAUTE-NORMANDIE, SOCIO-DEMOGRAPHIC CONTEXT AND HEALTH CHARACTERISTICS: INTEREST OF THE IMPLEMENTATION OF A PATIENT EDUCATION IN SICKLE CELL DISEASE

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Background: Sickle cell anemia (SCA) is a genetic disease causing a severe disease manifesting by painful crisis but which can also be marked by organ complications. Mortality is still happening at a young age. Many of these complications may be better taken care of if treated early. The best way to manage this disease is probably through Patient Education (PE)
Aims: Patient Education for children with sickle cell anemia has been a subject of research, organized in France by association such as ROFSED, but PE in adult patients has been little studied. The main objective of this work was to evaluate SCA patients followed in Haute-Normandie, from a sociodemographic, health and socio-demographic perspective in order to establish a PE program. The secondary objective was to give them the opportunity to express their expectations of such a program.
Methods: We did an observational multicenter study. A self-questionnaire of 39 items was sent to all patients suffering from SCA followed in Haute-Normandie.
Results: Fifty patients (male / female ratio 0.92) out of 123 (40.6%) responded, mean age 33±10.5 years (SS genotypes [66%], SC[25%], S-beta-thalassemia [9%]). 56% of them were born outside of Metropolitan France, 36% came from French speaking African countries. Aerage age was 18±10.9 years. Despite the fact that their education has been disrupted by the disease for the majority (69.4%), the level of education was “satisfactory”: 68% of patients had graduated from high school or achieved a higher level, 18% had graduated from professional education, 10% had a primary / middle school level and 4% were illiterate. 68% of the patients had a job or were students. 48% of patients reported to practice physical activity at least once weekly. Tobacco was consumed on a daily basis by 14%, alcohol 2% and 4% for cannabis. Self-assessment of health status was 6.9 / 10, self-assessment of morale of 7.9 / 10 and impact of the disease on daily life was estimated at 5.4 / 10. The mean age at at which specialized follow-up was started was 11±9 years. 88% of the subjects stated that they understood everything the doctor said during consultation. Missed appointments were reported by 26% which was justified by forgetfulness, lack of will or physical incapacity. Regarding sources of information regarding SCA, patients declared asking their specialist first and then looking on the internet. 68% of subjects had a first-degree relative suffering from the same disease, 71% were able to talk about the disease with their family. While the triggers of crises and the management of crises were well-identified by patients (average scores of 13.8 and 12/20), “standards” were not met with chronic complications, prenatal diagnosis, and long term treatment (mean scores respectively of 7.4; 4.2 and 2.2 / 20). Average score on the whole questionnaire was 9/20. Most patients showed interested in PE (52.1%) *vs* 31.3% that claimed were not interested, 17.7% did not decide.
Summary/Conclusions: A majority of SCA adults followed in Haute-Normandie are first-generation migrants. Even if the disease has heavy impact on everyday life and school access, their education level appeared correct. PE sessions will need to focus on chronic complications, prenatal diagnosis, and the long term treatment. The majority of adults with SCA are motivated by PE, we will have to adapt to a heterogeneous population in terms of educational level, ethnic origin and knowledge of the disease.

PB2151

DELAYED HAEMOLYTIC TRANSFUSION REACTIONS: A MASQUERADE OF SICKLE CELL COMPLICATIONS

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Background: Patients with sickle cell disease (SCD) may require repeated red blood cells (RBCs) transfusion, putting them at risk from minor blood group alloimmunization and the development of delayed haemolytic transfusion reactions.
Aims: Reported a prevalence of recognized DHTR syndrome in patients with SCD.
Methods: We reviewed the cases of (DHTR) in SCD patients in a 5-year period (2010- 2016). A total of 10 patients had a clinical picture compatible with DHTR and underwent treatment with high dose steroids, intravenous immunoglobulins (IVIG) or erythropoietin. Any patient received Rituximab.
Results: The most common indications for transfusion were anemia due to vasococlusive sickle cell crisis or preoperative anaemia optimization. The cohort received partial exchange transfusion and phenotypically matched RBCs. Before transfusion the median of Hb level was 69 g/L (baseline range 80g/L) and the nadir at haemolysis episode was 38 g/L, Ht was 21.9%, WBC was 17.3 × 10^9 cells/L and mean LDH 1290 IU/L). The median time to develop DHTR was seven days after the transfusion and approximately 6 days after the surgical interventions (range: 4–12 days) and all cases presented with symptoms of anaemia, jaundice, tiredness and tachycardia. The median age was 29 years with female predominance (6:4). Blood cultures were negative in 80% of patients and only positive in 2 cases. 30% of patients tested positive for viral infection on PCR. Mortality rate in our series was low (zero). Pain episodes and other complications associated with DHTR was treated as required and four cases were successfully monitored in HDU. One patient required noninvasive ventilations and inotropic support. Two patients received RBCs transfusion (median 1.5 unit of packed RBCs). Possibly as their presentation mimics an acute vaso-oclusive crisis. In all cases haemoglobin stabilized and improved, symptoms resolved and patients were discharged on small course of oral antibiotics (median admission 6 days).
Summary/Conclusions: The symptoms of DHTR can easily be mistaken for other SCD complications, including infection and vaso-occlusive crisis.
The diagnosis of DHRT is based on clinical suspicion, when there is a rapid Hb drop after a recent RBC transfusion with clinical signs of haemolysis. To support the diagnosis, laboratory tests (serial FBCs, haemolysis screen, DAT, measurement of Hb S levels) and exclusion of other aetiologies are useful. Whenever a DHTR is suspected, further RBC transfusion should be withheld unless absolutely necessary, as it may precipitate acceleration of the hemolytic reaction. Patients in whom the diagnosis of DHTR is missed may receive repeat transfusions, which may contribute to the complications associated with SCD. The use of more extensive phenotypic matching of blood and minimizing RBC transfusion help to prevent DHTR. The present series emphasize the importance of early recognition of symptoms and signs in correlation with a recent history of RBC transfusions, as DHTR can be a potentially life-threatening complication.

PB2152

HBS MONITORING ON TOSOH G8 IN VARIANT HBA1C MODE IN CASE OF URGENT RCE

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Background: Pre- and post-transfusion HbS levels are used to document the efficacy of red blood cell exchange (RCE) in patients with sickle cell disease (SCD). In case of urgent RCE a 24/7 STAT analysis, with the ability to identify and quantify hemoglobin (Hb) S, is warranted.
Aims: We evaluated the use of Tosoh Automated Glycohemoglobin Analyzer HLC-723G8 (Tosoh Europe, Amsterdam, The Netherlands) for this purpose, using the variant HbA1c mode. Results were compared to our routine CZE Minicap Flex Piercing (Sebia, Lisses, France).
Methods: Within- and between-run imprecision were assessed using a sickle cell trait and a sickle cell anemia sample, aliquoted and stored at -80°C, twice daily in duplicate for ten days. A linearity study was performed using duplicate measurements of a dilution set of 11 samples (HbS range: 0% - 88%). Additionally, a comparison study was conducted between TOSOH G8 and Minicap Flex Piercing using 32 whole blood left-over HbS samples (HbS range: 9% - 93%). Data analysis was performed using Microsoft Excel Analyze-it version 4.65.3 and differences were considered as statistically different if the P-value was <0.05.
Results: Within- and between-run imprecision were <2% and an acceptable linearity was observed. Passing-bablok regression analysis comparing TOSOH G8 and Minicap Flex Piercing showed an acceptable correlation coefficient of 0.998 (>0.95) and a slope and intercept of 0.94 (95%CI: 0.92 to 0.98) and 0.057 (95% CI: -2.5 to 1.3), respectively. Differences in HbS results between TOSOH G8 and Minicap Flex Piercing ranged from -8.76% to +0.36% (mean difference: -3.54%). More specifically, for samples with a HbS concentration <25% HbS results on TOSOH G8 differed between -0.34% to +0.36% compared to Minicap Flex Piercing. For samples with a HbS concentration >25%, differences in HbS results ranged from -8.76% to -0.43%.
Summary/Conclusions: In our clinical laboratory, TOSOH G8 is used in variant HbA1c mode to quantify HbA1c. Previous studies demonstrated reliable HbS identification using TOSOH G8 in variant HbA1c mode. Our study showed a good analytical performance for HbS quantification using TOSOH G8. Good correlation with Minicap Flex Piercing system was found, although results were statistically not interchangeable. Our results suggest that TOSOH G8 in variant HbA1c mode generates lower HbS results in samples with a high HbS concentration (>25%) compared to our routine analyzer. However, the goal of RCE is to achieve a post-transfusion HbS level of 30% or less. Therefore, results obtained with TOSOH G8 are clinically acceptable to monitor post-transfusion HbS levels. Importantly, HbS on TOSOH G8 can only be requested in case of urgent RCE. Our routine hemoglobinopathy screening will still be performed using CZE Minicap Flex Piercing in combination with CE-HPLC Variant ^{II}TM.

PB2153

GENDER DIFFERENCES IN THE DEVELOPMENT OF CMR ABNORMALITIES AND CARDIAC COMPLICATIONS: A MULTICENTRIC PROSPECTIVE STUDY IN A COHORT OF SICKLE CELL DISEASE PATIENTS

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Background: No data are available in literature about the relationship between gender and the development of CMR abnormalities and/or cardiac complications in sickle cell disease (SCD).
Aims: This prospective and multicentre study aimed to assess if there was an association between gender and risk of cardiac iron overload, heart dysfunction and dilation, left ventricular (LV) hypertrophy, and myocardial fibrosis, assessed by Cardiovascular Magnetic Resonance (CMR), and of cardio-vascular complications in sickle cell disease (SCD) patients.
Methods: We considered 115 SCD patients (58 females, 34.79±13.26 years), consecutively enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) Network. Myocardial iron overload was assessed by the multislice multiecho T2* technique. Biventricular function parameters and atrial areas were quantified by cine images. Late gadolinium enhancement (LGE) images were acquired to detect myocardial fibrosis.
Results: Table 1 shows the comparison between sexes in the development of cardiac outcomes. Males and fameles showed a similar risk of accumulating cardiac iron, but both patients with cardiac iron were females. Compared to females, males showed a significant lower risk of developing LV hypertrophy, although having a similar risk for biventricular dilation and dysfunction and for myocardial fibrosis. No patients with less than 31 years developed LV hypertrophy and age at the CMR was significantly higher in patients with LV hypertrophy *versus* patients without it (41.24±75.98 years *versus* 34.47±13.37 years; P=0.003). We recorded 12 (10.4%) cardiac events: 4 ischemic strokes, 5 arrhythmias (4 supraventricular and 1 ventricular), two pulmonary hypertensions and one pulmonary embolism. No prospective association was detected between gender and cardiac complications.

Table 1.

	N(%) with positive outcome	Cox Regression	
		HR (95%IC)	P
Global heart T2<20 ms:*			
Female sex	2 (3.4)	Reference	0.506
Male sex	0	0.02	
Ventricular dilatation			
Female sex	10 (17.2)	Reference	0.162
Male sex	4/55 (7.3)	0.44 (0.14-1.39)	
LV hypertrophy			
Female sex	12 (20.7)	Reference	0.02
Male sex	1/55 (1.8)	0.09 (0.01-0.069)	
Ventricular dysfunction:			
Female sex	12 (20.7)	Reference	0.965
Male sex	11/55 (20.0)	1.02 (0.44-2.37)	
Myocardial Fibrosis:			
Female sex	7/39 (17.9)	Reference	0.338
Male sex	3/35 (8.3)	0.50 (0.12-2.05)	
Atrial dilatation			
Female sex	9/36 (25.0)	Reference	0.990
Male sex	7/32 (21.9)	1.01 (0.37-2.71)	
Cardiac complications			
Female sex	8 (13.8)	Reference	0.204
Male sex	4 (7.0)	0.42 (0.11-1.59)	

Summary/Conclusions: In SCD male and female seem to show a comparable risk in developing cardiac complication, although compared to females, males showed a significant lower risk of developing LV hypertrophy. There are no specific guidelines for SCD patients and, as a consequence, the cardiovascular follow-up is conformed to that one of thalassemia patients (complete cardiac evaluation performed annually for both genders). Our data not support a different follow up time based on the gender.

PB2154

SICKLE CELL PAIN IN CHILDREN: TARGETS FOR ADMINISTRATION OF ADEQUATE INITIAL ANALGESIA

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Background: Acute pain is a hallmark presentation in sickle cell disease (SCD) and frequently requires attendance to the emergency department (ED).
Aims: Here we report our findings following a complete retrospective audit cycle, documenting the timeliness of analgesia administration and post-treatment pain review as per National Institute of Clinical Excellence and College of Emergency Medicine guidelines, in children with SCD presenting to a single inner city London ED over a 14 month period.
Methods: In 2014, we evaluated 48 patient records of children presenting to the ED, with respect to mild, moderate and severe pain scores, time of analgesia administration and pain review. Completing the audit cycle, 97 records were re-audited in 2015. A total of 145 admission records were evaluated.
Results: In 2014 the ED met CEM criteria for the timeliness of analgesia administration in 100% of severe and 95% of the moderate pain category; however fell 33% short of NICE standards. Pain review was poorly performed, identifying an area for improvement. Proportions meeting the aforementioned criteria fell significantly in 2015, except review of moderate pain, which increased by 25%.
Summary/Conclusions: We conclude CEM guidelines promote timely administration of analgesia in patients with severe pain; however mild pain may be overlooked. NICE avoids this discrimination. Thus we recommend combining the mild and moderate pain categories to acknowledge the fluctuating nature of sickle pain and its tendency to rapidly escalate. In addition, we reiterate the need for regular pain reviews. This is important in ensuring analgesia is closely titrated to pain level.

PB2155

DIAGNOSTIC CHALLENGES IN A POPULATION WITH INCREASED IMMIGRATION: HEMOGLOBINOPATHIES IN THE NEW CENTURY

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Background: The diagnosis of hemoglobinopathies (Hbpts) has changed in recent years due to immigration, with an increase in structural Hbpts. In our region, Asturias, population census is 1,061.756 habitants; 48,097 out of them are immigrants.
Aims: Review the incidence of structural Hbpts and thalassemias in our region in the last 10 years.
Methods: A retrospective analysis was performed with 1202 hemoglobin (Hb) studies at Hospital Universitario Central de Asturias between January 2006 and March 2016. The studies came from medical applications, the finding of abnormal Hb patterns in the HbA1c test or suggestive results of thalassemia on hematimetry test. Studies were performed by high-performance liquid chromatography (HPLC) with the Variant Hemoglobin Testing machine of Bio Rad, and Hb electrophoresis with Paragon plates until May 2013. Since then, it has been performed by capillary electrophoresis (CE) with the MINICAP System of the Sebia laboratory.
Results: We analyzed 1202 patients, 49% were males and the median age was 33 years (range 0-85). We found 562 patologic studies. - Thalassemia were detected in 390; 337 were β or δβ (86.4%); 54% came from Spain. The cases of β-thalassemia were: 5 intermedia, 3 major, 1 δβ-homozygote and the remainder were minor (97%). All thalassemia major, 3 intermediate and 74%of minor were Caucasian. Anemia was found in all of major thalassemia (median Hb 6.2g / dL, range 6-6.5), in 3 of intermedia (11.4g / dL, range 6-10), and in 197 of minor (10.87g / dL, range 6.9-11.9). - Structural Hbpts were found in 170, the predominant was Hb S (n=125). Only 17 sickle cells (Hb SS and Hb SC). Most of them black (n=14) coming from Africa (n=10) and South America (n=5). Eighty six percent (n=108) were heterozygous (Hb SA), mainly from Africa (n=56) and South America (n=23). Anemia were seen: 4 Hb SC (median Hb 10.5g / dL, range: 9.4-11.2), 9 Hb SS (7.94g / dL, range 5.2-9.7) and 37 heterozygotes (10.15g / dL, range 6.7-11.9). There are two peaks of higher incidence of structural Hbpts, in 2008 coinciding with the creation of the Tropical Diseases Unit and since 2013 when detection increases with the introduction of HbA1c test. The increase in thalassemias was due to the decision to extend studies due to pathological findings in hematimetry results (Figure 1).

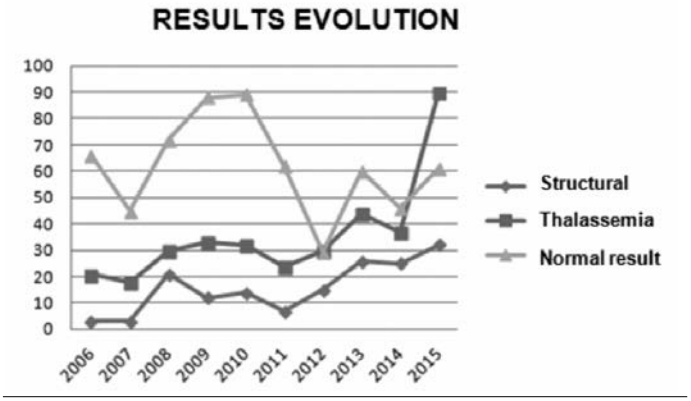

Figure 1.

Summary/Conclusions: In our area there is a predominance of β-thalassemia minor. Structural Hbpts are the main diagnosis in immigrants. The incidence is still small, although increasing in the last 3 years, so a neonatal screening program is being implemented. Both HPLC and EC are simple, fast and efficient methods in the diagnosis of Hbpts. In our area there is a predominance of β-thalassemia minor. Structural Hbpts are the main diagnosis in immigrants. The incidence is still small, although increasing in the last 3 years, so a neonatal screening program is being implemented. Both HPLC and CE are simple, fast and efficient methods in the diagnosis of Hbpts.

PB2156

EFFECT OF SUSTAINED-RELEASE SUPPLEMENTATION OF L-ARGININE AMONG CHILDREN WITH SICKLE CELL DISEASE IN FEDERAL TEACHING HOSPITAL GOMBE, NORTH EASTERN NIGERIA

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Background: Sickle cell disease is a global public health problem. As of 2013 about 3.2 million people have sickle-cell disease with 176,000 deaths
Aims: In this present study, we investigated the effect of 8 weeks, low dose supplementation of sustained-release of nitric oxide generating L-arginine supplement (350mg) given two times daily on the full blood count, L-arginine, nitric oxide, Pantothenic acid, plasma malondaldehyde, glutathione and total antioxidant capacity of children with sickle cell disease.
Methods: This study included children with sickle cell disease (HbSS) aged 1-14 years with mean age 7.4500±0.50613 years presenting to the sickle cell clinic unit of Federal Teaching Hospital Gombe, Gombe State. Subjects received sustained release oral L-arginine supplementation of 350mg twice daily for 8 weeks.
Results: L-arginine and nitric oxide levels were significantly higher among sickle cell disease children. There were no statistically significant differences between the baseline and post L-arginine supplementation in the PCV, WBC, RBC and LYM levels of subjects ($p>0.05$). There was a statistically significant difference between the baseline and post L-arginine supplementation in the MCV, MCH, MCHC, PLT, NEU, EOS, MON and RDW-SD levels of subjects ($p<0.05$). The L-arginine and nitric oxide levels was significantly higher post supplementation compared to baseline levels ($p=0.002$ and 0.000 respectively). The pantothenic acid level was significantly higher at baseline compared to post supplementation levels ($p=0.00$). The L-arginine, nitric oxide and Total Antioxidant Capacity was significantly higher post supplementation compared to baseline levels among sickle cell disease subjects with vaso-occlusive crisis ($p= 0.001$, 0.01 and 0.05 respectively). The pantothenic acid and malondaldehyde levels at baseline were significantly higher than the post supplementation levels among subjects with vaso-occlusive crisis ($p=0.002$ and 0.000 respectively). The Total Antioxidant Capacity and Glutathione levels were significantly higher post supplementation compared to baseline levels among the sickle cell subjects ($p= 0.05$ and 0.000 respectively). The baseline plasma malondaldehyde level was significant higher that the post supplementation levels among the sickle cell disease subjects. There is need for more effort and resources to be dedicated to research especially in supplementation studies involving a larger population aimed at establishing specific treatment for sickle cell disease. It is recommended that L-arginine supplementation be included in the management of patients with sickle cell disease particularly those with vaso-occlusive crisis. We observed a statistically significant negative correlation between the L-arginine levels and the red cell count among sickle cell disease subjects ($r= -0.350$, $p=0.043$).

Summary/Conclusions: L-arginine supplement should be made available in the paediatric emergency unit, clinic and pharmacy department in high risk communities to obviate the negative effects during vaso-occlusive crisis and potentially reduce the length of stay in the hospital. L-arginine, nitric oxide, total antioxidant capacity, malondaldehyde and glutathione levels should be routinely monitored in sickle cell disease patients particularly those presenting with vaso-occlusive crisis.

Stem cell transplantation - Clinical

PB2157

THE EFFECT OF BODY MASS INDEX ON OUTCOME AFTER UMBILICAL CORD BLOOD TRANSPLANTATION IN PEDIATRIC PATIENTS WITH ACUTE LEUKEMIA ON BEHALF OF EUROCORD, PDWP

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Background: Body mass index (BMI) may influence outcome after allogeneic transplantation. Previous studies have demonstrated that being obese or underweight may have a detrimental effect on survival rates after chemotherapy induction in children with acute leukemia. However, the impact of BMI of transplanted patients on survival is still not clear, with conflicting results being reported on this issue.
Aims: To analyze the effect of BMI on UCBT outcomes in children with acute leukemia
Methods: We retrospectively analyzed 517 patients aged from 2 to 20 years with acute leukemia who underwent umbilical cord blood transplantation (UCBT) from 1990 to 2015. Patients were classified according to BMI as normal (5^{th}-85^{th} percentile), underweight (<5^{th} percentile), overweight (85^{th}-95^{th} percentile) and obese (>95^{th} percentile) by using growth charts for age and gender.
Results: Sixty-one percent (n=314) of patients were in the normal category, 12% (n=63) were underweight, 15% (n=80) overweight and 12% (n=60) obese. All patients received single-unit UCBT after a myeloablative conditioning regimen. Diagnosis was acute lymphoid leukemia in 70% (n=363) and acute myeloid leukemia in 30% (n=154). Median age at UCBT was 7.4 years (range 2-19.6). Cytomegalovirus (CMV) serology was positive in 45% patients; 60% of patients were male. Most patients (92%) were in complete remission at UCBT. Median follow-up was 52 months (range 2-201). Total body irradiation (>6 Gy) was used in 58% of cases; antithymocyte globulin (ATG) in 68% of cases. Median infused total nucleated cell (TNC) dose was $4.2x10^7$/Kg (0.3-17.8); 56% of patients received a graft with 0-1 HLA mismatch donor. Four-year overall survival (OS), leukemia-free survival (LFS) and graft-*versus*-host disease-free, relapse-free survival (GRFS) were 45±2%, 43±2% and 35±2%, respectively. Cumulative incidence function (CIF) of neutrophil engraftment was 88.6% (85.9-91.4%). CIF for acute GVHD was 34% (30.1-38.4%) at 100 days. At 4 years chronic GVHD was 19.1% (15.7-23.3%), relapse incidence was 34.5% (30.1-38.9%) and non-relapse mortality (NRM) was 22.8% (19.2-26.7%). In univariate analysis, no statistically significant difference in OS, LFS, GRFS, neutrophil engraftment, NRM and chronic GVHD between the 4 groups identified according to BMI was identified. Conversely, acute GVHD was 44.3% (33.3-58.8%) for underweight, 36% (31-41.8%) for normal, 26.2% (18.1-38%) for overweight and 23.3% (14.7-37.1%) for obese (p=0.03). Among patients underweight who experienced acute GVHD (n=27), 37.5% had grade III-IV acute GVHD with gut involvement. In multivariate analysis, infused TNC dose>$4.2x10^7$/Kg was associated with higher neutrophil engraftment (HR=1.46, CI 95% 1.18-1.82, p=0.001). Positive CMV serology (HR=1.5, CI 95% 1.04-2.26, p=0.03) and female gender (HR=1.5, CI 95% 1.03-2.23, p=0.03) were associated with higher NRM. ATG use (HR=1.6, CI 95% 1.05-2.31, p=0.03) was associated with higher relapse incidence. Moreover, ATG use and a positive CMV serology were associated with worse OS (HR=1.6, CI 95% 1.15-2.17, p=0.04 and HR=1.3, CI 95% 1.01-1.69, p<0.001, respectively) and LFS (HR=1.6, CI 95% 1.17-2.16, p<0.001 and HR=1.34, CI 95% 1.04-1.72, p=0.02, respectively). Infused TNC >$4.2x10^7$/Kg (HR=1.5, CI 95% 1.07-2.14, p=0.02), lack of ATG in the conditioning (HR=2.72, CI 95% 1.6-3.1, p<0.001) and BMI <5^{th} percentile (HR=1.8, CI 95% 1.19-2.78, p<0.001) were associated with higher incidence of acute grade II-IV GVHD.
Summary/Conclusions: In conclusion, we did not find association of obesity with transplant outcomes in this study population. However a BMI <5^{th} percentile at UCBT was found to be associated with higher risk of acute GVHD, highlighting the importance of nutritional status before UCBT.

PB2158

PROSPECTIVE PHASE STUDY OF REDUCED TOXICITY CONDITIONING CONSISTED OF HIGH DOSE-CYTARABINE, FLUDARABINE, CYCLOPHOSPHAMIDE +/- TOTAL BODY IRRADIATION FOLLOWED BY ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: Allogeneic hematopoietic stem cell transplantation (allo-SCT) using reduced intensity conditioning (RIC) has been widely applied to elderly or frail patients who are not eligible for conventional conditioning regimen. However, benefit provided by reduced toxicity has been often offset by increased incidence of relapse. So far, the optimal conditioning for those patients has not been established.
Aims: Here, to investigate whether addition of high dose cytarabine (AraC) to RIC regimen consisting of fludarabine (Flu) and cyclophosphamide (Cy) +/- total body irradiation (TBI) can be available for elderly or frail recipients, phase II study has been designed.
Methods: This study was conducted from April 2011 to December 2015. The protocol was approved by each institutional review board (Trial identifier: UMIN000007281). Patients aged from 55 to 70, or patients who have some organ damage or a history of SCT aged from 20 to 54 with hematologic malignancies were enrolled after obtaining written informed consent. Bone marrow (BM), peripheral blood (PB), or cord blood (CB) was used as stem cell sources. Pretransplant conditioning regimen consisted of 30 mg/m^2 of Flu for 5 days (total 150 mg/m^2), 4 g/m^2 of AraC for 2-4 days (divided by 2 daily, total 8-16 g/m^2) and 50mg/kg of Cy for a day. Four gray of TBI was used for all CB transplant recipients, whereas 2 gray of TBI was used in other stem cell sources except a matched related donor according to each institutional policy. Calcineurine inhibitors (cyclosporine or tacrolimus) and short term methotrexate were used as GVHD prophylaxis. Donor cell engraftment and 60 day-survival were assessed as a primary end point to evaluate feasibility of this protocol.
Results: Thirty nine patients including 7 recipients with a history of SCT were enrolled. Median age was 61 (28-69), 21 were male, and 18 were female. Nineteen were acute myeloid leukemia, 11 myelodysplastic syndrome, 6 malignant lymphoma and 3 acute lymphoblastic leukemia. Donors were 4 matched related PB, 8 matched unrelated BM, 5 1-Ag/allele-mismatched unrelated BM, and 22 ≤2-Ag-mismatched CB. Thirty seven (94.9%) patients have passed 60-day-point post-transplant. In 38 (97.4%) recipients, engraftment was obtained, a patient died before engraftment due to sepsis caused by enterococcus faecium (male CB recipient, 55y, day15). Median neutrophil recovery to over 500/μl was obtained on day 19 (16-38). Fourteen blood stream infections (13 bacteremias and 1 candidemia) judged as grade 3 toxicity and 2 cases (1 sepsis and 1 endotoxemia) of grade 4 toxicity were observed within 60 days post-transplant. There were 2 deaths of post-engraftment due to cerebral bleeding (1 female CB recipient, 64y, day 46) and GVHD (1 male CB recipient, 60y, day 77) within 100 days. Although no relapse was observed up to day 60, 7 relapses were observed up to 1 year. Overall survival and disease-free survival were estimated to be 82.1% and 73.8% at 1 year post-transplant, respectively.
Summary/Conclusions: RIC using Flu/high dose AraC /Cy +/- TBI was well tolerated with acceptable low toxicities and was sufficient to allow donor cell-engraftment post allo-SCT for elderly or frail patients with hematologic malignancies. Longer follow up and another prospective study enrolling more patients are required to evaluate the eventual survival benefit by reducing relapse.

PB2159

LATE COMPLICATIONS OF CONDITIONING REGIMENS (CYCLOPHOSPHAMIDE - TOTAL BODY IRRADIATION *vs* BEAM) FOR AUTOLOGOUS STEM CELL TRANSPLANTATION IN NON-HODGKIN LYMPHOMA.

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Background: Autologous stem cell transplantation (ASCT) is a frequently used procedure for the treatment of patients with relapsed non-Hodgkin lymphoma (NHL). While chemotherapy-based regimens are now commonly administered, total body irradiation (TBI) was largely used in the past. The current conditioning regimen in our center is BEAM (a combination of carmustine (BCNU), etoposide, cytarabine and melphalan) allthough we also have a large experience with cyclophosphamide (CFM)-total body irradiation (TBI) since this was the usual conditioning until year 2000.
Aims: To analyze the cumulative incidence of secondary neoplastic complications and non neoplastic complications (grade 3-4 infections, cardiovascular and pulmonary toxicity) after the two conditioning regimens (CFM-TBI *vs* BEAM) for ASCT.
Methods: We performed a retrospective analysis of patients with NHL that received an ASCT between October 1992 and December 2012. The late complications were defined as those to other previous comorbidity or to aging. Statistical analysis was performed using the IBM SPSS Statistics version 21.0. Cumulative incidences were estimated using EZR version 1.27 (Saitama Medical Center, Jichi Medical University, Omiya, Japan), a graphical user interface for R (version 3.1.1).
Results: A total of 105 atografted patients were analyzed. Patient's characteristics are in Table 1. The median follow up since ASCT was 73 months (0 – 274 months). Thirty-one percent (n=33) of patients were conditioned with CFM-TBI. The overall 5-years survival (OS) was 68.3% (58-77% - CI 95%) and the 5-year disease free survival (DFS) was 52% (42 61% - CI 95%). There were no differences regarding OS and DFS between the two conditioning regimens. The 5-years cumulative incidence (CI) of relapse was 0.48 (0.37-0.57, CI 95%). We detected 10 secondary neoplasm (myelodysplasia n=1, skin carcinoma n=2, lung carcinoma n=3, oropharingeal carcinoma n= 1, intestinal adenocarcinoma n=1, renal neoplasia n=1, bladder neoplasia n=1). The median time for the neoplastic event was 10.5 years (0-18.5 years). The CI of secondary neoplasias (2nd neoplasia) at 10 years was 10% (1-20%, CI 95%) and at last point of follow up (18.5 years) was 40% (13%>63%, CI 95%). There were no differences in the CI of 2nd neoplasias between BEAM and CFM-TBI. Non-neoplastic complications were present in 10% of patients (n=11). Three cases were infections grade 3-4 related to ASCT. Six cases had cardiac complications (5 acute coronary syndrome, 1 myocardiopathy) and 2 had pulmonary toxicity. The CI of non-2nd neoplastic complications at 10 year was 10% (1 – 25%, CI 95%).No differences were detected between the two conditioning regimens regarding non-neosplastic complications. (see Figure 1).

Table. 1. Patient's characteristics.

	Frequency (n)	Percentage
Age (years)	51 years old (18-70 years)	
Gender	Male: 62	59%
Histology	Diffuse Large Cell Lymphoma: 40	38%
	Follicular Lymphoma: 65	62%
Prognostic Index (IPI or FLIPI) (when available, n=103)	Low risk: 23; Intermediate risk: 67; High risk: 13	22%/65%/13%
Response pre- ASCT	Complete Remission: 58	55%
	Partial Remission: 46	44%
	Stable disease: 1	1%
Number of Lines pre-ASCT	1 line: 11; 2 lines: 69; 3 lines: 18; 4 lines: 7	10%/66%/17%/7%
Conditioning	CFM-TBI: 33 ; BEAM: 74	31% / 69%

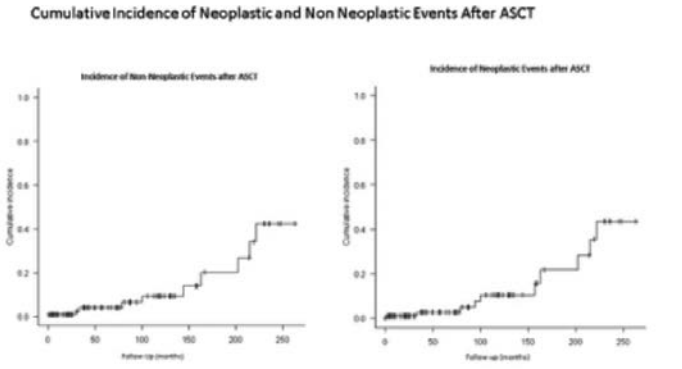

Figure 1.

Summary/Conclusions: Autologous stem cell transplantation offers long disease free survival for half of the patients with a high risk non-Hodgkin lymphoma. In our series, patients conditioned with BEAM or CFM-TBI had a comparable incidence of neoplastic and non-neoplastic events

PB2160

THE MANAGEMENT OF RELAPSED HODGKIN´S LYMPHOMA AFTER HAPLOIDENTICAL STEM CELL TRANSPLANTATION: DONNOR LYMPHOCYTE INFUSION AND BRENTUXIMAB.

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Background: Hodgkin's lymphoma, is an heterogeneous malignancy wich is posible to cure. For those patients who relapse, chemotherapy followed by an autologous transplantation (autoTPH) may conduce to a complete remission. Allogeneic transplantation (alo-SCT) is used for patients in relapse after auto-SCT or those with refractory advanced disease. Since 2012, with the experience of the Baltimore group, our Center has chosen the haploidentical family donor as a source for aloSCT in Hodgkin's disease. Despite the promising results, the rate of relapse is between 25 and 35%, and there is not standardized treatment for this situation.
Aims: To analyze the outcome of post-transplant relapse treatment of haploident donor haematopoietic progenitors (haploTPH).

Methods: We studied 18 patients with the diagnose of Hodgkins lymphoma in our center between August 2004 and July of 2013. All of them were submitted to haploSCT with a median follow-up of 495 days (455-1054).
Results: The median age was 32 years (21-60). 44% (8 patients) relapsed. 60% of then (5 patients) were nodular sclerosis histological subtype and 40% (3) lifocitic predominance. 2 patients (25%) were diagnosed in stage IV and 75% (6) stage II. The median of number of treatment lines received before transplantation was 5.5 (4-7); Compared to 7 in the non-relapsed group (4-11). 5 patients (60%) of the patients who relapsed had reached haploTPH in complete remission and 40% in partial remission, we did not observe differences related to the pre-SCT status. Peripheral blood was used as progenitors source in 75% (6) of the patients who relapsed and in 70% (7) in the non-relapsed group. 38% of the whole group of patients, had a donor/recipient KIR alloreactivity without differences between the two groups of the study. 88% (7) of the relapses occurred before 6 months of the SCT. The mean time to relapse was 316 days (range 181-446). Between the 8 relapsed patients, one was treated by another center with Vimblastine / Dexamethasone and died by infection, another patient died by abdominal sepsis before starting any treatment. Brentuximab was administered in 63% (5) of the patients. One of them received a single Brentuximab cycle with no tolerance, and changed to RT, GPD+Donnor lymphocyte infusions (DLI) and had reached complete response after 5 DLI. The rest (4) received between 3 and 7 doses with adequate tolerance. According to the the re-evaluation (PET-TC) after 3rd Brentuximab, 4 were in partial remission and one reached complete response. We associated Donor lymphocyte infusion in 6 patients. The mean of DLI received was 10; the median was 8, with a range between 22-3. Four patients reached complete remission, two of them maintain a partial response. All of them presented good tolerance to DLI. We observed Graft *versus* host disease in four patients, 3 of them presented moderate cutaneous affection, and one of them suffered hepatic graft *versus* host disease stage III, with adequate evolution after treatment.
Summary/Conclusions: Is posible to treat patients who relapsed after haploidentical stem cell transplantation with Brentuximab+DLI, with a very good tolerance. We observed cutaneous graft *versus* host disease in most of the patients who reached completed response after DLI. Despite this findings, we need multicentric studies to perform standarized treatments and protocols.

PB2161

CONDITIONING REGIMENS BEFORE AUTOLOGOUS STEM CELL TRANSPLANT FOR PATIENTS WITH MALIGNANT LYMPHOMA – LEED *vs* MCEC –

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Background: High-dose chemotherapy before ASCT has been established as an effective treatment option for high-risk patients with chemo-sensitive ML. Although the therapeutic efficacy of this strategy highly depends on the conditioning regimens before ASCT, the appropriate regimen has been controversial. Thus, we performed a multi-center retrospective study of ASCT recipients with ML to compare the safety and efficacy of the conditioning regimens LEED and MCEC, which are widely used in Japan.
Aims: The primary objective was to determine the preferable conditioning regimen before ASCT: LEED or MCEC.
Methods: This study analyzed 127 adult patients who underwent ASCT following LEED or MCEC as the conditioning regimen against chemo-sensitive ML at four institutions in Japan between 1997 and 2015. Any type of pathological diagnosis was considered. The LEED regimen consisted of 140 mg/m^2 L-PAM (day −1), 500 mg/m^2 etoposide (days −4 to −2), 60 mg/kg cyclophosphamide (days −4 to −3), and 40 mg/body dexamethasone (days −4 to −1). The MCEC regimen consisted of 200 mg/m^2 MCNU (days −8 and -3), 300 mg/m^2 carboplatin (days −7 to −4), 500 mg/m^2 etoposide (days −6 to −4), and 50 mg/kg cyclophosphamide (days −3 to −2). Fisher's exact test was used to compare binary variables. OS rates were estimated by the Kaplan-Meier method and compared using the log-rank test. Cumulative incidences (CIs) of relapse and non-relapse mortality (NRM) were compared using the stratified Gray test. The Cox proportional hazards regression model was used for multivariate analysis of OS. Values of p <0.05 were considered significant.
Results: Of the 127 patients, 76 were male and 51 were female, and the median age was 56 years (range: 18 to 68 years). Underlying diseases were DLBCL in 74 patients, mantle cell lymphoma in 16, other B-cell lymphoma in 14, Hodgkin lymphoma in 9, and T-NK-cell lymphoma in 14. The disease status at the time of transplant was first complete remission (CR) in 68, advanced CR in 27, and partial remission in 32. As the conditioning regimens before ASCT, 81 patients (64%) received the LEED regimen, and 46 (36%) received the MCEC regimen. No significant differences in patient characteristics, disease features, or transplant procedures were present between the two groups except for the following three factors: (1) ASCT in the later period (2007–2015) in the LEED group compared with the MCEC group (72% *vs* 13%; p <0.01); (2) more frequent administration of rituximab before ASCT in the LEED group (84% *vs* 59%; p <0.01); and (3) less frequent radiation therapy before ASCT in the LEED group (17% *vs* 37%; p=0.02). The 5-year OS rates were not significantly different between the LEED and MCEC groups (77% *vs* 68%; p=0.35). Likewise, both the 5-year CIs of relapse and NRM were similar in the two groups (relapse: 39% *vs* 33%; p=0.61, NRM: 1% *vs* 5%; p=0.71). In multivariate analysis that included the transplant periods, rituximab administration, and radiation therapy as independent variables, two or more prior regimens was extracted as an independent unfavorable prognostic factor for OS, but not conditioning regimens. Regimen-related toxicities within 100 days after ASCT were assessed. The incidences of grade 3-4 nausea (36% *vs* 78%; p <0.01), vomiting (4% *vs* 28%; p <0.01), diarrhea (36% *vs* 56%; p=0.02), and liver dysfunction (4% *vs* 36%; p <0.01) were significantly decreased in the LEED group. The 5-year CIs of secondary MDS/AML were similar between the two groups (4% *vs* 3%; p=0.62).
Summary/Conclusions: Our findings demonstrated that both the LEED and MCEC regimens showed sufficient anti-lymphoma effect as conditioning regimens before ASCT, with a 5-year OS rate of more than 70% in patients with chemo-sensitive ML. However, the LEED regimen is considered more preferable in comparison with the MCEC regimen based on the low frequency of severe regimen-related toxicities. A large-scale prospective study is warranted to confirm these findings.

PB2162

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR SECONDARY HAEMATOLOGICAL NEOPLASIA: A SINGLE CENTER EXPERIENCE

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Background: Therapy related haematological neoplasms (t-HN) occur due to direct mutational events of chemotherapeutic agents and radiotherapy. Disease latency, mutational events and prognosis vary with drugs categories.
Aims: The aim of this retrospective study was to assess the outcome of t-HN after hematopoietic stem cell transplantation (HSCT).
Methods: We describe a cohort of 31 patients, 19 females (61.3%) and 12 males (38.7%), with median age of 53 years (range, 20 to 64), who received an allogeneic HSCT in our Unit, between September 1999 and July 2016. Patients had a history of solid tumor in 15 cases (48.4%), haematological malignancies in 15 cases (48.4%) and both of them in one case (3.2%). All but one received a median of 2 (range, 1 to 6) lines of therapy. After a median of 36 months (range 12-190) from the first neoplasia, patients developed t-AML (n=19) (61.3%), t-Ph+ ALL (n=1) (3.2%), or t-MDS (n=11) (35.5%). Molecular abnormalities were detected in 7 (46.7%) out of 15 evaluable patients: BCR/ABL (1), ITD FLT3 (2), inv16 (1), NPM1 (2), NPM1+/ITD FLT3 (1). Karyotype aberrations were found in 18 (64.3%) out of 28 evaluable patients: 16.7% was favourable risk (n=3), 27.8% was intermediate risk (n=5) and 55.5% was adverse risk (n=10). The disease status at transplant was as follows: complete remission (n=13) (42%), refractory disease (N=10) (32%), stable disease (n=3) (10%). Patients received conventional chemotherapy in 14 cases (45.2%), azacytidine in 11 cases (35.5%), both of them in one case (3.2%), whereas 5 patients (16.1%) were untreated. The conditioning was myeloablative (MAC) in 20 patients (64.5%) or reduced intensity (RIC) in 11 patients (35.5%); the donor was a family member (REL) in 17 patients (54.8%) or unrelated (MUD) in 14 patients (45.2%). The hematopoietic cell transplantation comorbidity index (HCT-CI) was as follows: 14 patients (45.2%) had a score of 3 and 17 patients (54.8%) had a score of 4 or more. Overall survival was calculated with Kaplan-Meier method. Transplant-related mortality (TRM) and relapse-related mortality (RRD) rates were estimated by cumulative incidence (CI), both considering the opposite event as competing. Fine and Gray's method for CI of TRM and RRD was used to evaluate the risk factors on univariate analysis.
Results: Twenty-three patients were in remission on day +30, by bone marrow cytology, 3 patients were classified as resistant disease and five patients were not evaluable because of early death. Five patients (21.7%) relapsed after a median 6 months (range, 3 to 15). At the time of this analysis (December 2016) 14 patients were alive with a median OS of 53 months (range 8-190), while 17 patients died after a median of 4 months (range 1-27): RRD was 16% (n=5) and TRM was 39% (n=12). Non relapse causes of death were as follows: GvHD (n=3), infectious complications (n=8) and EBV-related PTLD (n=1). One patient experienced a third tumor (breast cancer) thirteen years from HSCT. TRM was higher for patients transplanted from MUD (66%) as compared to REL donor (16%) (p=0.01). The overall survival was 45.2% (Figure 1) and 58% maintained a complete remission.
Summary/Conclusions: This report confirms that allogeneic HSCT is a curative approach in approximately 50% of patients with therapy related haematological neoplasms, especially for those patients who benefit from a familial donor.

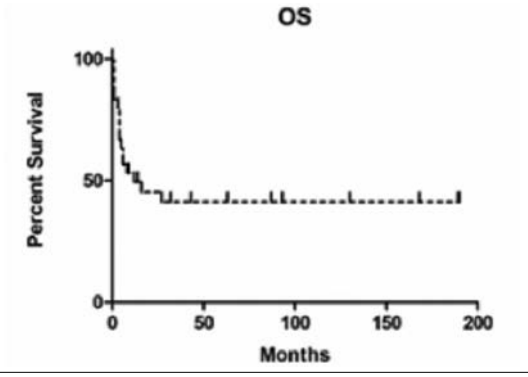

Figure 1.

PB2163

IMPROVEMENT IN BIVENTRICULAR CARDIAC MECHANICS NOTED IN PATIENTS UNDERGOING MYELOABLATIVE AUTOLOGOUS-HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR AL AMYLOIDOSIS

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Background: Primary amyloidosis (AL) is characterized by extracellular deposition of insoluble protein fibrils often with multisystem organ involvement. The Mayo staging model for determining prognosis in patients with cardiac amyloidosis takes into account troponin, NT-proBNP, and serum free-light chain difference in order to stage patients prior to undergoing autologous hematopoietic stem cell transplant (Auto-HCT). Since amyloidosis often involves the kidneys, serum biomarkers that require renal clearance are less reliable in the setting of significant renal dysfunction. 2D-echo and strain imaging offer non-invasive modalities for identifying early cardiac changes independent of renal function. These changes may also precede symptom improvement as assessed by NYHA classification.
Aims: Our hypothesis is that strain imaging is a feasible biomarker for cardiac respone after Auto-HCT in AL amyloidosis.
Methods: Seven patients with biopsy-proven AL amyloidosis who were treated with a Melphalan based myeloablative regimen and Auto-HCT were evaluated retrospectively. Each patient underwent 2D-echo up to 36-days prior to treatment followed by repeat 2D-echo within 14-months. Strain imaging was performed using EchoInsight®. Chart review was conducted to determine associated NYHA functional classification and Mayo staging. Statistical analysis was performed using SPSS.
Results: Of the 7 patients studied, 3 were Mayo stage I, 2 stage II, 1 stage III, and 1 stage IV. The median follow-up from transplant was 47.4 months. There was one death at 20.4 months. The mean NYHA classification at baseline was 2.3 and after transplant was 1.9. Longitudinal, radial and circumferential left ventricular strain (LV) were evaluated, but only the global longitudinal strain (GLS) showed an improvement (baseline -14.69%; follow-up -16.84%; mean absolute improvement 2.15%; p <0.05) across all four Mayo Stages. There was no difference in GLS within individual stages. In patients with stable NYHA classification after transplant, there was also a significant improvement in Right Ventricular Free-Wall Strain (RVFWS) with a mean absolute improvement of 6.2% (p <0.05). There was no significant change in left ventricular ejection fraction (LVEF) (Figure 1).

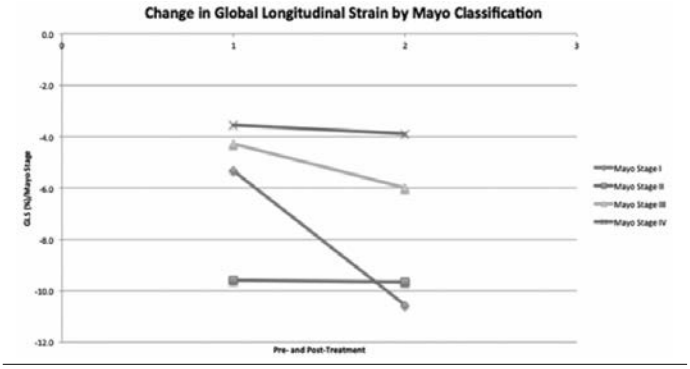

Figure 1.

Summary/Conclusions: We demonstrate that there is a clinically meaningful improvement in cardiac mechanics one year after Auto-HCT, despite no alteration in LVEF. This metric may prove useful in assessing organ response, especially when serum biomarkers are less reliable. Changes in left ventricular GLS occur independent of pre-transplant Mayo stage, although prospective studies are needed for confirmation. We further believe that improvements in RVFWS may predict clinical improvement.

PB2164

AN ABSOLUTE NUMBER OF CD34+ CELLS IN BLOOD AS A PREDICTOR OF A SUCCESSFUL HARVEST OF HEMATOPOIETIC STEM CELLS IN DIFFERENT MOBILIZATION REGIMENS

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Background: Autologous stem cells transplantation (ASCT) has become necessary part in therapy of hematological diseases. Transfusion of at least 2×10^6 CD34+ HSCs per kg of patient's weight allows achieving an adequate hematopoiesis after high-dose chemotherapy. The most optimal is to collect $\geq2\times10^6$ CD34+ cells/kg with single harvest apheresis. Different mobilization regimens lead to variations in white blood cell count (WBC) and the number of circulating HSCs. Deciding on the time of the first leukapheresis is very important.
Aims: The aim was to identify significant parameter predicting efficiency of CD34+ cells collection.
Methods: The study included 142 patients (pts) who undergo ASCT (80 m, 62 f, median age 53 y.o., 81 were diagnosed with multiple myeloma, 10 - Hodgkin's lymphoma, 51 – non-Hodgkin's lymphomas). WBC and absolute CD34+ number in the blood before the first apheresis and the number of CD34+/kg in the apheresis product were determined for each patient. There were three different mobilization regimens: 12 pts received 10 μg/kg/day G-CSF in the stable hematopoiesis for 5-6 days; 86 pts - 4 g/m^2 cyclophosphamide and 5-10 μg/kg/day G-CSF (Cph+G-CSF); 44 pts - DHAP: 40 mg dexamethasone, 100 mg/m^{2}cisplatin, 2g/m^{2}cytarabine and 10 μg/kg/day G-CSF (DHAP+G-CSF). CD34+ HSCs were evaluated with ISHAGE-protocol by BD FACSCanto II flow cytometer. Results are presented as mean±SEM. ROC-analysis was performed for WBC and the absolute number of CD34+ HSCs in the blood as the predictor markers for HSCs successful harvesting ($\geq2\times10^6$ CD34+/kg for fist apheresis).
Results: WBC mean was higher in pts with G-CSF mobilization scheme compared to Cph+G-CSF and DHAP+G-CSF (28.5±3.5 *vs* 10.4±0.9 and $9.0\pm1.8\times10^9$/l, respectively, p<0.0001), but the absolute number of CD34+ HSCs in the blood (26.3±9.3 *vs* 55.5±5.6 and 93.1±22.3/μl, p=0.03) and the number of CD34+/kg in the leukapheresis product (1.9±0.7 *vs* 5.2±0.6 and $6.9\pm1.3\times10^6$/kg, p=0.01) were lower. Differences between Cph+G-CSF and DHAP+G-CSF in all parameters were not found. There was not any relationship between WBC and the number of CD34+/kg: the area under ROC-curve (AUC) didn't differ from 0.5 for all mobilization regimens. Then absolute number of CD34+ in blood was investigated as predictor for harvest success, AUCs were 0.964, 0.938 and 0.979 (p<0.0001) for G-CSF, Cph+G-CSF and DHAP+G-CSF, respectively. In the ROC-analysis showed the optimal CD34+ number in blood than most likely to collect $\geq2\times10^6$ CD34+/kg for first leukapheresis. It was 29 CD34+cells/μl in G-CSF mobilization, 24 CD34+cells/μl – in Cph+G-CSF and 27 CD34+cells/μl - in DHAP+G-CSF. To calculate universal level of absolute CD34+ number all data from 142 pts was used. In this case AUC was 0.952 and a threshold of successful harvesting was 20 CD34+cells/μl in blood before apheresis with sensitivity of 96% and specificity of 81%.
Summary/Conclusions: Various mobilization regimens differ in count of leucocytes and CD34+ HSCs in peripheral blood: WBC was significant higher in G-CSF than in Cph+G-CSF and DHAP+G-CSF, but the absolute number of CD34+ cells was higher in chemotherapy-based mobilization and G-CSF than in G-CSF alone. The absolute number of leucocytes in blood before apheresis was not a predictor factor of harvest success in all variants of mobilization regimens. If there is at least 20 CD34+cells/μl in blood before apheresis it is possible to collect $\geq2\times10^6$ CD34+/kg for single leukapheresis with high sensitivity and specificity independent of mobilization regimen.

PB2165

QUANTIFICATION OF CD34+ CELL AND ITS VIABILITY OF FRESH OR CRYOPRESERVED NUCLEATED CELLS BY IMAGE-BASED CELL COUNTER IS COMPARABLE TO STANDARD FLOW CYTOMETER

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Background: As a standard method for quantification of CD34+ stem cells, flow cytometry has been widely used. However, it has some limitations such as

expensive instrumentation, high reagent costs, and poor reproducibility between technicians and laboratories.
Aims: We developed and assessed an instrument performance of a newly-developed image-based microscopic cell counter (ADAM II™) for enumeration of CD34+ cell and its viability.
Methods: We used samples of fresh and cryopreserved nucleated cells from G-CSF mobilized peripheral blood stem cells (PBSCs) as well as cord blood (CB). We assessed the reproducibility and linearity of the new device and compared numbers and viabilities of CD45+ cells and CD34+ cells determined with the ADAM II™ and flow cytometer.
Results: Each analysis used 10 aliquots from one sample to assess the reproducibility of ADAM II™, with expected values of 14.77~172.06 CD34+ cells/, 0.08~0.56 CD34(%)/CD45. The number of CD34+ cells determined by ADAM II™ was sufficiently accurate over the expected range, and the intra-assay coefficient of variation (CV) was ≤10.8%. The linearity of CD34+ cell counts was confirmed over a range of dilutions (0.58~280 cells/□ of sample). Linearity was satisfactory (R^2=0.99). The numbers and viabilities of CD45+ cell and CD34+ cell obtained with the ADAM II™ were highly correlated with those obtained with the flow cytometer (R^2>0.9841, p<0.0001). In all samples from fresh/cryopreserved PBSC and fresh/cryopreserved CB, there were no significant differences of total numbers and viabilities of CD45+ cell and CD34+ cell count analyzed from ADAM II™ as well as flow cytometer.
Summary/Conclusions: The newly developed image-based microscopic cell counter (ADAM II™) appears to be suitable for quantification of CD34+ cell and its viability of fresh or cryopreserved PBSCs or CBs.

PB2166

EXTRACORPOREAL PHOTOPHERESIS IN STEROID-DEPENDENT OR REFRACTORY ACUTE GRAFT-*VERSUS*-HOST DISEASE

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Background: Extracorporeal photopheresis (ECP) has been incorporated in the management of graft-*versus*-host disease (GVHD) post allogeneic haematopoietic cell transplantation (alloHCT) in many centres. The introduction of ECP as an early second-line treatment in steroid-dependent or refractory patients with acute GVHD (aGVHD) remains under study. The rationale of its early use is based on the low incidence of complete responses to corticosteroids and the profound immunosuppression caused by traditional secondary treatments.
Aims: Based on our long-lasting experience in chronic GVHD, we aimed to prospectively assess the role of ECP in this high-risk population.
Methods: We enrolled consecutive patients with steroid-dependent or refractory grade (gr) II-IV aGVHD post alloHCT from January 2013 to August 2016. All patients with unrelated or haploidentical donors received thymoglobulin (ATG) 5mg/kg as prophylaxis. Post-transplant GVHD prophylaxis included cyclosporine – methotrexate in myeloablative and cyclosporine – mycophenolate mofetil in reduced toxicity or intensity regimens. ECP was commenced after assessment of response to 5 days of steroid treatment according to our protocol: 2 sessions/week for 1 month, 1 session/2 weeks for 3 months, evaluation of response and 1 session/month for 6 months.
Results: We studied 20 patients, aged 35 (18-65), post alloHCT with myeloablative (14), reduced toxicity (4) and intensity (4) conditioning, from sibling (3), matched (8) or one locus mismatched (8) volunteer unrelated and haploidentical (1) donors. Disease risk index was high (10), intermediate (9) and low (1). Acute GVHD was observed at day +17 (8-50) in 15 patients, late-onset at+130 (110-160) in 4 patients and induced at +38 post donor lymphocyte infusion in a relapsed AML patient. Skin, intestine and liver involvement was evident in 6 patients, skin and intestine in 10 and skin only in 4 patients. Nine patients (2 with GrII, 7 with GrIII aGVHD) were steroid-dependent and 11 (8 with GrIII, 3 with GrIV) steroid-refractory. ATG was administered simultaneously with ECP initiation in 6 refractory patients that further developed EBV reactivation (p=0.032) treated pre-emptively with rituximab. ECP was commenced at day +51 for 16 (4-20) sessions. The majority of patients (16/20) presented partial (6), very good (9) or complete (1) response to ECP. With 9.3 (1.8-54.7) months of follow-up, immunosuppression was reduced in 10/20 and ceased in 1 patient. Clinically significant bacterial infections were found in 17 patients, fungal in 2, CMV and EBV reactivation in 14 and 13 respectively and other viral in 5 patients. Cumulative incidence (CI) of chronic GVHD was 77.4 at 1-year. 1-year CI of aGVHD-related mortality was 20%. 1-year overall survival (OS) was 53% and significantly increased in steroid-dependent *versus* refractory patients (76% *vs* 36%, p=0.041). Reduction of immunosuppression (p=0.026) and steroid dependence (p=0.023) were associated with improved OS, irrespectively of other factors.
Summary/Conclusions: Our study supports that ECP should be considered early in the course of steroid-dependent or refractory aGVHD, before significant irreversible end organ damage has been established. Optimal timing of intervention, frequency, duration and tapering schedule of ECP need to be investigated in future studies.

PB2167

RAPID RECONSTITUTION OF NK1 CELLS IS ASSOCIATED WITH THE LOWER INCIDENCE OF GRAFT-*VERSUS*-HOST DISEASE AFTER ALLOGENEIC TRANSPLANTATION

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Background: The balance between immunostimulation and immunoregulation in T cell immunity is achieved by a Th1/Th2/Th3/Tr1 and CD4+CD25+ regulatory T (Treg) cell paradigm.
Aims: We investigated the production of type1 (IFN-*gamma*, NK1), type2 (IL-13, NK2), type3 (TGF-*beta*, NK3) and regulatory cytokines (IL10, NKr) from human peripheral blood to discuss the cytokine paradigm of NK cells in human allogeneic hematopoietic stem cells transplantation (allo-HSCT).
Methods: Forty patients undergoing haploidentical (n=27) and HLA-identical sibling (n=13) allo-HSCT between August 2009 and December 2009 were enrolled in this analysis after being originally selected using a protocol exploring the association of reconstituted donor derived NK1/NK2/NK3/NKr cells to GVHD and CMV reactivation.
Results: Expansion of NK2 and NK3 were found post allo-HSCT compared to healthy donor. The levels of NKr reconstituted to donor's level since day 15 post allo-HSCT, and the levels of NK1 in recipients post transplantation were consistently lower compared to donors' levels until day 60 post allo-HSCT. Multivariate analysis showed that the higher levels of NK1 by day 15 were associated with lower overall acute GVHD (HR 0.157, 0.039-0.642, P=0.010) as well as II-IV acute GVHD (HR 0.260, 95%CI, 0.064-1.053, P=0.059). Meanwhile, the higher levels of NK1 by day 15 correlated with lower CMV reactivation (HR 0.040, 0.005-0.348, P=0.003).
Summary/Conclusions: These results indicate that rapid reconstitution of NK cells; especially NK1 cells would be help to prevent the development of graft-*versus*-host disease as well as CMV reactivation after allogeneic transplantation.

PB2168

BORTEZOMIB FOR STEROID-REFRACTORY RITUXIMAB AUTOIMMUNITY AFTER ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Therapy of post-transplant autoimmunity manifestations remains a challenge. Many cases are steroid and rituximab refractory and continuing intensified immune suppression increase the risk of infection in the post-HSCT patient. In our institution, we have used bortezomib as our third agent after failure of steroids or rituximab, or in cases of steroid-dependence since Bortezomib appears to be effective in cases with refractory autoimmunity.
Aims: In our series, we assessed the therapeutic response to proteasome inhibitor in 4 cases of post-transplant refractory autoimmunity
Methods: Three of the 4 cases received Bortezomib for autoimmune cytopenia (autoimmune haemolytic anaemia AIHA (n=2), AIHA with acquired red Cell Aplasia (n=1)). At least 2 therapy lines had failed to resolve the cytopenia. One to two courses of Bortezomib were administered at a dose of 1.3 mg/m2 at day 1, 4, 8, 11 each course. In two cases this treatment was combined with immunosuppressive agents: Mycophenolate mofetil (MMF) alone in one case and associated with sirolimus in the other case.
Results: Resolution of autoimmune cytopenia was observed in the three cases after a median of 33 days from the first day of administration. The fourth case received 1 course of Bortezomib for persistent anti-enzyme antibodies after allogeneic transplant for Wolman disease. Therapeutic response was obtained after 25 days reflected by a complete regression of circulating anti-enzyme antibodies. In all cases, no Bortezomib related toxicity was noticed. The response was maintained in all cases.
Table 1 summarizes the clinical data and the results of the four cases.

Table 1.

patients	Age at Allogeneic HSCT	Diagnosis	post-transplant bortezomib indication	Previous treatments	bortezomib courses (n)	Response	Time to response	bortezomib-related toxicity	loss of response at last follow up
1	14	Beta thalassemia	AIHA	IVIG, Sirolimus, Steroids, Rituximab, Cyclophosphamide, Splenectomy	2	Yes	33	None	No
2	91	myelodysplastic syndrome trisomy 8	AIHA	IVIG, Steroids, Rituximab, MMF	2	Yes	57	None	No
3	14	Mucopolysaccharidos type 1	AIHA with Acquired Red Cell Aplasia	Rituximab, MMF	1	Yes	14	None	No
4	25	Wolman disease	Anti-enzyme antibodies	None	1	Yes	25	None	No

Summary/Conclusions: Our study shows that Bortezomib is a promising therapeutic option for refractory post-transplant autoimmunity with high tolerance and no related toxicities.

PB2169

POST-THAW CELL COUNT PREDICTS ENGRAFTMENT RATE IN CORD BLOOD TRANSPLANTATION

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Background: The infused cell count in cord blood transplantation (CBT) is an important element for engraftment; however, this number in the prior reports has been based on the pre-thaw cell count. Therefore, the association between post-thaw cell count and engraftment rate, especially in pediatric patients, is unclear.
Aims: Tte Aim of this study is to reveal the association between post-thaw cell count and engraftment rate in pediatric patients in the setting of CBT at our institution.
Methods: We retrospectively reviewed the medical records of 78 patients who underwent CBT between June 1998 and April 2016. We excluded the cases of CBT that required rescuing after engraftment failure.
Results: Underlying disease was acute leukemia (AL) in 63 (ALL, 38; AML, 25) patients, chronic myeloid leukemia in one, malignant lymphoma (ML) in two, myelodysplastic syndrome (MDS) in three, aplastic anemia in one, and others (such as primary immunodeficiency syndrome) in eight. In terms of conditioning regimens, myeloablative conditioning was administered to 62 patients and reduced intensity conditioning was administered to 16 patients. The median age at CBT was 3 (range, 0–19) years, and the median follow-up period was 898 (range, 47–6236) days. The engraftment rate was 84.6%, primary engraftment failure was observed in 11 patients (AL,seven; ML, one; MDS, one; neuroblatoma, one; and others, one) and secondary graft failure was observed in one patient (severe congenital neutropenia). The 3-years overall survival rate was 55.1%, and 32 patients had died (cause of death: progressing disease in 19 patients). We analyzed the data on 34 patients of whom both of pre- and post- thaw CD34+ cell counts in the cord blood samples were available. The median pre- and post-thaw CD34+ cell counts was 1.67×10^5/kg and 1.51×10^5/kg, respectively, and they were significantly correlated with each other (r=0.73, p=0.52). In our study cohort, the engraftment failure occurred in five patients (primary in all patients). The median post-thaw CD34+ cell count was 1.60×10^5/kg in the patients who achieved engraftment and 1.01×10^5/kg in the patients who did not achieve engraftment. No statistically significant difference was observed between these two groups (p=0.30). When we defined the cut-off value of the pre-thaw CD34+ cell count as 1.2×10^5/kg in the patients who were infused with CD34+ cells more than the cut-off value, the specificity and sensitivity of graft failure was 79.3% and 60%, respectively. When we defined the cut-off value of the post-thaw CD34+ cell count as 0.7×10^5/kg in the patients who were infused with CD34+ cells more than the cut-off value, the specificity and sensitivity of graft failure was 96.6% and 40%, respectively.
Summary/Conclusions: We concluded that the risk of graft failure is more precisely predicted by the post-thaw than pre-thaw CD34+cell count and that if the post-thaw CD34+ cell count is more than 0.7×10^5/kg, the risk of graft failure is very low.

PB2170

COLONYFORMING CAPACITY OF HEMATOPOIETIC STEM CELLS MOBILIZED INTO PERIPHERAL BLOOD WITH VINIRELBINE AND GRANULOCYTE COLONY STIMULATING FACTOR

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Background: One of the alternative method to mobilize stem cells from bone marrow to peripheral blood is using of vinorelbine with granulocyte colony stimulating factor (G-CSF). The specific features of vinorelbine are absence of hospitalization necessity and predictability of leukocytapheresis' optimal time. But there is not enough data to conclude whether vinorelbine is safe for haematopoietic stem cells.
Aims: The aim of the study was to determine the colonyforming capacity of haematopoietic stem cells mobilized into peripheral blood with vinirelbine and G-CSF.
Methods: Data of 11 patients with multiple myeloma (MM) and 1 patient with Hodgkin lymphoma (HL) were analyzed. Vinorelbine was injected IV in dose 50-70 mg (35 mg/m^2). Daily lenograstim dose was 10 mcg/kg. The number of BFU-E, CFU-GM, CFU-GMME and CFU-Macrophage was studied in 14-days culture: $1x10^5$ cells of leukocytapheresis product were set into Petri dish with MethoCult H 4435 full medium. Control group was consisted of hematopoietic stem cells donors' data in which G-CSF monotherapy was used for mobilization.
Results: The median patients' age was 55 (43-64) y. Induction courses for MM treatment included borteszomibe (11 patients), lenalidomide (5 patients) and carfilsomibe (1 patient) in combination with steroids. A patient with HL was treated with ABVD scheme. Leukocytaphereses were started on 6-8 day, herewith in 9/11 (75%) patients on 7 day. The number of gained CD34$^+$ was 1.7-7.8x10^6/kg (Me 3.3x10^6/kg). Median number of BFU-E, CFU-GM, CFU-GMME and CFU-Macrophage in patients' group was 207, 180, 14 and 9 accordingly. The results were not significantly different from control group data: 168, 170, 10 and 12 accordingly; p<0.05.
Summary/Conclusions: We conclude that mobilization regimen with vinorelbine in combination with G-CSF does not damage colonyforming capacity of hematopoietic stem cells.

PB2171

URIC ACID LEVEL MIGHT BE A PROGNOSTIC INDICATOR FOR SURVIVAL IN PATIENTS WHO UNDERWENT ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION. SINGLE CENTER EXPERIENCE

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Background: Uric acid (UA) is an abundant aqueous antioxidant that accounts for almost two thirds of all free-radical-scavenging activity in human serum. It is released from injured cells during conditioning for allogeneic hematopoietic stem cell transplantation (AHSCT).
Aims: The aim of this study was to evaluate the prognostic impact of pre transplantation uric acid levels on survival and mortality in allogeneic HSCT patients.
Methods: We retrospectively analyze 273 patients with hematologic diseases undergoing AHSCT. The patients were categorized as patients with acute leukemia, myelodysplastic syndrome, lymphoma patients and other hematologic disease diagnoses. A serum uric asid concentration 3.4 mg/dl was considered hypouricemia. Pretransplantation uric asid, creatine, total protein and albumin were analyzed. Univariate, multivariate Cox regression models and Kaplan–Meier curves were performed to uric asid, creatine, total protein and albumin associated with disease-free survival (DFS) ,over all survival (OS), early non relaps mortality (+30 day) and late non relaps mortality (+100 day).
Results: Pretransplantation low uric acid levels were detected in 57 (%20.8) patients. Low UA levels were significantly associated with DFS (HR: 0.52; p= 0.027) . None of the creatine, total protein and albümin were significantly associated with DFS (HR:0.98; p= 0.98, HR :0.87 p=0.60, HR: 1.15; p= 0.66). There was no significant association between UA, creatine, total protein and albümin levels and overall survival (HR: 0.84; p= 0.46, HR: 2.10; p= 0.057, HR :0.88; p= 0.52, HR: 0.78; p= 0.26), early relapse mortality (HR: 1.38; p=0.54, (HR: 2.16; p= 0.29, HR: 0.61; p= 0.25, HR: 0.53; p= 0.13) and late non-relapse mortality (HR:0.57; p=0.35, HR: 0.21; p=0.29, HR: 1.04; p= 0.94, HR: 1.07; p= 0.92).
Summary/Conclusions: Uric asid is a natural antioxsidant compound. UA reacts with oxygen-derived free radicals and becomes oxidized. Since humans are unable to catabolize UA to the more soluble compound allantoin due to lack of urate oxidase or uricase, the serum UA concentration is higher in humans than almost all other mammals. However, this high UA level in humans has been regarded as being beneficial in the presence of elevated oxidative stress. Our study supports that the uric asid is a antioxidant compound. BecauseDisease-free survival is lower in patients with low uric acid levels before transplantation. This is the first report demonstrating a positive association between UA levels and survival analyses in allogeneic HSCT patients. Our findings are potentially clinically relevant. Confirmation in independent cohorts and further investigations into underlying mechanisms, such as reduced antioxidative capacity in hypouricemia, are warranted. In thecoming years, as a result of increased works on this subject, uric asid may be considered a possible prognostic marker in allogeneic hematopoietic stem cell transplantation.

PB2172

RISK FACTORS FOR HERPES SIMPLEX VIRUS-1/2 VIREMIA AND CLINICAL OUTCOMES FOLLOWING UNMANIPULATED HAPLOIDENTICAL HAEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Herpes simplex virus(HSV)-1/2 can still be reactivated after allogeneic haematopoietic stem cell transplantation (allo-HSCT) even when the prophylactic acycloviris used. However, the risk factors for HSV-1/2 viremia and the clinical outcomes following unmanipulated haploidentical HSCT remain unknown.
Aims: The aim of this study was to explore the risk factors for HSV-1/2 viremia and to evaluate clinical outcomes following haplo-HSCT.
Methods: Nineteen patients with HSV-1/2 viremia and fifty-seven patients without HSV-1/2 viremia which were selected using the case-pair method after haploidentical HSCT were enrolled. We analysed the risk factors for HSV-1/2 viremia and compared clinical outcomes between the two patient groups.
Results: The risk factors for HSV-1/2 viremia included HLA disparity ≥2 loci (p=0.049) and cytomegalovirus (CMV) reactivation (p=0.028). The incidences of platelet engraftment, oral mucositis and severe haemorrhagic cystitis (HC) in patients with and without HSV-1/2 viremia were 77% and 94% (p=0.003),

78% and 13% (p=0.000), and 25% and 6% (p=0.04), respectively. Moreover, the median time to platelet engraftment in patients with and without HSV-1/2 viremia was 25 d(range,11–80 d) and 17 d(range, 8–67 d) (p=0.004). In a multivariate analyses, HSV-1/2 viremia was associated with delayed platelet engraftment(p=0.038), a higher incidence of oral mucositis(p=0.000) and severe HC (p=0.038). However, HSV-1/2 viremia was not associated with non-relapse mortality (34.0% *vs* 31.5%, p=0.26),leukaemia-free survival (60.9% *vs* 57.9%, p=0.46) and overall survival (61.2% *vs* 60.7%, p=0.37) (Figure 1).

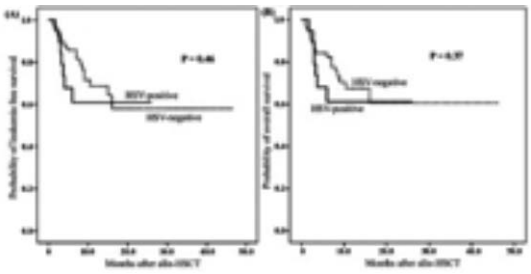

Figure 1.

Summary/Conclusions: Based on our study results, we recommend that HSV-1/2 PCR should be performed on clinical suspicion.

PB2173

FACTORS PREDICTING GRAFT-*VERSUS*-HOST DISEASE-FREE, RELAPSE-FREE SURVIVAL AND OUTCOMES AFTER REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION FOR ACUTE LEUKEMIA OR MYELODYSPLASTIC SYNDROMES

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Background: Reduced intensity allogeneic stem cell transplantation (RIST) is now commonly applied for elderly patients with acute leukemia (AL) or myelodysplastic syndromes (MDS). However, the factors affecting graft-*versus*-host disease-free, relapse-free survival (GRFS) and overall survival (OS) remain obscure.
Aims: To identify such factors and to clarify the clinical significance of RIST with various graft sources, we retrospectively analyzed patients with AL or MDS who received RIST in our hospital.
Methods: The study included patients with acute myeloid leukemia (n=73), acute lymphoid leukemia (n=31) or MDS (n=25), who received fludarabine (Flu)/melphalan (Mel)-based RIST between 2004 and 2015 as the first transplantation.
Results: There were a total of 129 patients, including 3 in low risk (L), 74 in intermediate risk (I), 36 in high risk (H) and 16 in very high risk (V), classified by the refined disease risk index (rDRI). The median age was 58 years (range: 18-68 years), with 73 males and 56 females. Conditioning regimens contained Flu (125mg/m^2) combined with Mel (80mg/m^2, n=21 or 140mg/m^2, n=108). Total body irradiation (4Gy) was used in 96 patients who received transplantation from unrelated donors or HLA mismatched related donors. Bone marrow (BM) or peripheral blood stem cell (PB) from related donors was used in 40 patients, BM or PB from unrelated donors in 33 and cord blood (CB) from unrelated donors in 56. Primary graft failure occurred in 7 patients and death before engraftment was observed in two. After a median follow-up of 46 months (range: 15-144 months) for the survivors, the 1-year GRFS, disease free survival (DFS) and OS were 57%, 61% and 70%, respectively. On univariate analysis for all patients, pre-transplant factors associated with the 1-year GRFS included stem cell sources (BM/PB *vs* CB: 44% *vs* 68%, p=0.005), donors (related *vs* unrelated: 38% *vs* 62%, p=0.012), disease (AL *vs* MDS: 60% *vs* 28%, p<0.001) and rDRI (L/I *vs* H/V: 65% *vs* 38%, p=0.003). On multivariate analysis, BM/PB (HR 2.0, 95% CI 1.0-4.0, p=0.039), MDS (HR 2.6, 95% CI 1.5-4.6, p=0.001) and H/V rDRI (HR 2.1, 95% CI 1.2-3.5, p=0.006) were associated with a worse GRFS. The 5-year OS, cumulative incidence of relapse (CIR) and non-relapse mortality (NRM) were 55%, 36% and 18%, respectively. On univariate analysis, significant prognostic factors were hematopoietic cell transplantation-specific comorbidity index (HCT-CI) (score 0 vs >= 1: 78% *vs* 48%, p=0.007), disease (AL *vs* MDS: 59% *vs* 40%, p=0.004) and rDRI (L/I *vs* H/V: 64% *vs* 43%, p=0.003) for the 5-year OS, donors (related *vs* unrelated: 53% *vs* 27%, p=0.005) and rDRI (L/I *vs* HV: 27% *vs* 48%, p=0.005) for CIR, and age (<60 *vs* >= 60: 10% *vs* 28%, p=0.021), donors (related *vs* unrelated: 8% *vs* 23%, p=0.034) and disease (AL *vs* MDS: 13% *vs* 36%, p=0.003) for NRM. On multivariate analysis, HCT-CI score >= 1 (HR 3.1, 95% CI 1.3-7.4, p=0.009) and MDS (HR 2.4, 95% CI 1.3-4.5, p=0.005) were adversely associated with OS, so were H/V rDRI (HR 2.5, 95%CI 1.4-4.7, p=0.003) and MDS (HR 3.7, 95%CI 1.6-8.8, p=0.002) for CIR and NRM, respectively.

Summary/Conclusions: Our data suggest that Flu/Mel-based RIST was a promising strategy for patients with hematologic malignancy, irrespective of (?) donor or stem cell sources. However, GRFS and OS of MDS were significantly worse than those of AL, and MDS is strongly associated with high NRM even with RIST. This indicates that we should pay more attention to NRM in MDS.

PB2174

INCIDENCE AND RISK FACTORS FOR THE DEVELOPMENT OF HEMORRHAGIC CYSTITIS ON HAPLOIDENTICAL TRANSPLANTATION

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Background: Hemorrhagic cystitis (HC) is a serious complication ocurring after allogenic hematopoietic stem cell transplantation (HSCT) more frequent on haploidentical (haplo) HSCT, with an incidence of 10% to 70%(Silva et al Haematologica 2010;95(7):1183–1190) associated mainly with the effect of cytotoxic agents such as Cyclophosphamyde (Cy). The conditioning regimen, BKPyV infection and graft *versus* host disease have an implication in the incidence. Other authors related the reactivation of CMV and a previous transplantation as risk factors to HC development(Ruggeri et al Transplant Infectious Disease 2015:17:822–830).
Aims: With this study we aim to describe the HC incidence and risk factors in all haplo-HSCT performed in the Canary Islands.
Methods: We analyzed all consecutive haplo-HSCT from family donors performed at our Hospital between 2013 and 2016. The conditioning regimen used for the transplant was the Hopkins haplo protocol with high dose Cy (50 mg/kg on days 3 and 4) posttransplantation (PTCy). We used as HC prophylaxis intense hydratation on the Cy administration day and the following 24 hours (using bladder wash only in 1 patient with cardiac dysfunction) and perfused MESNA at 100% of Cy dose beginig 15 minutes before the Cy administration on 16 pts and at 20% of the last dose at 0, 4 and 8 hours on all pts. We used SPSS V.23 to determine the cumulative incidence (CI) of HC.
Results: We performed 20 haplo-HSCT, of which 10 were males (1 was transplanted 3 times) and 8 were women. The mean age was 40 (range 16-64). The pts presented the following diagnosis: AML (10), ALL (1), EH (5), NHL (3), AM (1). 45% of pts received the haplo-HSCT in remission, 50% with refractory disease and 5% of pts did not receive previous treatment. 6 pts developed HC (36.5% CI at day +80) (Figure 1a) with a median time from haplo-HSCT to onset of 23 days (range 3-42), 1 (17%) was grade I, 4 (66%) grade II and 1 (17%) grade IV. The grade I case did not received the MESNA infusion like most of the other pts. No pts died due to HC and all cases resolved without sequelae. 12 pts received Cy pre- and post-transplant and only 8 pts received PTCy. The CI at day +80 for the pts with PTCy was 33.3% and for Cy pre- and post-transplant 38.3% (Figure 1b). We found no statistically significant difference on the CI of HC between these two groups. The development of HC was related to Cy in 1 patient, who suffered from this complication on the second and third haplo-HSCT. For the rest of the pts (after day +30) the HC was related to BKPyV infection, as a consequence of the immunosuppression state of the patient, we also observed all these pts had positive serum viral load for CMV.

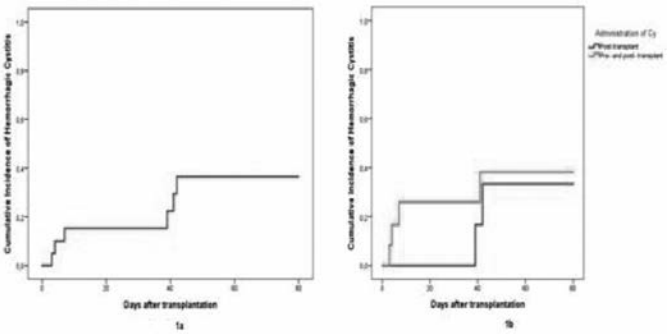

Figure 1.

Summary/Conclusions: The incidence of HC associated to post-HSCT high Cy dose in our series is 15% lower than other ones. Most of them on grade 1 or 2 and without mortality associated. The risk of HC is high, particularly in the setting of highly pre-treated patients (especially those undergoing a 2nd transplant). The development of HC after day +30 is evidently associated to BKPyV as a contributing factor for continuous inflammation and CMV reactivation (as an immunosuppression marker). In our study, HC did not have an impact on mortality of high-risk patients after haplo-HSCT. The HC remains frequent with a high morbidity in particular when it is severe, often causing prolonged hospitalization and resource use. We need further studies to recognize the at-risk population early.

PB2175

OUTCOME OF CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS UNDERGOING NON-MYELOABLATIVE ALLOGENIC STEM CELL TRANSPLANTATION AFTER TREATMENT WITH THE BRUTON TYROSINE KINASE INHIBITOR IBRUTINIB

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Background: Although the Bruton tyrosine kinase (BTK) inhibitor ibrutinib significantly improves the prognosis of CLL patients (pts), allogeneic hematological stem cell transplantation (HCT) remains the only curative option for the underlying disease. Data on pre-transplant treatment of CLL with ibrutinib are very limited.
Aims: Here we present our experience of HCT in pts previously treated with ibrutinib.
Methods: 11 CLL pts (median age at HCT 57 years [y], range 52-66 y) treated between 2014 and 2016 in our unit with non-myeloablative (nma) HCT after ibrutinib were included. Ibrutinib treatment lasted median 4.03 months (range 1 - 28). Conditioning regimen was Fludarabin 30 mg/m^{2}on day -4 to -2 followed by 2 Gy total body irradiation. Disease status at HCT was Binet B (n=3) or Binet C (n=8). Two pts had Richter's transformation (RT) diagnosed before nma-HCT. Ten pts were in partial remission (PR) at nma-HCT (PR1 n=4; PR2 n=3; PR3 n=2, PR4 n=1) while one was in first relapse. Donors were human leukocyte antigen (HLA) matched related (n=3, MRD) or HLA-matched unrelated (n=8, MUD). Pts received median 3 lines of therapy (range 1-6) including ibrutinib before transplantation. Classical cytogenetic analysis and fluorescence *in situ* hybridization (FISH) was carried out for every pt. Five pts had a deletion (del)(17p13) and one a del(11q22.3).
Results: The average overall survival (OS) for all pts was 471 days (range 36-812) (Figure 1). The average OS of patients with del(17p13) was 379 days (range 66-628) compared to 456 days (range 36-812) for those without del(17p13, p=0.98). OS was not significantly influenced by the stem cell source (MUD *vs* MRD, p=0.63) or remission status PR1 *vs* >PR1 (353 *vs* 472 days, p=0.79). Non-matched CMV-Status (negative recipient and positive donor or positive recipient and negative donor) had an OS comparable to that of matched CMV-Status (p=0.73). Pts above the median age had a lower OS although this didn't reach significance (p=0.39). EFS was median 125 days (range 26-628). Pts with or without a TP53 alteration had a similar EFS (p=0.91). Pts undergoing MRD-HCT had better EFS than those undergoing MUD transplantation (p=0.055). CMV-Status or age>median had no prognostic influence on the EFS (p.0.83 and p=0.39 respectively). Non-relapse mortality (NRM) was 32% at 10 months (Figure 1), which was consistent with a previous publication from our group (30% at 4y). The acute GvHD Grade 3-4 was present in 3 pts (27.2%).

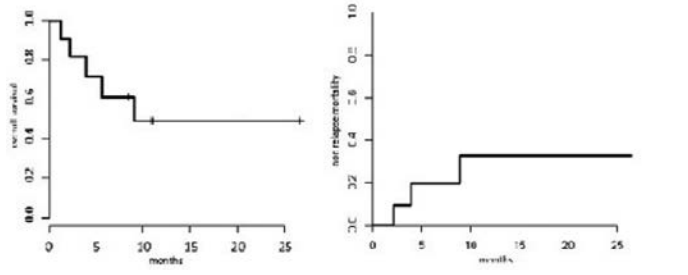

Figure 1.

Summary/Conclusions: The outcome in this small group is comparable to studies of CLL-pts undergoing nma-HCT without receiving ibrutinib upfront. Previous data from our unit (Hebenstreit *et al.*, Leuk Lymphoma 2014) showed OS 51% and NRM 30% at 4y. Ibrutinib appears to be a feasible option in a transplantation setting, although further testing with larger numbers of patients and a longer follow up is required.

PB2176

SHORT-TERM CHIMERISM IN T-HELPER CELL SUBSETS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Despite the fact that almost all studies in transplant biology dedicate T-cells the chimerism in T-helper (Th) cells and its subsets such as T-regulatory (Treg) cells after allogeneic hematopoietic stem cell transplantation (allo-HSCT) has never been evaluated.
Aims: To evaluate Th, Treg and bone marrow cell short-term chimerism in allo-HSCT patients.
Methods: Between May 2015 and November 2016 there was 109 transplants in our center. The research included 24 patients with hematological malignancies (AML =14,ALL =7,MDS =2,CMML -1). The median age of patients was 33,5 (range 19 to 60) years old, female=16, male=8. Myeloablative conditioning regimen was used for 11 patients. The other 13 patients underwent reduced intensity conditioning regimen. Peripheral blood stem cells (PBSCs) as graft source was used in 9 patients, BM in 15 patients. 9 patients were transplanted from HLA-identical related donor ,15 - from unrelated matched. Chimerism was evaluated at +30, +60, and 90-day in blood and bone marrow. Peripheral blood mononuclear cells (PBMC) were isolated using standard protocol. Cells were sequentially incubated with CD4-biotin and anti-biotin microbeads (Milteney Biotec, Germany). Next pure fraction of Treg cells (CD4$^+$CD25^{high}) was obtained by positive selection with the use of anti-CD25 microbeads. DNA was isolated by AmpliSens DNA-sorbB nucleic acid extraction kit. Chimerism was assessed by the STR-PCR analysis (polymerase chain reaction with a panel of primers for loci of short tandem repeats).
Results: For detailed result see Figure 1. 18 patients didn't have any signs of relapse, graft failure or acute graft-*versus* host disease at all observation time. In this group on day 30% of cells with donors genotype was - 97,17±0,75; on day 60 – 95,75±2,15; on day 90- 98,21±0,80. On day 30 T-helper – 87,51±3,12; on day Th 60- 90,43±3,18; on day 90 Th – 93,71± 3,03. On day 30 T-regulatory – 77,36±4,50; on day 60Treg – 82,08± 5,94; on day 90Treg – 97,71± 1,18. Four patients were diagnosed with relapse at +4 and +6 months after allo-HSCT. Two patients were diagnosed with acute GVHD.

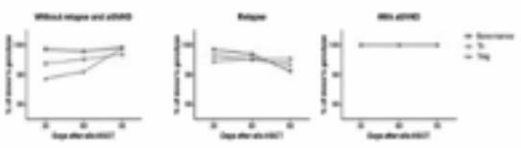

Figure 1.

Summary/Conclusions: Impact of chimerism in different T-helper subsets still need further investigation. We will continue our research and further results will be reported later.

PB2177

ADIPOSE TISSUE CHANGES IN LYMPHOMA PATIENTS IN THE PERI TRANSPLANTATION PHASE

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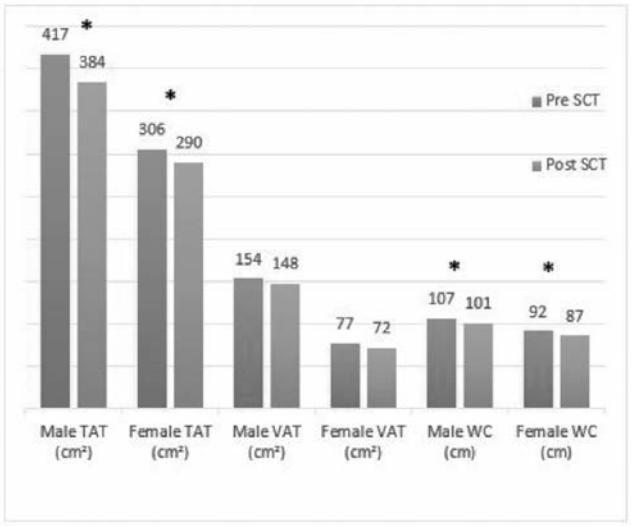
Background: Abdominal Visceral Adipose Tissues (VAT) have been shown to have inflammatory activity and have been used to predict cancer outcomes. The ratio of VAT/Total Adipose Tissues (TAT) is a negative predictor of progression free survival in Lymphoma patients on chemotherapy.
Aims: Assess the changes in adipose tissues among stem cell lymphoma recipients in the peri-transplantation phase.
Methods: Institutional Review Board approved this retrospective study for adult patients (age>16 years) having B and T lymphoma who underwent Stem Cell Transplantation (SCT). Each patient was imaged by PET/CT scan pre-SCT and in the first 3 months post transplantation. A cross sectional image was analyzed at the level of the L3 to calculate TAT, VAT and Waist Circumference (WC). Data was analyzed by gender since body composition parameters differed significantly between the two categories in the literature.
Results: The study sample consisted of 91 patients [mean age: 37.7±13.5 years, n=52 (57%) males, n=81(87%) autologous SCT, n=12(13%) allogeneic SCT, median overall survival in months: 12 in males and 19 in females]. Death was observed in 6 (11.5%) males and 1(2.4%) female. Patient characteristics were similar across gender categories except for weights (kg) and Body Mass Index (kg/m^2): 88.1 and 28.6 *vs* 65.2 and 25.0, in males and females respectively (p<0.05). Changes from pre-SCT to 3 months post SCT revealed that TAT and VAT decreased with mean differences of 33±56 cm^2 (p<0.01) and 7.0±36 cm^2 (p=0.17) in males and 16±44 cm^2 (p<0.01) and 4±14 cm^2 (p=0.056) in females, respectively. Waist circumference decreased significantly with mean

differences of 3.9±4.9 cm and 2.8±4.4 cm in males and females, respectively (p<0.01). VAT/TAT withnissed a slight increase in males and reduction in females (p>0.05). In multivariate analysis, no significant associations were shown with mortality and progression rates (Figure 1).



* p value<0.05

Adipose Tissue Changes in the peri transplantation phase presented by gender.

TAT: Total Adipose Tissues; VAT: Visceral Adipose Tissues ; WC: Waist Circumference

Figure 1.

Summary/Conclusions: This study provides data on the evolution of adipose tissues parameters in the peri-transplantation phase. TAT, VAT and WC decrease 3 months post transplantation. Future studies should evaluate the associations of these parameters with major outcomes on larger sample sizes.

PB2178

NON RELAPSE MORTALITY (NRM) ANALYSIS IN 93 UNRELATED DONOR TRANSPLANTATION - SINGLE CENTRE EXPERIENCE - HLA HAPLOTYPE ROLE?

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Background: Unrelated donor stem cell transplantation has a curative potential against haematological malignancies. However there are concerns about associated risk of non-relapse mortality. We performed a retrospective single centre study of causes of non-relapse mortality over four year period - 2012-2016.
Aims: Purpose of the study was to analyse non-relapse mortality (NRM) in patients subjected to unrelated donor transplantation in four-year-period: 2012 to 2016 - 93 transplant procedures in 86 patients.
Methods: Study cohort was analysed - relapse rate and non-relapse mortality were assessed. Causes of both - early and late NRM were studied.
Results: There were 23 relapses in the group of assessed patient cohort (24,7%). 7 patients undergone the second transplant - five patients - because of AML relapse, later two - because of secondary graft failure. Out of re-transplanted 7 patients - 3 patients are alive - 2 patients with graft failure and one with post-transplant AML relapse in 2nd CR. Out of 93 procedures of unrelated donor transplantation there were 16 cases of death - assumed as non relapse mortality NRM (17%). There were 9 early deaths (before day +100) - 6 cases in patients with relapsed/refractory acute leukaemia without remission after conventional chemotherapy. These patients were subjected to sequential conditioning with cytreduction phase. Active disease and highly active antileukaemic treatment can be reason for higher treatment related toxicity and elevated risk of death. Later two patients developed infectious bacterial complications with septic shock. In one patient - antiviral treatment refractory CMV encephalitis with massive macrophage activation syndrome was diagnosed. Analysis of NRM after day100 revealed 7 affected patients. All these patients GVHD 2-4 was diagnosed previously, accompanied by transplant associated microangiopathy (TAM) and infections - both viral and fungal. Additionally to factors connected to NRM - age, comorbidity score, patient/donor HLA allelic and antigen and sex mismatches, HLA patient/donor haplotypes were analysed. It was possible to categorise 15 out of 16 NRM patients into 5 HLA class II haplotype groups connected with autoimmune diseases in Caucasian population - rheumatoid arthritis and lupus erythematosus: DRB1 01:01 DQB1 05:01 (5 patients), DRB1 03:01 DQB1 02:01 (4 patients), DRB1 11:01 DQB1 03:01 (3 patients), DRB1 15:01 DQB1 06:02 (2 patients), DRB1 04:01 DQB1 03:02 (1 patient).
Summary/Conclusions: Based on these results we create working hypothesis that HLA class II haplotype may predispose to severe post-transplant infectious or/and non-infectious complications and affect the risk of NRM. Because small number of analysed patients and documented high frequency of these haplotypes in population, further analysis is required.

PB2179

HAPLOIDENTICAL STEM CELL TRANSPLANTATION WITH HIGH DOSE CYCLOPHOSPHAMIDE POST-TRANSPLANT IN HIGH RISK HEMATOLOGIC MALIGNANCIES: RISK FACTOR AND OUTCOME ANALYSES IN OUR CENTER

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Background: Allogenic hematopoietic stem cell transplantation (allo-HSCT) is an effective therapy for a variety of hematological malignancies. However, a lack of HLA-identical sibling donors or unrelated donors has restricted the application of allo-HSCT in hematological malignancies. Haploidentical HSCT (Haplo-HSCT) offers the benefits of rapid and nearly universal donor availability and, in the past decade, has been accepted worldwide as an alternative treatment for patients with hematological malignancies who do not have an HLA-identical sibling donor or who require urgent transplantation.
Aims: The purpose of this study was to investigate the incidence, causes and factors influencing overall and transplant-related mortality after Haplo-HSCT.
Methods: We analyzed all consecutive patients receiving Haplo-HSCT from family donors at our hospital from 2013 to 2016. The conditioning regimen used for the transplant was the Hopkins haplo protocol with high dose Cy (50 mg/kg on days 3 and 4) posttransplantation. We classified the patients before the Haplo-HSCT according to disease risk index (DRI), ECOG, Sorror score and EBMT risk score to evaluate the correlation between the physical state of the patients before the transplant and the survival (overall mortality (OM) and transplant-related mortality (TRM)). We used SPSS V.23 to calculate the cumulative Mortality incidence by the KM test and the Cox proportional hazards model.

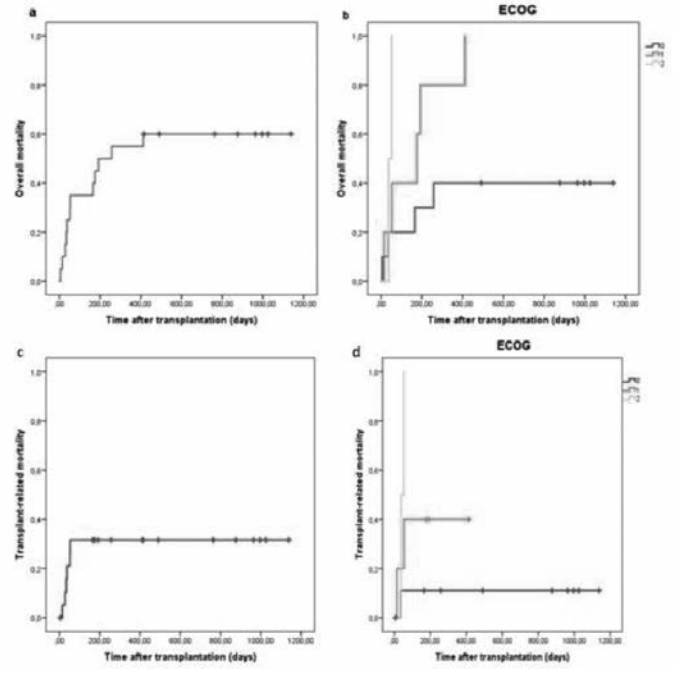

Figure 1.

Results: We performed 20 haplo-HSCT, 10 were males (1 was transplanted 3 times) and 8 were females mean age of 40 (range 16-64). Diagnosis: AML (10), ALL (1), EH (5), NHL (3), AM (1). Forty five percent of patients received the haplo-HSCT in remission, 50% with refractory disease and 5% of patients did not receive previous treatment. Of the 20 patients from our series, 12 died post transplant with an OM of 60%. The cumulative incidence (CI) of OM was 15% at 1 month (m), 35% at 3 m, 45% at 6 m, 55% at 1 year, and 40% at 2 and 3 years (Figure 1a). When we analyzed the OM depending on the different physical status scores we found no statistically significant differ-

ence between OM from the different states of EBMT (p=0.356) and DRI (p=0.07), however we found a statistically significant difference for ECOG (p=0.028) (Figure 1b) and Sorror (p=0.016). On a pairwise analysis of the OM we found no statistically signification for EBMT, and found a statistically significant difference between the patients with low-high DRI (p=0.01), intermediate-high DRI (p=0.01), ECOG 0-1 (p=0.046) and Sorror 0-5 (p=0.003). The multivariate analysis showed that ECOG 2 *vs* 0 (p=0.013, HR=46.59), Sorror 2-3 *vs* 0-1 (p=0.041, HR=19.55) and Sorror 4-5 *vs* 0-1 (p=0.005, HR=282.48) were significantly related with a higher incidence of OM. Five patients died of infection (41.67%), 3 of disease progression (25%), 1 of relapse (8.33%) and 3 of other causes (25%). Six patients died of TRM (50%). The CI of TRM was 10.5% at 1 m and 31.6% at 3 m, 6 m, 1, 2 and 3 years (Figure 1c). When we analyzed the TRM depending on the different physical status scores we only found a statistically significant difference between TRM incidence from the different states of ECOG (p=0.038) (Figure 1d) and no statistically significant difference for EBMT (p=0.386), DRI (p=0.372) and Sorror (p=0.073). On the pairwise analysis we also found statistically significant differences between ECOG 1-2 (p=0.018) and EBMT 1-5 (p=0.046), for Sorror we found a marginal statistical signification between 0-1 (p=0.052), 0-2 (p=0.052) and 0-5 (p=0.052), for DRI we found no statistically significant difference. On the multivariate analysis we found no statistically significant correlation between TRM and the physical status scores.

Summary/Conclusions: Despite the fact that Sorror, EBMT and DRI scores are widely validated to establish the risk of patients undergoing HSCT, in our experience ECOG remains a useful score for assessing the risk of TRM on patients receiving Haplo-HSCT. We think further studies with a larger sample would be necessary to confirm our results.

PB2180

A SIMPLIFIED METHOD OF CRYOPRESERVATION OF PERIPHERAL BLOOD STEM CELLS WITH OVER 10% GRANULOCYTE CONCENTRATION FOR LESS THAN 36MONTHS

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Background: The long-term stability of cryopreserved peripheral blood stem cells (PBSCs) is an important concern for patients experiencing disease relapse. However, the quality of long-term cryopreserved PBSCs stored at -80°C by using simplified method has not been elucidated in detail. Cryopreserved PBSCs undergo cell damage and decrease in viability, and those containing granulocytes might influence cell loss.
Aims: The aim of this study was to evaluate the effect of cryopreservation for less than 36 months and the number of granulocytes in the cryopreserved PBSC products on $CD34^+$ cells.
Methods: We examined the effects of cryopreservation on the viability of CD34+ cells that were stored for less than six months and those stored for 7–24 months, and 25-36 months, and the change of CD34+ cell viability with higher granulocyte content. We also evaluated the correlations between the number of granulocyte in the cryopreserved PBSC products and the time to engraftment of leukocyte or platelet. Informed consent was obtained prior to the procedure from all the patients following institutional guidelines.
Results: A total of 65 PBSC samples were collected. We compared three groups based on the cryopreservation period: (1) less than 6 months, (2) 7–24 months, and (3) 25-36 months. The median (range) viability of CD34+ cells after thawing was 81.8% (58.2–94.4). 80.5% (56.6–92.8), and 76.1% (54.5–89.6) in the three groups, respectively. No significant difference in the viability of the cells in either frozen period was observed (p=0.14, respectively). We compared the effect of granulocyte concentration (over 10% concentration against less than 10% concentration) on CD34+ cells viability. The median (range) viability of CD34+ cells containing >10% granulocytes was 76.6% (54.5–93.0%), and that for cells containing <10% granulocyte was 82.1% (59.1–94.4%), respectively. There was significant difference in the viability of CD34+ cells between the two groups (p=0.02, respectively). Second, we analyzed 81 autologous PBSC transplants after stored at -80°C by using simplified method. We compared two groups based on the granulocyte concentration (>10% concentration against <10% concentration). No significant difference in the days to leukocyte >1.0x10^9/L and to platelet >20x10^9/L in either granulocyte concentration was observed. However, the median (range) time to platelet >50x10^9/L containing >10% granulocytes was 27.2(12-87), and that for cells containing <10% granulocyte was 20.3(10-51), respectively. There was significant difference in the day to platelet >50x10^9/L between the two groups (p=0.04, respectively).
Summary/Conclusions: Long-term cryopreservation represents a means of holding a potential therapeutic modality in reserve for use at a future date. In this study, PBSCs can safely be stored for at least36 months by the simplified method at -80°C. The loss of the viability of CD34+ cells was greater when the granulocyte content was over 10% than in cells with less than 10% of granulocytes. The effect of reduced CD34+ cells viability seems important for engraftment. Difference in the day to platelet >50x10^9/L between the two groups based on the granulocyte concentration (>10% concentration against <10% concentration) was observed. Thus, a lesser granulocyte content could give a more reliable graft with better quality. Further research is necessary to observe the effect of long-term cryopreservation period and granulocyte content on the viability of stored CD34+ cells.

PB2181

LYMPHOCYTE RECONSTITUTION AFTER ALOGENIC TRANSPLANTATION, DOES EARLY RECOVERY HAVE ANY INFLUENCE IN SURVIVAL RATES?

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Background: Immune reconstitution after AloTPH has significant influence on the procedure final success. Studies have established that early lymphocyte recovery can influence survival rates, associated to a reduction in mortality unrelated relapse (NMR) and, in some studies, also to a reduction in relapse rate.
Aims: Analyze our patients survival rates in terms of lymphocyte reconstitution in absolute value on day+30 and+60 post-HSCT. Check if there is any relationship between the number of transfused CD34+ progenitors and LT3+ and see if that possible link affects the speed of recovery after transplant t- lymphocyte count.
Methods: Analysis of the lymphoid recovery in a retrospective study of 63 of 71 patients transplanted (ALO, and Haplo Unrelated Donor) by AML and ALL between 2008- 2015. (8 died before the day+60). Table 1 shows the characteristics of the pre-transplanted patients and analyze the influence of the parameters of the infused product (CD34x10^6 and LTx10^8/kg r), type of transplant, GVHD presentation. treatment and reactivation of CMV on the recovery of absolute lymphocyte numbers in s+30 and +60 days post transplantation using as cutt off <0.3x10^3/ml. We have analyzed the ratio of the number of lymphocytes on day +60 with survival after transplantation. It has made a statistical - analysis of OS and DFS in relation to the number of lymphocytes on day +30 and day +60 with Kaplan Meier compared the results with long-rank test and subsequent analysis of the variables collected with Cox Regression.
Results: After analyzing the product infused we observe a relationship between LT and lymphocyte recovery on day+30 (p: 0097, cor: 0223) and day +60 (p=0.059, cor: 0257) but not with the CD34+/Kg r. Table 2 shows the patient characteristics in lymphocyte absolute count in the day +60. We analyzed the overall survival (OS) and disease - free survival (DFS) and a decrease in OS with statistical difference was evident in patients with <.300 (p=0.0029) on day +60 and day+30 (p: 0.05), a decline also in DFS, with no statistically significant difference (p=0.1). Multivariate analysis to determine which factors could influence the lymphoid recovery on day +60 and SG, we observed that the type of unrelated donor, myeloablative conditioning and ATG administration can influence a delay in this recovery. No differences were observed in the rest of the variables.

Table 1.

CHARACTERISTICS OF THE PATIENT S:

SEX:	
- Male: 44 (62%)	
- Female: 27 (38%)	
AGE: Median: 41 (14-69)	
DIAGNOSTICS:	
- LLA: 22 (31%)	
- LMA: 47 (66.2%)	
- THE Biphenotype: 1 (1.4%)	
- Bilinear: 1 (1.4%)	
STATE OF ILLNESS TO TRANSPLANTATION:	
- RC: 55 (77.4%)	
- RP / EMR + 10 (14%)	
- Refractoriness: 6 (8.45%)	
STEM CELL:	
- SP: 66 (92.95%)	
- MO: 2 (2.8%)	
- SCU: 3 (4.2%)	
DONOR:	
- DE: 48 (67.6%)	
- DNE: 20 (28.165)	
- Haploidentical: 3 (4.2%)	
CONDITIONING:	
- MIELOBLATIVO: 46 (64.8%)	
- Low Intensity: 25 (35.2%)	

Summary/Conclusions: A delay in lymphocyte recovery is associated with a decrease in survival rates in our patients. Measures favoring an accelerated lymphocyte recovery (judicious use of thymoglobulin, adequate donor selection, and transplantation modality) could affect the post-transplant survival. It appears that the amount of infused product could play an important role in reconstitution, so it would be a factor to take into account prior to infusion.

PB2182

SUCCESFULL AUTOLOGOUS STEM CELL TRANSPLANTATION AFTER VELCADE-BASED REFRACTORY MULTIPLE MYELOMA PATIENTS

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Background: The optimal induction treatment for Newly Diagnosed Multiple Myeloma Patients needs combinations with Bortezomib-Based (Bor-Based) schemes. Primary Refractory patients include patients with progressive disease or rapid (<60 d) relapse after these optimal induction approach have a very bad prognosis. Lenalidomide-Dexamethasone (LenDex) were usually the next step in the treament of these patients, until the recent introduction of triplets combination LenDex-based. Autologous Stem Cell Transplantation (ASCT) have proven efficacy in NDMM younger patients that have got at least a partial response (PR) after the induction therapy. There are few data about toxicity and response of ASCT in primary refractory patient that can obtain a response with LenDex rescue treatment.
Aims: Analysis of tolerance, response and overall survival of ASCT-candidates that are primary refractory to Bor-Based induction treatment.
Methods: Retrospective analysis of our database. From 2010 to Nov-2016, 53 ASCT-Candidates (for 1st or 2nd ASCT procedures) were included. Median Age for diagnosis was 62 (46-71). Median Age for ASCT procedure was 63 (46-72). 12 of these 53 patients (22.6%) were considered primary refractory and considered candidates to get Len-Based rescue treatment. 6 of them (50%) were woman. Characteristics of Disease: IgG kappa (4), IgG-lambda (3), IgA kappa (3), IgA lambda (1), Light Chain lambda (1). ISS I/II/III): 5/2/5. Induction treatment: VelDex (4), VTD (6), VCD (2). Median of cycles administered: 6 (2-8). Best Response to induction treatment: >PR (6), Minimal Response (1), progressive disease (5) Len-Based (12). Plus CFM in 3 of them to optimize response. Median number of cycles administered: 6 (3-12). 3 of them didn´t responde. Of the other 9, 6 of them were considered candidate to intensificate treatment with high doses chemotherapy supported with an ASCT (2 of 6 to a 2nd ASCT procedure). The other 3 patients are in treatment or in preASCT evaluation. Characteristics of the after-rescue ASCT-patients. Median age at ASCT: 62 (49-70). All of them received Melphalan 200 mg/m2 as conditioning treatment.
Results: Morbidity or mortality (M&M) (0%) of ASCT procedure in refractory patients is similar to non-refractory patients. After a median follow up of 46 months from diagnosis for all ASCT-candidates group, the refractory patients get an overall survival of 46.2 months (3-72 m). Any of them have relapsed yet. 2 of them are in biological relapse without need of treatment.
Summary/Conclusions: Patients refractoriness to induction may receive ASCT after a rescue treatment LenDex based, as is effective in this group conventionally considered to have a very bad outcome. New combinations (triplet) with new drugs with LenDex-based treatment may improve the responses rates and overall survival before and after of ASCT procedure in these group.

PB2183

SAFETY AND EFFICACY OF TBF CONDITIONING IN PATIENTS UNDERGOING ALLOGENEIC STEM CELL TRANSPLANTATION.A RETROSPECTIVE SINGLE CENTER EXPERIENCE.

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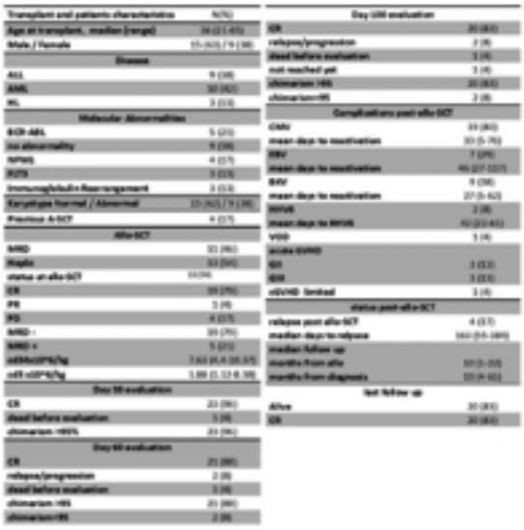
Background: The optimal intensity of myeloablation with a reduced-toxicity conditioning (RTC) regimen to decrease relapse rate after allogeneic stem cell transplant (allo-SCT)without increasing non-relapse mortality (NRM), has not been well established.
Aims: In this retrospective study at the American University of Beirut medical center (AUBMC) we aimed to evaluate the outcomes of patients who underwent allo-SCT with thiotepa, busulfan and fludarabine (TBF) as RTC.
Methods: We included twenty fourconsecutivepatients with hematological malignancies who received TBF as conditioning forallo-SCTfrom January to December 2016**.** All patients and transplant characteristics are listed in Table1. All patients received the myeloablative conditioning regimen consisting ofthiotepa(5mg/kg/day)infused on day -7 and -6, fludarabine(30mg/m^2/day) was infused on day -5 to day -2; and busulfan(130mg/m^2/day) was infused on day -5 to day-3. All patients received 2.5mg/kg/day intravenousrabbit antithymocyte globulin (ATG) on days -2 and -1. GVHD prophylaxis for patients transplanted from haploidentical donors consisted of post-transplant cyclophosphamide 50mg/kg/day on day +3 and day +5, cyclosporine started at 3 mg/kg/day on day+6 and readjusted according to level, and mycophenolate mofetil 500mgx4/day started on day+6 to +28. Patients transplanted from matched related donor, received cyclosporine as of day +1.
Results: Twenty three patients(96%) engrafted, with 14 days (range, 10-18) and 13 days (range, 8-48) as median time for neutrophil and platelet engraftment respectively. One patient who underwent haploidentical donor transplant with persistent disease for AML (karyotype 45,XY,-7) failed to engraft and died due to disease progression on day+22.After a median follow up of 10 months (range, 1-22) post-allo-SCT, the cumulative incidence of GradeII-IV acute GVHD (aGVHD) was 26%. One patient developed chronic limited GVHD (cGVHD).All the complication post allo-SCT are listed in table 1. Five patients (24%) relapsed post allo-SCT at a median of 163 days (range, 55-384), of which three (13%) died due to disease progression, and two were successfully salvaged and are in complete remission (CR) with full donor chimerism (FDC) at last follow up. Two patients developed JC virus progressive multifocal leukoencephalopathy, one of them made a full recovery and the other died in CR. The day 100 NRM was 0%. At last follow up 20 patients (83%) are alive in CR, with negative minimal residual disease and FDC.

Table 1.



Summary/Conclusions: Our results show that This TBF conditioning regimen appears to be safe,allows high rate of engraftment and low NRM rate among high-risk patients and can lead to a long-term disease control.

PB2184

COMPLETE REMISSION STATUS BEFORE AUTOLOGOUS STEM CELL TRANSPLANTATION AS PROGNOSTIC FACTOR IN PATIENTS WITH NON-HODGKIN LYMPHOMA

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Background: High dose chemotherapy (HDC) followed by autologous stem cell transplantation (ASCT) is commonly used for treatment of relapsed or refractory non-Hodgkin's lymphoma (NHL), as well as for first-remission consolidation in patients with mantle cell lymphoma. Disease status before ASCT is variable and is unclear whether complete response before ASCT or after ASCT correlates with better survival.
Aims: To evaluate the prognostic effect of disease status before ASCT - complete remission (CR) *vs* partial remission (PR) - in a cohort of patients with NHL.
Methods: Retrospective analysis of patients with NHL treated with HDT and ASCT between 2007 and 2016 in a single institution. All patients received peripheral blood stem cell support after conditioning with BEAM regimen (carmustine 300mg/m^2, etoposide 800mg/m^2, Ara-c 1600mg/m^{2}and melphalan140 mg/m^2). Response was assessed according to The Lugano Classification. The Kaplan-Meier method was used to estimate progression free survival (PFS) and overall survival (OS) and comparison between risk groups was performed by using the log-rank test. Univariate analysis was performed and significant predictors at the level of 0.05 were used to adjust a multivariate Cox regression model.
Results: We included 83 NHL patients, mainly males (72.3%) with a median age at diagnosis of 51 years (18-65). The most prevalent histological subtypes were diffuse large B cell lymphoma (53.0%), mantle cell lymphoma (36.1%) and follicular lymphoma (15.7%). The median number of therapeutic lines was 2 (1-5). Patients with diffuse large B cell lymphoma and follicular lymphoma were mainly treated with R-CHOP/R-CVP (82.5%) at first-line. For those who did not achieve a CR or relapsed after first-line treatment, (R)-ESHAP/DHAP/ICE (78.8%) was performed as second-line followed by ASCT as salvage therapy in order to achieve and consolidate CR. The majority of patients with mantle cell lymphoma received R-CHOP/R-DHAP (55.0%) followed by consolidation with ASCT in first remission. With a median follow-up time from ASCT of 39.66 months (0.3-117.6), OS at 2 and 5 years was 84.8%

and 74.5% and PFS was 76.8% and 58.2%, respectively. Before ASCT, 60 patients (72.3%) were in CR and 23 (27.7%) were in PR. After ACST, 4 patients were not assessed for response due to early death by toxicity. Of the remaining, 70 (88.6%) achieved a CR, 4 (5.1%) a PR and 5 (6.3%) failed to respond. Patients in CR before ASCT presented significantly longer PFS compared with those in PR (107.9 *vs* 44.0 months, p=0.01). Besides that, patients that obtained CR after ASCT also had longer OS and PFS compared with those in PR (107.9 *vs* 8.0 and 107.9 *vs* 7.3 months, p<0.001). However, these patients had significantly lower PFS compared to patients that continued in CR after ASCT (45.3 *vs* 107.9 months, p=0.041). Univariate analysis indicated that remission status pre-ASCT (CR *vs* PR) is a significant predictor of PFS after ASCT (HR 0.39; 95% CI 0.19-0.82, p=0.013). Multivariate Cox regression model showed that this factor retains prognostic value after adjustment for age, histological subtype, Ann Arbor stage and number of previous lines of treatment.

Summary/Conclusions: Our results highlight the relevance of the obtained CR after ASCT in the OS. Furthermore, we conclude that patients with NHL who are in CR before ASCT have a better PFS than those in PR before ASCT. Additionally, continued CR after ASCT may also be an important prognostic factor. Our results suggest that the use of more effective induction regimens in order to improve initial response may be advantageous in terms of clinical benefits post-ASCT.

PB2185

AUTOLOGOUS STEM CELL TRANSPLANTATION FOR MANTLE CELL LYMPHOMA:SINGLE CENTER EXPERIENCE

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Background: Mantle cell lymphoma accounts for relatively small proportion (3%>10%) of non-Hodgkin lymphoma. High-dose chemotherapy (HDT) and autologous-stem cell transplantation (ASCT) has played a critical role in the treatment of mantle cell lymphoma. Regardless of that, mantle cell lymphoma remains largely a relapsing/remitting disease.

Aims: Our aim is to present our mantle cell lymphoma patients who underwent ASCT.

Methods: We retrospectively evaluated our 21 mantle cell NHL patients. The patients were followed after ASCT for relapse

Results: Patients were followed by a median time of 56.9 months (range, 6-170 months).The median age at diagnosis was 45 (range, 18-69), female to male ratio:5/16. The stages and MIPI scores at diagnosis were as follows: 5% stage II, 19% stage III, 76% stage IV; Low MIPI 29%, intermediate MIPI 48% and high MIPI 23%. First line treatments were R-CHOP for 6 cycles in 6 patients (29%) and R-CHOP for 3 cycles followed by R-DHAP in 15 patients (71%). The median time to ASCT was 20 months (range, 7-48 months). All patients were in at least partial remission at the time of ASCT. The transplant conditioning regimen was CVB in 5 patients (24%) and R+/-ICE in 5 patients (24%), R+/-BEAM in 11 patients (52%). Six patients (29%) achieved complete remission. Four patients (19%) died within three months of ASCT due to infection. Eleven patients (52%) was relapsed with a median time of 39 months (range, 4-123 months). Ten patients received BORID (bortezomib, rituximab, dexamethasone) and 1 patient received lenalidomide as salvage therapy and six of them achieved complete remission. Three patients underwent allogeneic hematopoietic stem cell transplantation as well as two patients received ibrutinib as fourth line therapy and followed in remission. The 3 year overall survival was 71%.

Summary/Conclusions: ASCT is a part of initial treatment strategy in fit patients with mantle cell lymphoma however%19 patients in our series had transplant related toxicity. Today, novel agents may present a less intensive approach for achieving response.

PB2186

ALLOGENEIC STEM CELL TRANSPLANTATION IN CHILDREN WITH AUTISM

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Background: Autism Spectrum Disorders (ASD) are severe heterogeneous neurodevelopmental abnormalities characterized by dysfunctions in social interactions and communication skills, restricted interests, repetitive, and stereotypic verbal and non-verbal behaviors. The etiology of ASD remains unknown, but recent studies suggest a possible association with altered immune responses and ASD. Inflammation in the brain and Central Nervous System has been reported with microglia activation and increased cytokine production in post-mortem brain specimens of individuals with ASD. Other studies have established a correlation between ASD and family history of autoimmune diseases, associations with MHC complex haplotypes, and abnormal levels of various inflammatory cytokines and immunological markers in the blood. The paracrine, secretome, and immunomodulatory effects of stem cells would appear to be the likely mechanisms of application for ASD therapeutics.

Aims: Evaluation the benefits of HSCT in patients with ASD.

Methods: We describe two cases of patients with ASD who underwent HSCT for acute lymphoblastic leukemia (ALL) and whose symptoms were markedly decreased like an improvement of social interaction, communication, and behaviors.

Results: The first patient is an 11-year-old girl with ASD who was diagnosis with Ph-positive ALL in October 2011 (at the end of treatment, BCR-ABL remained positive). She underwent a matched sibling HSCT in March 2015. The conditioning regimen was total body irradiation (TBI) and cyclophosphamide. During the 20-month follow-up period, we observed improvement in social interaction, communication, and behaviours. According to The Childhood Autism Rating Scale – CARS, prior to HSCT, she had a score of 39 (Severe Symptoms of ASDisorder), and she currently scores 30 (Mild-to-Moderate Symptoms of ASD). The second case is a 7-year-old boy with ASD, Asperger Syndrome, who was diagnosis with ALL in September 2012. He presented with bone marrow and testicular relapse in May 2015 and underwent a matched unrelated HSCT in November 2015. The conditioning regimen was Etoposide, ATG and TBI. During the 12-month follow-up period, we observed improvement in social interaction, communication, and behaviours. According to CARS, prior to HSCT he had a score of 30 (Mild-to-Moderate Symptoms of ASD), and he currently scores 24 (Minimal-to-No Symptoms of ASD). There is no treatment for ASD thus every effort to minimize the symptoms are valuable. In both cases, social interaction was significantly increased, and the aggressive behaviors decreased. Clinical cases have reported responses in autistic children receiving cord blood CD34+ cells.

Summary/Conclusions: Several incurable neurological disorders have shown benefits with cellular therapy. Thus, autism should be explored as an indication. Clinical studies are an immediate need to fully explore its potential in autism.

PB2187

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PEDIATRIC MYELODYSPLASTIC SYNDROMES: A SINGLE CENTER EXPERIENCE FROM TURKEY

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Background: Myelodysplastic syndrome (MDS) in childhood is a rare disorder and hematopoietic stem cell transplantation (HSCT) is the only known curative treatment option. However, there exist few reports regarding the outcome of transplantation for children with various types of MDS.

Aims: We analyzed the outcome of pediatric patients who underwent HSCT in our center.

Methods: We reviewed retrospectively 14 pediatric MDS patients who received HSCT at a single center. Median age at time of HSCT of the patients was 4.3 years and disease duration from diagnosis to transplantation ranged from 3 to 36 months with a median of 10 months. Five patients had primary and one had secondary MDS. Four patients had juvenile myelomonocytic leukemia (JMML) and 4 patients had myelodysplastic related acute myeloid leukemia (MDR-AML). Diagnostic cytogenetics included monosomy 7 (n=2), trisomy 8 (n=3), KRAS (n=1) or normal/other (n=8). Patients received a median of $6.8x10^6$ CD34+cells/kg. Eight patients received a bone marrow, 5 had peripheral blood graft and one an unrelated cord blood (UCB) transplant; five patients were transplanted from a matched sibling donor (MSD), 5 from a matched unrelated donor (MUD) and 4 from haploidentical donor. Conditioning regimen consisted of busulfan/cyclophosphamide in MSD/MUD patients. The patients transplanted from MUD and UCB also received antithymocyte globulin (ATG) for 3–5 days pretransplantation. Haploidentical transplantation was performed with RIC regimen and TCRαβ /CD3 depletion.

Results: Graft failure occurred in three patients with JMML (n=1), secondary MDS (n=1) and MDR-AML (n=1). Except one, all of the JMML patients relapsed at a median 83.5 days post-transplant and two of them died. One patient with MDR-AML underwent second transplantation from another MUD one year after first transplant and died from GVHD. Ten patients are alive with a median follow-up of 19.5 months (range 3-61). All patients with primer MDS are alive and well. Four patients died from transplant-related toxicity (n=2) and relapse (n=2). For the entire group, estimated five-year relapse-free survival (RFS), event-free survival (EFS) and overall survival (OS) were 78.6%, 64.3% and 70.7%, respectively.

Summary/Conclusions: These data demonstrate that especially children with primer MDS may achieve encouraging OS and RFS following HSCT. Relapse remains the main cause of treatment failure in children with JMML given HSCT. All children with MDS should be referred for allogeneic HSCT soon after diagnosis.

Thalassemias

PB2188

RELATIONSHIP BETWEEN URIC ACID LEVELS AND CARDIAC FINDINGS IN A LARGE COHORT OF B-THALASSEMIA MAJOR: GENDER-RELATED DIFFERENCES

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Background: Iron overload, secondary to recurrent transfusions and ineffective erythropoiesis, induces oxidative stress in thalassemia (TM). Uric acid (UA), a major blood antioxidant, may act either as an antioxidant or pro-oxidant.
Aims: Our aim was to evaluate the role of UA in TM and its association with cardiac iron, dysfunction, fibrosis, and complications, and cardiovascular risk factors in a large cohort of TM patients of both sexes.
Methods: 397 TM patients (200 men, mean age 32±8 years) enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) Network were considered. Myocardial and hepatic iron burdens were quantified by the T2* technique. Atrial dimensions and biventricular function were quantified by cine images. Late gadolinium enhancement (LGE) images were acquired to detect myocardial fibrosis.
Results: As expected, UA resulted significantly higher in male respect to female TM patients (4.74.7±1.3 *vs* 4.0±1.0 mg/dL; P<0.0001).
UA levels directly correlated with BMI (R=0.25, P=0.0003), and triglycerides (TG) (R=0.20, P=0.005) in female patients. Moreover, female which presented myocardial fibrosis showed higher levels of UA (4.4±1.3 *vs* 3.9±0.9 mg/dL, P=0.03). The multiple regression model identified BMI (T-value 3.7, P=0.0003), TG (2.1, P=0.04) and cardiac fibrosis (2.5, P=0.01) as independent correlates of UA level in women. In men, UA levels were positively correlated with BMI (R=0.17, P=0.02), TG (R=0.38, P<0.001), and inversely with HDL (R=-0.20, P=0.006) and glycemia (R=-0.15, P=0.04). Interestingly, UA was also directly correlated with global heart T2* values (R=0.3, P<0.001). After multivariate analysis adjustment, global heart T2* (T-value 2.6, P=0.01), TG (4.7, P<0.001), and BMI (1.9, P=0.05) remained as independent determinants of UA in male TM patients.
Summary/Conclusions: UA levels correlates with factors related to metabolic dysfunction in TM patients of both sex, while a more strong correlation between UA and cardiac fibrosis was observed only in females, and a direct relationship between UA and T2* global heart only in males. The differences in male and female TM patients imply some gender-specific mechanisms, providing biochemical basis for the epidemiological differences between sexes.

PB2189

CHARACTERIZATION OF HEMORHEOLOGICAL ALTERATIONS IN THALASSEMIA BY A CHEMOMETRIC APPROACH

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Background: Several studies reported a high incidence of thromboembolic events in β - thalassemia, more frequent in thalassemia intermedia than in regularly transfused thalassemia major. In these patients a chronic hypercoagulable state is evident and the red blood cells exhibit impaired flow properties that facilitate micro-circulatory disorders.
Aims: Since many abnormalities described in thalassemia may determine rheological alterations, in this study we have investigated the viscoelastic profiles of red blood cells from patients with β-thalassemia. The hemorheological profiles of blood samples obtained from healthy subjects and thalassemic patients were studied by chemometric tools in order to develop a model of prediction of circulatory disorders according to the viscoelastic behaviour.
Methods: Blood samples from 45 β-thalassemia patients and 48 healthy individuals, after informed consent, were analyzed. Hemorheologic profiles were investigated at 37 °C at native and normalized hematocrit. The evaluation of RBCs viscoelastic properties was performed by determining storage modulus G', loss modulus G" and complex modulus G* in oscillation mode as a function of angular frequency ω in the range 0.1-10 Hz. Multivariate statistical analysis was performed on the resulting G', G'' and G* curves and Principal Components Analysis was used as display method.
Results: The hemorheological profiles of patients affected by ß-thalassemia and healthy subjects showed significant differences and the chemometric analysis demonstrated a clearly identification of anemic status according to viscoelastic profile. Increased G', G'' and G* modula were observed in thalassemic patients demonstrating a reduction in deformability and impaired flow properties.
Summary/Conclusions: In this study a characterization of haemorheological alterations in thalassemia patients has been performed by a chemometric approach. The achieved results permit to consider the viscoelastic properties as promising predictive new indices of microvascular damage in β-thalassemia and to explain the increased incidence of vascular complications in these disorders.

PB2190

HEPATITIS E IN TRANSFUSION-DEPENDENT THALASSEMIA PATIENTS, IN GREECE. A SINGLE CENTER EXPERIENCE

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Background: Hepatitis E (HE) is nowadays considered an emerging disease that may be a threat in both developing and industrialized countries all over the world. The causal agent is an RNA virus, transmitted mainly through the fecal-oral route. Nevertheless, there are additional patterns of transmission, including the transfusion of infected blood products. The risk of developing chronic HE infection following transfusion of infected blood–derived products is higher among immune-compromised individuals. Transfusion-dependent Thalassemia patients consist a distinct category of immune-compromised patients, but the data regarding transfusion-transmitted HE infection are limited for this group of patients. Accordingly, there is, as yet, no consensus on whether blood products should be systematically screened for markers of the HE virus
Aims: The aim of this study was to assess the status of Hepatitis E infection in 96 transfusion-dependent Thalassemia patients, followed up in a single Thalassemia Unit, in Northern Greece.
Methods: Over a one-month period, we retrospectively evaluated 96 consecutive patients, from a registry of 150 adult TDT patients followed at a single Thalassemia Unit, in Northern Greece. The mean age of the study population was 36±10 years, 42% were male and 58% female. According to the patients' blood transfusion history, the participants had been transfused with 47.376 blood units during the last 14 years, whereas during the last year the same patient population had been transfused with 3.384 blood units. The detection of HEV RNA was performed by Real-Time RT-PCR method (hepatitise2@ceeramTools kit, Applied Biosystems ABI), according to the instructions. The detection of HEV was based on the identification of the "a" region of ORF2. The detection of IgG anti-HEV antibodies and their titration were performed in 92/96 samples using a commercially available enzyme-linked immunosorbent assay kit (CUSABIO BIOTECH kit), according to the manufacturer's instructions. The cut-off value was calculated according to the manufacturer's instructions.
Results: HE RNA was not detected in any of the 96 samples, whereas the IgG anti-HEV antibodies were also negative in all measured samples. The negative HEV RNA, in all the participants of this study, indicates the absence of an active HE infection, whereas the negative IgG anti-HEV antibody titre implicates that there was no history of previous HE infection. According to the literature, IgG antibodies may be detectable following an HE infection for a time period that varies from one year to 14 years.
Summary/Conclusions: This is the first assessment of the HE virus seroprevalence in the population of TDT patients in Greece, over the last two decades. Our results suggest that TDT patients are not at a high risk for HE infection. Further studies are necessary to evaluate the clinical importance of the transfusion-transmitted HE infection in TDT patients and clarify whether screening of blood donors is necessary for countries with a lower or higher prevalence of HE.

PB2191

Abstract withdrawn.

PB2192

A PRELIMINARY STUDY OF THE CARDIAC EFFECT OF PPAR GAMMA IN BETA THALASSEMIA MAJOR WITH IRON OVERLOAD

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Background: Peroxisome proliferator–activated receptor (PPAR)-gamma is a transcription factor belonging to the same family of nuclear receptors as steroid and thyroid hormone receptors. PPAR-gamma is a master transcriptional regulator involved in the expression of probably hundreds of genes. One of PPAR gamma gene polymorphisms is Pro12Ala which is present in at least 80% of humans. Pro12Ala polymorphism may reduce the risk of cardiovascular complications. Consistently, Ala12 allele carriers were found to have lower carotid intima- media thickness and reduced risk of myocardial infarction in type 2 diabetes patients. Pharmacological agonists of PPAR-gamma leads to a molecular

switch providing alleviating myocardial injury through modulating oxidative, inflammatory and apoptotic signaling pathway.
Aims: Our aim was to investigate the frequency of Pro12Ala polymorphism (substitution of proline to alanine at codon 12 in exon B of PPARγ gene in Egyptian β-thalassemia major (β-TM) with iron overload. Untreated transfusion induced iron overload in thalassemia major is fatal, usually as a result of cardiac complications.
Methods: 30 β-TM patients and 10 healthy volunteer matched for age, sex and body weight were involved in this study. β-TM patients followed up was in the "outpatient clinic of Hematology unit, at Alexandria main university hospital". Seventeen were males and thirteen were females with ages ranging from 16 – 39 years (21.53±5.44). Blood samples from β-TM patients and healthy controls were analyzed for PPARγ gene polymorphism using polymerase chain reaction-restriction fragment length polymorphism.
Results: The mean value of serum ferritin in β-TM was 4976.30±2216.41 ng/L which was significantly higher than that in controls (102.60±12.69 ng/L). The mean value of ejection fraction were 62.23±3.46% and 63.80±4.34 in cases and controls respectively. Pro12Ala polymorphism was present in 2 out of 30 (6.67%) β-TM patients with osteoporosis. One patient had heterozygous 12Ala polymorphism and the other had homozygous 12Ala polymorphism. Both had normal body mass index, lipid profile, ejection fraction and elevated serum ferritin (4923 ng/l in heterozygous patient and 4886 ng/l in homozygous patient). Ejection fraction was 70% in heterozygous patient and 68% in homozygous patient. Only one male control (10%) has homozygous 12Ala polymorphism (Table 1).

Table 1.

Comparison between the two studied groups according to PPAR gamma genotyping

Parameter	Cases (n = 30)		Control (n = 10)		χ^2	MCp
	No.	%	No.	%		
Genotyping						
Normal	28	93.3	9	90.0		
Heterozygous	1	3.3	0	0.0	1.509	0.585
Homozygous	1	3.3	1	10.0		

Data of the two cases with PPAR gamma polymorphism

Parameter	Homozygous	Heterozygous
Age (years)	20	18
Sex	male	Female
Body mass index	19.83	20.20
Ferritin (ng/ml)	4886	4923
FBG (mg/dl) kg/m^2	79	87
Triglyceride (mg/dl)	128	97
Total cholesterol (mg/dl)	130	161
HDL (mg/dl)	8	25
LDL (mg/dl)	80	71
Ejection fraction (%)	68	70
BMD	-6	-2.4

Summary/Conclusions: This study suggests that Pro12Ala polymorphism may have a cardioprotective effect in Egyptian thalassemic patients since we find the highest value of ejection fraction among the two positive cases. Further studies on a larger population of patients are still needed to confirm this finding.

PB2193

THALASSEMIA MAJOR AND INTERMEDIA IN PATIENTS OLDER THAN THIRTY-FIVE YEARS - FROM A FATAL TO A CHRONIC DISEASE

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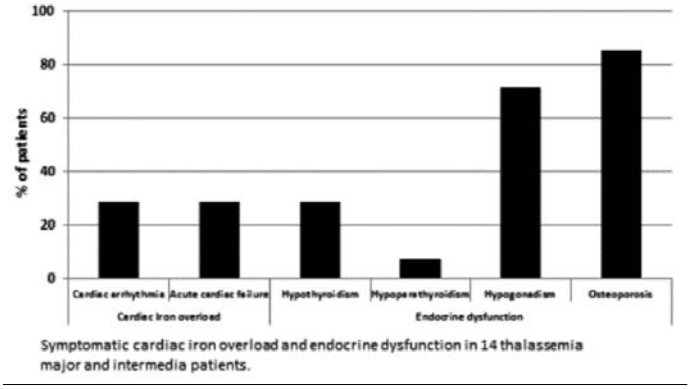
Background: During the past four decades beta thalassemia major (TM) and beta thalassemia intermedia (TI) have transformed from a universally fatal disease at a young age, into a chronic disease, with a constantly increasing life expectancy. This is attributed, amongst others, to the use of improved chelation therapy. Since prolongation of life expectancy has occurred only in recent years, there is little data regarding the older population with TM and TI.
Aims: We aimed to characterize disease and patients' characteristics in patients above 35 years of age in an adult thalassemia center in Israel.
Methods: We conducted a retrospective analysis of 14 adult patients over the age of 35 years with TM (N=10) and TI (N=4) treated in a single center, specializing in the care of adult thalassemia patients. We used descriptive statistics to describe characteristics of disease and patients and the Mann-Whiteny test to compare between patients with TI and patients with TM.
Results: Between 2006 and 2016, 14 adult patients older than 35 years with TM (n=10) & TI (n=4) were followed and treated in our center. Median patients' age was 37 (range, 35-51) years, with 66% males and 50% of Arab ethnicity. Most of the patients had at least high school education (85%), and 78% were employed. Thirteen patients (all TM patients and 3 out of the 4 TI patients) were treated regularly with blood transfusions. All patients received chelation treatment. Median hemoglobin (Hb) levels and mean corpuscular volume (MCV) levels were lower in patients with TI compared to TM (8.1 *vs* 10 g/dl, p=.002 and 72.4 *vs* 84 fl, p=.004, respectively). Median LDH levels and indirect bilirubin levels were higher in patients with TI compared to TM (603 *vs* 330 u/L, p=.004 and 2.02 *vs* 1.1 mg/dl, p=.06, respectively) indicating increased hemolysis. All patients underwent splenectomy and had secondary thrombocytosis. All but two patients were treated with at least two different chelation modalities, either as single agent, including subcutaneous (SC) or intravenous (IV) deferioxamine (DFO), deferiprone (DFP), or deferasirox (DFX), or as various combination therapy options. The median number of chelation treatment lines was 3. All patients treated with chelation suffered from at least one adverse event, necessitating temporary discontinuation and usually substitution of treatment. The median number of adverse events was 1.5 per patient. Nine patients (64.2%) had good compliance with current chelation therapy. Four patients with acute heart failure secondary to cardiac iron overload, and all four improved with intensified chelation treatment. Four TM patients (40%) were hypothyroid, half of them requiring thyroid hormone replacement therapy. All TM patients had hypogonadism. All females had amenorrhea and were treated with hormone replacement therapy, and none of them tried to conceive. Six of the seven male TM patients were treated with monthly testosterone injections, and three of them fathered children. All TM patients had osteoporosis, and three TI patients (75%) had metabolic bone disease. Figure 1 shows the relative rates of symptomatic cardiac iron overload and endocrine dysfunction in the cohort. Three patients (21.4%) had significant liver overload according to liver T2* MRI, necessitating chelation treatment intensification. None of the patients in our cohort underwent allogeneic hematopoietic stem cell transplantation and none developed secondary malignancy during follow-up.



Symptomatic cardiac iron overload and endocrine dysfunction in 14 thalassemia major and intermedia patients.

Figure 1.

Summary/Conclusions: Advances in the treatment of thalassemia patients have enabled the majority of these patients prolonged survival into adulthood. However, this has brought a new set of challenges for both patients and healthcare. This study delineates the challenges faced while treating adult patients with TI and TM in the new era.

PB2194

EVALUATION OF LIVER IRON CONCENTRATIONS IN CHILDREN WITH BETA THALASSEMIA INFECTED WITH HEPATITIS C VIRUS BEFORE AND AFTER SPIRULINA THERAPY BY MAGNETIC RESONANCE IMAGING

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Background: Magnetic resonance imaging (MRI) assessment of liver iron concentration (LIC) is necessary for quantitative staging of iron overload in children with β-Thalassemia. There is no enough evidence about the effect of spirulina therapy on LIC.
Aims: To assess LIC by MRI in multitransfused β-Thalassemic children infected with HCV before and after Spirulina Therapy.
Methods: Thirty multi-transfused β-thalassemic children infected with HCV, were subjected to clinical evaluation, appropriate laboratory investigations and assessment of LIC by MRI. They were classified according to LIC into mild

(group 1) and moderate to severe group (group 2). In addition to standard packed red cell transfusion, Spirulina therapy was given orally for 3 months, after which re-evaluation of these children was performed by repeating the same investigations.
Results: There was significant increase in LIC associated with significant changes in other MRI parameters (significant decrease in T2* and significant increase in R2*) in patients with β-Thalassemia of moderate to severe group as compared to those of the mild group before treatment. The mean values of serum ferritin (SF) was statistically insignificantly higher among patients of mild group. There was no significant correlation between different MRI parameters and SF level. There was negative correlations between LIC and T2* and positive correlation between LIC and R2*. There was significant decrease in values of LIC accompanied with significant improvements in SF after spirulina therapy as compared to their pretreatment values in patients of the moderate to severe group.

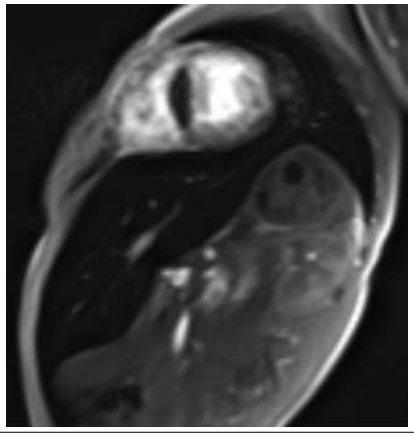

Figure 1.

Summary/Conclusions: Spirulina therapy may have favorable effects on lowering the values of LIC in children with β-Thalassemia infected with HCV.

PB2195

COMBINATION OF DEFERASIROX AND DEFEROXAMINE - A SUCCESSFUL CHELATION THERAPY IN B-THALASSEMIA MAJOR PATIENTS

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Background: Frequent transfusions required for β- thalassemia major patients cause iron overload. Without the appropriate chelation therapy, iron toxicity can cause significant heart, liver and endocrine morbidity.
Aims: In this case series we estimated the safety and efficacy of iron chelation with the combination of deferasirox (DFX) and deferoxamine (DFO) in transfusion dependent thalassemia (TDT) patients attending the Thalassemia Unit in a tertiary hospital in Athens, Greece.
Methods: 10 TDT patients were treated with a combination chelation therapy of DFX (30 ±10mg/kg/d) and DFO (44±12mg/kg/d for 2-6 days/wk in 12hr or 24hr infusion rates). Reasons for starting this combination treatment included: 1) treatment with one chelating agent did not succeed in decreasing heart and liver iron, 2) agranulocytosis or severe neutropenia due to deferiprone (DFP) treatment and 3) adverse events recorded with increased doses of one of the chelating agents. The efficacy of the treatment was estimated through MRI measurements of heart and liver iron ($T2^*_{Heart}$, and LIC) in combination with serum ferritin levels. Liver enzymes (ALT, AST) and serum creatinine were used to monitor safety of the treatment.
Results: Five of the 10 patients had significant liver hemosiderosis (LIC >15 mg Fe/gr d.w.) and 3 had heart iron overload, of which one significant (T2* 1.9 msec) (Table 1).

Table 1.

	Before starting DFX/DFO combination therapy	**12mos into** the DFX/DFO combination therapy
Serum Ferritin mean	3,360 ng/mL (range 1,700- 6,200)	2,450 ng/mL (range 800-4,900)
Liver Iron Concentration (LIC) mean	19.0 mg Fe/gr d.w. (range 6.2-46.4)	9 mg Fe/gr d.w. (range 1.6- 28.4)
Cardiac T2* mean	24.1 msec (range 1.9-36)	32.3 msec (range 5.2 -39.5)

The combination treatment was well tolerated without adverse events or effects on liver and kidney function.
Summary/Conclusions: The combination chelation with DFX/DFO can decrease iron overload and represents a safe and effective option when monotherapy is not effective or not well tolerated.

PB2196

EVALUATION OF THREE AUTOMATIC DEVICES FOR HEMOGLOBINOPATHY DIAGNOSTICS IN MULTI-ETHNIC POPULATIONS

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Background: We have tested three different dedicated haemoglobin separation devices for their capacity of performing the diagnostics of hemoglobinopathies. These involve the Variant II ™ HPLC (BioRad), the Capillarys2 capillary electrophoresis (Sebia) and the most recently introduced HPLC, Premier Hb9210™ High Resolution HPLC of Trinity Biotech (Menarini).
Aims: As the latter device is new to the market a multisite precision study was performed testing the reproducibility of the system across three test sites (Leiden, Genoa and London) using the same set of samples for several following days. The results between these three sites were compared and evaluated. Moreover we have tested the capacity to detect the most common structural haemoglobin variants, such as HbS, HbC, HbD, HbE and less common Hb variants important to be diagnosed in multi-ethnic populations found in the U.K., The Netherlands and Northern Italy as well as elevated HbA_2 , as indicator for beta-thalassemia carriers.
Methods: tHb variant separation using he Variant II ™ HPLC (BioRad), the Capillarys2 capillary electrophoresis (Sebia) and the most recently introduced HPLC, Premier Hb9210™ High Resolution HPLC of Trinity Biotech (Menarini). Molecular analysis to verify the hemoglobin variants found.
Results: We present the data of the comparison studies using the replicates of the three different sites for the Premier Hb9210™ and of 100 normal samples and 217 patient samples for a variety of beta-thalassemia trait and haemoglobin (Hb) variants, including the molecular data of the beta-thalassemia mutations and Hb variants.
Summary/Conclusions: All three apparatuses identified the common Hb variants and beta-thalassemia trait in carriers, homo- , hetero- and compound heterozygotes with the expected sensitivity and specificity. The Premier Hb9210™ High Resolution HPLC of Trinity Biotech shows comparible separation and quantitation on the three different sites using the same samples and is suitable for the analysis of samples suspected of having hemoglobinopathy and the diagnosis of beta-thalassemia trait or Hb variants.

PB2197

RED BLOOD CELL EXTENDED PARAMETERS IN HAEMOGLOBINOPATHIES

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Background: Sysmex® XE-5000 analyzer incorporates new research Red Blood Cell (RBC) parameters, derived from flow fluorescence cytometry technology, including%HYPO-H*e*, which indicates the percentage of RBC with haemoglobin (Hb) content <17 pg, and%MicroR which indicates the percentage of RBC with mean cell volume <60 fL.
Aims: The aim of this study was to establish the reference range of our Laboratory for the parameters%HYPO-H*e* &%MicroR, to investigate their values in haemoglobinopathies and their correlation, if any, with Hb A_2 levels in heterozygous β-thalassaemia.
Methods: Reference ranges were obtained from 175 healthy adult subjects (27 men, median age of 34 years & 148 women, median age of 30 years); control group (group A). 89 haemoglobinopathie heterozygotes (32 men, median age of 29 years & 57 women, median age of 30 years) were included in the study and classified into three groups; group B: β-thalassaemia heterozygotes, N=46, group C: α-thalassaemia heterozygotes, N=21 and group D: Hb O-Arab heterozygotes, N=22. We retrospectively recorded the results of full blood count analysis on Sysmex® XE-5000 analyzer including%HYPO-H*e* &%MicroR, of Hb pattern analysis (TOSOH®, G7) and ferritin levels (Roche®, cobas *e*411). All subjects included in the study presented ferritin levels within the normal range for age and gender. Statistical analysis: one-way ANOVA (Tukey post-hoc), Mann-Whitney, Pearson's correlation tests were applied. Reference ranges were calculated as the mean±2SD of the distribution. P value <0.05 was considered to be statistically significant. Data refer as median (percentiles).
Results: The reference ranges of our Laboratory for the parameters%HYPO-H*e* &%MicroR are 0.0 – 0.6% & 0.2 – 2.9%, respectively, and they are independent of gender and age (P=0.715, P=0.168 & P=0.073, P=0.843). There was a statistically significant difference between the groups as determined by one-way ANOVA for both parameters (all P <0.0001). Heterozygous β-thalassaemia presents statistically significantly higher%HYPO-H*e* values [11.6 (4.2-27.6)] as compared to groups A [0.3 (0.2-0.3)], C [1.9 (0.6-4.6)], D [0.6 (0.4-0.8)] (all P <0.0001), while there was no statistically significant difference of%HYPO-H*e* values between heterozygous Hb O-Arab and groups A and C (P=0.965 & P=0.134, respectively) based on Tukey post hoc test. Heterozygous β-thalassaemia presents statistically significantly higher%MicroR values [41.5 (22.9-58.7)] as compared to groups A [1.5 (1.1-2.0)], C [10.8 (7.9-20.5)] and D

[6.1 (4.2-9.0)] (all P <0.0001). Both parameters%HYPO-H*e* &%MicroR are correlated positively in a statistically significant degree with Hb A_2 levels in heterozygous β-thalassaemia (r=0.567, P <0.0001, y=0.050x+5.317 & r=0.680, P <0.0001, y=0.042x+4.371, respectively).
Summary/Conclusions: β-thalassaemia heterozygotes present higher values of%HYPO-H*e* &%MicroR compared to α-thalassaemia and Hb O-Arab heterozygotes.%HYPO-H*e* &%MicroR are possibly useful parameters for evaluating the relative severity of different genotypes in heterozygous β-thalassaemia because of the statistically significant positive correlation with Hb A_2 levels.

PB2198

PERFORMANCE OF THE ALPHA-GLOBIN STRIPASSAY® AND MLPA® FOR THE DIAGNOSIS OF ALPHA-THALASSAEMIA
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Background: Diagnosing α-thalassaemia requires second line diagnostics involving DNA analysis. Multiplex ligation probe amplification® (MLPA®) is a molecular technique introduced as a diagnostic tool for α-thalassaemia. This semi-quantitative technique determines the relative copy number of up to 60 DNA sequences and is able to detect deletions and duplications in a DNA sample. A novel commercial tool, the α-Globin StripAssay®, aims to detect the most common α-thalassaemia deletions and point mutations. The test involves three steps: DNA isolation, PCR reaction and a hybridization step to test strip containing allele-specific oligonucleotide probes immobilised as an array of parallel lines.
Aims: Our objective was to evaluate the α-Globin StripAssay® as a useful alternative for MLPA® in second line α-thalassaemia diagnostics.
Methods: Eight samples, including 7 known deletions (_ _SEA, _ _THAI, _ _MED, homozygous and heterozygous - $\alpha^{3.7}$, heterozygous - $\alpha^{4.2}$, $(\alpha)^{20.5}$) and 1 mutation (Hb Constant Spring) were analysed using multiplex Gap-PCR (deletions) and Sanger sequencing (point mutation) at the Leiden University Medical Center. These samples were anonymised and analysed in duplicate by MLPA® and α-Globin StripAssay® at our center. A comparison of diagnostic performance, interpretation, turnaround time (TAT) and costs (reagent and labour) was done.
Results: There are no significant differences between the MLPA® and the α-Globin StripAssay® results and each identification corresponded to the result of the reference lab in Leiden. MLPA® however provided additional information about underlying polymorphisms. Interpretation of the α-Globin StripAssay® was easier and faster compared to MLPA®. The α-Globin StripAssay® proved to have a shorter TAT, but on the other hand, the costs for MLPA® were significantly less.
Summary/Conclusions: Despite its straightforward interpretation, shorter TAT and the ability of detecting both (known) deletions and point mutations, the significantly higher costs of the α-Globin StripAssay® may hinder it's routine use. Specialised laboratories are usually acquainted with the MLPA technique and in these settings the ability to detect both known and unknown deletions is a plus for research purposes.

PB2199

CARDIAC AND HEPATIC IRON ASSESSMENT OF YOUNG ADULTS WITH TRANSFUSION DEPENDENT THALASSEMIA: TIME TO THINK BEYOND FERRITIN
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Background: With the improvement in availability of blood transfusion practices and progress in chelation therapy, there is an increasing population of thalassemic patients surviving into adulthood in developing countries. However, there is scarcity of clinical, biochemical and radiological data showing cardiac and hepatic iron assessment in these chronically transfused individuals.
Aims: 1. Cardiac and hepatic iron assessment in young adults with TDT. 2. Compare the ferritin level with T2* MRI finding.
Methods: in this prospective observational study we analysed demographic details, clinical features and cardiac and liver iron assessment of young adults with (TDT) at recently established adult thalassemia clinic at PGIMER, Chandigarh. For cardiac and liver iron assessment serum ferritin, ECG, 2D Echo, MUGA scan, Liver function test, Fibroscan (if indicated) and T2* MRI of Liver and heart was done. All patients who were diagnosed in childhood and referred to adult haematology unit at age ≥18 years and had received more than 20 blood transfusions were included in the study.
Results: A total of 53 patients (n=53) were analysed. The mean age was 23 yrs. Majority of patients (56%) were male. The average age at diagnosis and at first transfusion was 7 months & 11months respectively. The average years of PRBC transfusion was 23 yrs. The average number of transfusion in last two years prior to registration was 24 PRBC units. The mean age at start of chelation was 10.0 yrs. Mean duration of chelation was 14 yrs. Majority (88%) had growth failure with mean height of 159.6 cm & mean weight of 51.5 kg respectively. Splenomegaly was present in 47% and hepatomegaly in 25%patients. Twenty-eight percent have undergone splenectomy at an average age of 12.6 yrs. The mean of highest ferritin levels was 6131 ng/mL and the mean ferritin at the time of registration was 2919 ng/mL. LFT were deranged in 25% of patients. Evidence of cardiac dysfunction (ECG/MUGA) was present in 22% of patients. Iron overload in liver and heart as measured with T2* MRI was present in 56% & 28% respectively (Figure 1).

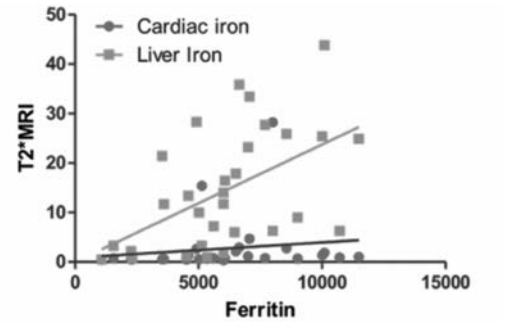

Figure 1.

Summary/Conclusions: majority of patients registered in our clinic are living a healthy life. All of them were on iron chelation therapy and the dose was being adjusted as per the serum ferritin level. Amongst asymptomatic individuals with no evidence of cardiac or hepatic dysfunction, evaluation by T2*MRI picked up evidence of hepatic and cardiac iron overload. Therefore, its prudent to monitor patients with T2*MRI and accordingly escalate or de-escalate chelation therapy.

PB2200

THALASSEMIA IN MADRID: A PICTURE OF THE CURRENT SITUATION
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Background: Diagnosis of thalassemia (Thal) in a Mediterranean country as Spain, could be thought as endemic, but few data are available so far. Moreover, attention to hemoglobinopathies is focused on sickle cell disease.
Aims: The aim of our study was to find out the prevalence of Thal and clinical significant hemoglobinopathies other than sickle cell diseases in a referral center for newborn sickle screening, in addition to the demographic characteristics of these patients. The secondary objectives were to obtain the frequency of specific treatments or prophylaxis accomplished by these patients, and the reasons for loss to follow-up.
Methods: The study is observational, unicentric, descriptive and retrospective, carried out in December 2016 in a tertiary hospital in the Community of Madrid, Spain. All patients diagnosed with Thal and other not sickle-hemoglobinopathies who had attended at least once to the hematology clinic were included. Demographic characteristics (date of birth, gender, country of birth) and clinical ones (genotype or Thal type, therapy and update in follow up, like alive, deceased or lost patient) were collected. Written informed consent was signed by patients or legal guardians in accordance with the Declaration of Helsinki. The study was approved by the hospital Ethical Committee. Statistical analyses were performed using SPSS version 18.0. Quantitative variables were reported as median or mean value and range, while categorical variables were expressed as absolute value and percentage.
Results: The total number of patients included was 31 (9 Thalassemia Major (TM), 1 Thalassemia Intermedia (TI), 21 other not sickle-hemoglobinopathies). The center follows 209 sickle patients, which leads to a ratio sickle/not sickle of 6.74 (Table 1). Ratio boy/girl is 1.21 for all group. Most of patients were born in Spain (90.32%), although 6.45% were born in Asia and one patient was born in Rumania. Considering the parents, 32% were born in Europe, 29% from Africa, 25% from Asia, and 12% from America. 92% of those patients born in Spain were detected in their first days of life due to universal screening detection implemented in Madrid since 2003. Median age at first diagnosis was 0.70 years (0-16.35). Median age at the end of inclusion was 9.39 years (range 1.90 to 35.44). 35% of them had molecular genotyping for diagnostic confirmation. Two out of 10 patients with Thal had HLA identical siblings. Quelation treatment was added to standard treatment to all the patients with Thal: 7 received deferasirox, 3 were treated with deferoxamine and 2 with deferiprone; 2 of the patients required double quelation. Two out of 10 patients with Thal underwent

splenectomy. None of these patients had sepsis or meningitis. Three Thal patients underwent progenitor stem cell transplantations and they remain on complete chimerism in the present moment. Patients lost to follow-up summed up 14: 3 emigrated to other countries, 2 continue the monitor of their diseases in other centers or in adults units and 7 for unknown reasons. There was one death (3.22%) for a cause unrelated to his illness.

Table 1.

Hemoglobinopathies	n (%)
Heterozygote β-Thal + Another variant of Hb (C, D, Andrew-Minneapolis, J Baltimore)	6 (28.57)
Hb CC	4 (19.04)
Hb EE	2 (9.52)
Heterozygote Hb O-Arab	2 (9.52)
Hb OO	1 (4.76)
Hb DD	1 (4.76)
Hb S + Hb Korle-Bu	1 (4.76)
Hb H	1 (4.76)
Hb DE	1 (4.76)
Hb CE	1 (4.76)
Hb OC	1 (4.76)

Not sickle-hemoglobinopathies

Summary/Conclusions: Early diagnosis derived from universal neonatal screening for sickle cell disease allows an effective health education and prompt therapy to other hemoglobinopathies, and a correct and thorough follow-up of these patients.

PB2201

PREVALENCE AND CAUSES OF CLOTTING TIMES PROLONGATION IN PATIENTS WITH TRANSFUSION DEPENDENT BETA THALASSEMIA

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Background: Thalassemia is traditionally known to be a thrombophilic, rather than hemorrhagic, disorder. In spite of this, prolongation of clotting times are often reported. Understanding if there is a real risk of bleeding, and what this risk can be associated to, is crucial, especially in relation to the frequent referral to surgery (*e.g.* for splenectomy, cholecistecomy). Hepatopathy due to iron overload or HCV infection has been addressed as a main cause of this finding, even though disorders in the clotting profile are often reported also in patients with no alterations of hepatic function. The impairment of factors XI and XII often reported has been hypothesized to be secondary to intravascular haemolysis or multiple transfusions *(Caocci et al, Acta Haematol 1978, Mcfadyen et al, Ann Hematol 2014)*, but no data are available to confirm this supposition.
Aims: To determine the prevalence of clotting disorders in a group of Transfusion dependent Thalassemia (TDT) patients and to assess the correlation with hepatopathy, degree of the hemolysis, transfusion frequency, erythroblastosis, iron chelation.
Methods: TDT patients followed at our center for whom clotting tests were available were included. From chart review data were collected regarding clotting times, demographics, disease history, comorbidities and concomitant medications, iron chelation therapies, iron overload (serum ferritin, LIC, cardiac T2*), liver function tests, hemolysis parameters, hemocromocitometric values. Patients on anticoagulation therapy were excluded.
Results: 56 TDT patients (female 55,35%) were enrolled in our study, mean age 26,02±13,38 years, 17 of them were pediatric. In 20/56 patients (35,71%) prolongation of clotting time was found: this included both prolonged INR (23,21%) and prolonged aPTT ratio (25%); 7 patients (12,5%) had both prolonged INR and aPTT. Subgroup with clotting disorder (group A) was compared to subgroup with clotting times within normal ranges (group B) using T-Test. No differences were found in terms of sex, age, genotype, transfusion interval, hemolysis degree, comorbidities, HCV infection included, iron overload, liver function, erythroblastosis and platelets levels, nor in history of thrombotic complications. No patients had history of hemorrhagic disease. Pretransfusion Hb was lower in patients with prolonged clotting times (p=0,045); none of the patients in Group A was splenectomized (p=0,042).
Summary/Conclusions: In our population clotting disorders were not correlated with hepatic disease, nor hemolysis or transfusions. The mild correlation with lower Hb values and with the lacking splenectomy could be consistent with the known effect of low Ht on lab procedures for clotting tests. In relation to this observation in patients with altered coagulation tests the repetition of clotting test after blood transfusion could be advisable to overcome the low Hb effect.

PB2202

COMPOUND HETEROZYGOSITY FOR HAEMOGLOBIN ADANA AND A-THALASSAEMIA IN GREECE. CLINICAL PHENOTYPE AND GENETIC COUNSELING

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Background: Haemoglobin (Hb) Adana (HBA2qC.179>A) in interaction with deletional and nondeletional α-thalassaemia mutations leads to HbH or, less commonly to thalassaemia intermedia with clinical manifestations varying from asymptomatic forms to severe anemia. First line screening tests are unable to detect the highly unstable variant. **Aims:** We report two cases of Hb Adana co-inheritance with the a-thalassaemia 3.7 kb deletion - the only α- thal and Hb Adana double heterozygosity cases diagnosed in subjects of Greek origin.
Methods: The first case concerns a 3 year old girl, born from parents referred for genetic counseling at the 11th week of a second gestation. The mother showed an Hb of 10.7g/dl,RBC 4.04 X 10^9 /L, MCV 80,7 fl, MCH 26.4 pg, Hb A2 2.8% and Hb F 1%,with positive inclusion bodies, and her ethnic (Greek) and regional background was of high risk for thalassaemia. The partner came from the same region, and he showed an Hb of 13.8g/dl, RBC 5.88 X 10^9/L, MCV 73,1 fl, MCH 23.5 pg, Hb A2 2.4% and Hb F1% and inclusion bodies were found positive. DNA analysis was requested and, routine investigation was performed in their first offspring. The girl had an Hb of 8.2 g/dl, RBC 3.82 X 10^9/L, MCV 70 fl, MCH 22 pg, Hb A2 1.9% and Hb F 2.3%, while her ferritin levels were 226ng/ml and inclusion bodies were found. On clinical examination she was found to be of normal weight and height for her age, but presented with paleness, icteric sclera and mild splenomegaly. Genetic analysis revealed that the mother carried the α-thalassaemia 3.7 kb deletion defect. The father carried the rare non deletional Hb Adana. As suspected from the haematological data, their offspring was a compound heterozygote for Hb Adana variant in trans to a 3.7 α+ thal deletion.The second case concerns a17-year-old boy, diagnosed with Hb Adana co-inheritance with the a-thalassemia 3.7 kb deletion at the age of 8 years. At diagnosis, findings were compatible with a very mild phenotype and growth was not impaired. The boy retained a mild hypochromic microcytic anemia (Hb~10g/dl, MCV 71 fl, MCH 23 pg, RDW 18.6%, reticulocyte count 5.1%), until adolescence but at the age of 11 transfusion inititation was decided due to marked splenomegaly and limited weight and height gain. For the following years he was transfused approximately once a month, necessitating chelation therapy. Weight, height and pubertal development were normal by the age of 15, but splenomegaly persisted. Splenectomy was decided and transfusions were stopped shortly afterwards. During the following months the boy retained an Hb of 9.5 g/dl, however, he complained of constant fatigue and impaired physical activity and asked to get back on a transfusion program.
Results: In both cases diagnosis was incidental highlighting the mild phenotype. However, the co inheritance of Hb Adana with the 3.7 kb α+ thal deletion is rare, with only the presenting cases in Greece, and in a few other families in Turkey, Southeast Asia, Philippines and Albania. The clinical phenotype of the combination seems to be a mild disease with a non-transfusion-dependent thalassaemia intermedia phenotype. Nonetheless, clinical severity prediction is always a difficult issue and phenotypes may change overtime as demonstrated by the second case described above.
Summary/Conclusions: Long follow-up of such rare cases is necessary in order to gain as much information as possible, so as to offer the best management to the patients and the most accurate genetic counseling.

Thrombosis and vascular biology

PB2203

ANTITHROMBOTIC EFFECTS OF PEPTIDE PGPL IN EXPERIMENTAL THROMBUS FORMATION

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Background: Previously, it was established that proline- and glycine-containing peptides have fibrinolytic, anticoagulant activity, inhibit platelet aggregation and thrombin activity *in vitro* and *in vivo*. Besides, it is known that short peptides of this family also have antithrombotic effects.
Aims: To study the influence of Pro-Gly-Pro-Leu (PGPL) and amino-acid leucine on fibrinolytic and anticoagulant blood activity, platelet aggregation and to estimate their possibility to reduce the formation of experimental blood clots.
Methods: Experiments were carried out on white rats (200-250 g) according to the ethical principles of the Helsinki Declaration. Peptide PGPL (1 mg/kg), leucine (0.33 mg/kg - equivalent to its content in PGPL) and saline (control rats) were intranasal entered to rats within 3 days. 1 hour after the last drugs administration we induced the formation of thrombus in v.jugularis (Wessler model). The degree of thrombus formation was estimated on thrombus weight. Fibrinolytic activity and activity of tissue-plasminogen activator (t-PA) of blood plasma were measured by fibrin plate method. Anticoagulant activity (APTT-test) and ADP-induced platelet aggregation were detected by standard methods.
Results: Our experiments demonstrated that preliminary intranasal administration of PGPL (before formation of thrombus) leads to increase of APTT, fibrinolytic and t-PA activity on 18%, 62%, 35% accordingly from control rats. Besides, we observed the decrease of platelet aggregation. Also we indicated the reduction of thrombus weigh in PGPL-treated rats on 68.5% comparatively with control rats. The thrombus weigh after leucine treatment decreased on 30% compared with control rats. But administration of leucine did not change of haemostasis system parameters.
Summary/Conclusions: Thus administration of PGPL enhanced of anticoagulant, fibrinolytic and antiplatelet activity in rats blood plasma. PGPL pretreatment lead to prevention of experimental venous thrombus formation. Therefore, PGPL may be used as perspective anticoagulant and fibrinolytic agent with direct antithrombotic effect.

PB2204

TREATMENT AND OUTCOME OF THROMBOTIC MICROANGIOPATHY IN MALAYSIA

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Background: Thrombotic Thrombocytopenic Purpura (TTP) is a potentially lethal disease that there is still no promising cure in this era. The ADAMTS-13 deficiency or defect in the disease has enabled clinician to recognize another entity which is Thrombotic Microangiopathy (TMA). This entity includes TTP, typical Haemolytic Uraemic Syndrome (HUS), Cancer associated TMA, Atypical HUS, Pregnancy TMA, SLE related TMA and Transplant TMA.
Aims: This study is to focus on the treatment among the TMA and the outcome of the disease.
Methods: The data was collected from year 2012 to 2016 from Ampang Hospital via the electronic hospital information system (EHIS) and external records traced from Haemostasis laboratory in Ampang Hospital as well as from other hospitals nationwide.
Results: There were total of 243 suspected TMA cases, encompassing 97 (39.9%) males and 146 (60.1%) females. The median age for this cohort was 34 years. Only 54 (24.15%) patients were diagnosed as TTP based on ADAMTS-13 activity ≤10%. Treatments were evaluated by using complete case details from Ampang Hospital cohort (69 cases). From this cohort, only 59 cases had ADAMTS-13 activity testing. There were 24% Primary Acquired TTP, 5% typical HUS , 3.4% atypical HUS, 3.4% Pregnancy TMA, 3.4% SLE related TMA, 20.3% Transplant TMA , 1.7% Cancer associated TMA and 37% TMA of other causes. The average plasma exchange was 8.4 cycles, and was higher in patients with ADAMTS-13 activity of ≤10% (11.4 cycles) as compared to those with ADAMTS-13 >10% (7.7 cycles). No infectious diseases were transmitted as a result of plasma exchange or plasma infusion. Treatments used in the patients included immunosuppressant therapy like methylprednisolone (85.5%), monoclonal antibody like rituximab (36.2%), bortezomib (11.6%), cyclophosphamide (10.1%), cyclosporine (10.1%), and vincristine (26.1%). The survival outcome seemed to be worse among the transplant TMA in comparison to other groups (log-rank, p<0.0001). Transplantation was also associated with higher odd of death among TMA cases (OR: 14.8571, 95% CL: 1.7385, 126.9707). Those with confirmed TTP was inevitably doing better than the others in terms of overall survival (log-rank, p=0.0299). The odds of death was 4.36 times higher in patients with ADAMTS-13 activity >10% (OR: 4.36, 95% CL: 1.0961, 17.3714), indicating secondary TTP may have inferior treatment and disease outcomes than primary TTP like congenital or acquired TTP. Besides, the complications of the disease were also evaluated which revealed 26.9% of renal failure and 52.2% of neurological deficit. Furthermore, 8.7% were complicated by Venous Thromboembolism, either provoked or spontaneous. The odds of relapse is 2.9 times higher given the ADAMTS-13 activity ≤10% to ADAMTS-13 activity >10%.
Summary/Conclusions: This study illustrated that the standard treatment like plasma exchange and immunosuppressant therapy are only effective in genuine TTP whereas those masquerading TTP (TMA) would be more challenging to be tackled in terms of improving the outcome. The task to investigate other types of TMA prospectively will be highly desirable in the future.

PB2205

ANTIPHOSPHOLIPID ANTIBODY PROFILE AND ORGAN INVOLVEMENT IN CRITALLY ILL PATIENTS WITH AUTOIMMUNE DISEASES

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Background: Antiphospholipid antibodies (APA) are a group of proteins directed against the phospholipids of cell membranes, such as cardiolipins or phospholipid binding proteins. APA presence provokes microvascular, arterial or venous thrombotic events indicating somehow the relationship between the immune system, the hemostatic system, and the inflammatory response. It has been suggested that their presence in a critically ill patient is related to thrombotic manifestations, organ dysfunction, and death.
Aims: The aim of this study was to evaluate the prevalence of antiphospholipid antibodies in critically ill patients with autoimmune diseases and the rate of organ involvement.
Methods: Retrospective and descriptive study of patients admitted to the intensive care unit of Hospital Universidad de la Samaritana between 2008 and 2016, in Bogotá, Colombia.
Results: A total of 79 patients were found to have systemic lupus erythematosus (SLE), antiphospholipid syndrome and vasculitis. 17 patients (22%) were positive for antiphospholipid antibodies. Of these, 76% were women and mean age was 38 years (18-63 years). APA profiles showed positivity with this distribution: one positive antibody, n=9 patients (53%) (lupus anticoagulant antibody being the most common), two positive antibodies in n=4 patients (23%) and three positive antibodies in n=4 patients. Anemia (100%), monocytosis (64%), thrombocytopenia (40%) and prolonged INR (17%) were found in 88% of patients on admission to the ICU. In descending order, other organ involvement was found to be: pulmonary and renal dysfunction (70%), shock (53%), central nervous system involvement (41%), cardiovascular (23%), and gastrointestinal (22%). 82% of this cohort had positive anti-nuclear antibodies (ANA) and 23% anti-cytoplasmic antibodies (ANCA). 100% of patients had elevated C-reactive protein (CRP), and APACHE II score average was 11 points (Table 1).

Table 1.

n	Gender	Age (years)	Lupus anticoagulant	Anticardiolipin antibodies	Antiphospholipid antibodies	Anti - B2 microglobulin
1	Female	26	Not available	IgG	Not available	Not available
2	Female	25	Positive	IgM	No available	Not available
3	Female	63	Negative	Negativo	IgM	Not available
4	Female	19	Positive	Negativo	IgG	Not available
5	Male	63	Positive	Negative	Not available	Not available
6	Female	61	Positive	IgG	Not available	Negative
7	Male	62	Positive	Negativo	Not available	Negative
8	Female	30	Positive	Negative	Not available	Negative
9	Male	53	Positive	Negative	Not available	Not available
10	Male	53	Positive	IgM/IgG	Not available	Negative
11	Female	18	Positive	IgM	Negative	Negative
12	Female	29	Not available	Not available	Not available	IgG
13	Female	18	Negative	IgM	IgG	IgG
14	Female	20	Positive	IgG	IgG	Not available
15	Female	49	Negative	IgG	IgG	IgG
16	Female	22	Positive	Negative	Negative	Not available
17	Female	35	Positive	Negative	Negative	Negative

Demographic data and antiphospholipid antibody distribution.

Summary/Conclusions: Hematologic, renal and pulmonary involvement are the most commonly compromised in patients with antiphospholipid antibodies positivity in patients with autoimmune diseases in the ICU. Based on these results, a prospective study is proposed in order to evaluate the presence of APA and their impact on mortality and multi-organ dysfunction in these patients.

PB2206

PREVALENCE OF ANTIPHOSPHOLIP ANTIBODY AND HBA1C IN T2DM WITH DIABETIC VASCULAR COMPLICATIONS

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Background: Antiphospholipid antibodies (APLS) have been implicated in vascular (arterial, venous or both) thrombosis Diabetes Mellitus (DM), as a disease entity has been associated with hyper-coagulable and pro-thrombotic states, with studies showing an increased procoagulant state and thrombotic events especially in poorly controlled Type 2 Diabetes Mellitus (T_2DM).
Aims: The aim of the study is to assess the APLS and HbA1c levels and evaluate the correlation between APLS levels and HBA1c in T_2DM patients with diabetic vascular complications.
Methods: This was a cross-sectional study of subjects with T2DM attending the diabetic clinic of University of Nigeria Teaching Hospital. A total of two hundred and ten (210) subjects were recruited for this study. There were grouped into three (complicated T2DM, uncomplicated T2DM and health control. Each had 70 subjects matched for sex and age. Lupus anticoagulant (LA) was assayed using DRVVT (technoclone GmbH Austria) IgGβ2GPI-ACA was assayed using ELISA test kit (Genway Bio-tech San Diego USA), HbA1C was assayed using D10^{TM} haemoglobin analyzer. Ethical clearance was obtained from ethical committee UNTH.
Results: The prevalence of LA was 7.1%, 4.3% and 4.3% for complicated T2DM, uncomplicated and healthy control subjects respectively, while the prevalence of IgG-B2GPI ACA was 4.3% in all groups. The mean HbA1C were 8.2(1.5), 8.0 (1.7) , 5.6 (0.38) for complicated ,uncomplicated T2DM and control subjects respectively. ANOVA showed a significance difference in mean HbA1C among complicated uncomplicated T2DM and healthy controls. Post hoc analysis showed this difference was between complicated T2DM and healthy controls (p<0.001, 95%,CI-3.0 to -2.1) and in uncomplicated T2DM and healthy control subjects (p<0.001,95%,CL-2.8 to -2.0) there was a significant correlation between HbA1C and IgGβ2GPI-ACA for complicated T2DM(r=0.316),P=0.008) and uncomplicated T2DM (r=0.316),P<0.001)
Summary/Conclusions: The study did not find any causal or other association between T2DM and the occurrence of APLS positivity, however, APLS may be simply an aggravating factor for vascular complications especially in poor controlled T2DM

PB2207

VWF THR789ALA GENETIC VARIANTS CORRELATE WITH DISEASE PHENOTYPE IN EGYPTIAN PATIENTS WITH ACUTE CORONARY SYNDROME

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Background: von Willbrand factor antigen level (vWF: Ag) was shown to contribute to the risk of cardiovascular disease. vWF Thr789Ala single nucleotide polymorphism is thought to affect factor level and function.
Aims: This study aimed to investigate the impact of genetic variants at that position on the risk of acute coronary syndrome (ACS).
Methods: The study included 112 patients of ACS; 31 with unstable angina (UA) and 81 with myocardial infarction (MI) as well as 118 healthy controls. vWF: Ag level was measured by ELISA. The gene analysis was carried out by polymerase chain reaction using restriction fragment length polymorphism (RFLP-PCR) principles.
Results: vWF: Ag levels were significantly higher in MI (111.68±24.77 IU/dl) and UA (110.27±23.44 IU/ml) patients compared to healthy controls (71.13±13.72 IU/dl), p <0.001 for both groups, The majority of patients with UA (80.6%) were Ala789 homozygous, 6.5% were Thr789Ala heterozygous and 12.9% were Thr789 homozygous. Regarding the MI group, Ala789 genotype was present in 34.6%, Thr789Ala genotype was the predominant genotype and was seen in 48.1% of patients and Thr789 homozygous was present in 17.3% of patients. The genotype frequency in the control group was as follow; 47.4% were Ala789 homozygous, 33.1% were heterozygous and 19.5% were Thr789 homozygous. The genotype distribution was significantly different among the 3 groups, p<0.001, and between the groups with UA and MI, p <0.001. Ala789 homozygous genotype was an independent risk factor for UA while the Thr789Ala genotype was shown as an independent risk factor of MI.
Summary/Conclusions: vWF Thr789Ala genotype is independent risk factor for UA and has significant impact on the type of myocardial ischemia. It should be incorporated in a risk assessment model to identify individual patient risk and guide the management plan.

PB2208

THE INFLUENCE OF FIBRINOGENASE ISOLATED FROM THE ANTARCTIC SCALLOP ON BLOOD COAGULATION

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Background: At the present time, cardiovascular diseases such as acute myocardial infarction, ischemic heart diseases, and stroke are the most important causes of the human mortality around the world. Thrombosis is probably the most common symptom among cardiovascular diseases. Thrombolytic agents have been extensively used in the therapeutic treatment of thrombosis. But most of them have some serious shortcomings, including limited efficacy, short plasma half-life, large therapeutic dose or allergic response. Considering the global burden, the search continues for a safe and cheap thrombolytic agent to treat cardiovascular diseases. To date, many investigators have been trying to improve the safety and efficacy of thrombolytic therapy. Fibrinogenolytic enzymes or fibrinogenases because of their role in dissolving of blood clots as well as prevention their formation have attracted special medical and scientific attention. Enzymes that affect hemostasis have been isolated from different sources. In recent years, special attention is paid to the hydrobionts from the Antarctic region which are poorly explored and potentially can be a valuable source of new bioactive compounds, in particular enzymes.
Aims: The main goal of current research was to test the effect of fibrinogenase from marine hydrobiont the Antarctic scallop *Adamussium colbecki* on platelet aggregation and blood coagulation.
Methods: Fibrinogenase from the crude tissue extract of *A. colbecki* was isolated by three-step procedure (affinity chromatography on SBTI-sepharose following affinity chromatography on Blue-Sepharose and size exclusion chromatography on Superdex 75-PG). Platelet aggregation was determined by AT-02 aggregometer (Medtech, RF). The platelet count was adapted to $2.5x10^5$ platelets/μL with platelet-poor plasma. Then, fibrinogenase (12.5 μg/mL or 6.25 μg/mL) was added 2 min before the addition of the platelet aggregation inducer ($5x10^{-6}$ M ADP). The changes in light transmittance were continuously monitored during 8 min, and the percentage of aggregation, in the presence of the samples, was calculated comparing the transmittance against the controls. Activated partial thromboplastin time (APTT) and prothrombin time (PT) were measured in CLOTimer analyzer.
Results: According to our result investigated enzyme inhibited ADP-induced platelet aggregation and the inhibition increased with increasing concentration of the enzyme. Data analysis revealed a significant (p<0.05) inhibition of ADP-induced platelet aggregation by 80.5% for 12.5 μg/mL of fibrinogenase and by 31.8% for 6.25 μg/mL of fibrinogenase. Isolated fibrinogenase may inhibit platelet aggregation by hydrolyzing the Aα-chain of fibrinogen to prevent fibrinogen from combining with fibrinogen receptor on platelet membrane. In addition, fibrinogen degradation products may act as competitive inhibitors of platelet–fibrinogen bridging formation. We also investigated the effect of fibrinogenase on blood coagulation by determination of APTT and PT. According to our result APTT increased in 2.25 and 1.25 times at the concentration of fibrinogenase of 12.5 μg/mL and 6.25 μg/mL, respectively. The fibrinogenase also prolonged PT from 18 s to 68 s when the concentration of enzyme was 6.25 μg/mL.
Summary/Conclusions: Further investigations of fibrinogenase from A. colbecki are interesting and would probably help to develop new therapeutic agents to treat thrombotic disorders.

PB2209

IMPORTANCE OF MONITORING PATIENTS WITH DIRECT ORAL ANTICOAGULANTS

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Background: A major advantage of these agents is the lack of a requirement for monitoring, due to less variability in drug effect for a given dose, however it´s recommended monitoring the drug for Rivaroxaban Apixaban and Endoxaban use anti-Xa chromogenic studies and for Dabigatran Hemoclot thrombin inhibitor and Ecarin clotting time (DTI test).
Aims: Determine the effectiveness of laboratory tests to monitor patients treated with direct oral anticoagulants.
Methods: We conducted a retrospective study with 227 patients who received direct oral anticoagulants (DOACs) between January 2015 and December 2016. One hundred eighteen patients (52%) receive Rivaroxaban, fifty patients (22%) receive Dabigatran and fifty nine patients receive Apixaban (26%). We analyzed the variables that's increases the bleeding risk such as age, weight, prothrombin time (PT) and activated partial thromboplastin time (aPTT), therapeutic range of the drug, and measurement of serum creatinine.
Results: We found 10% of toxicity with Dabigatran, a 7% with Rivaroxaban and a 3% with Apixaban. Thirty-five patients (15%) developed bleeding of which 11% patients had a minor bleeding and a 4% of patients had a mayor bleeding, we also found that 6% of patients with Dabigatran, 2.5% with Rixaroxaban and 1.5% with Apixaban developed thrombotic episodes. Twenty percent of patient didn't have therapeutic range of the drug. For each DOACs is shown in Table 1. When we analyzed the patients who had hemorrhage we found that all patients with Dabigatran prolonged aPTT and the PT in 80%, for other DOACs is shown in Table1. A retrospective case-matched analysis was performed comparing 35 patients who developed bleeding with an equal number of patients who did, case and control groups were matched according to age, weight and measurement of serum creatinine we didn´t found significant difference.
Summary/Conclusions: In our series, in patients with dabigatran and who suffered bleeding, we found a significant prolongation of aTTP and PT, demonstrating the importance of laboratory tests prior to the administration of these agents and in emergency situations, for these reason should be include PT

and aPTT, therapeutic level of the drug and creatinine measurement, within the emergency and control laboratory tests in patients that receive DOACs.

Table 1.

Variable	Apixaban	Rivaroxaban	Dabigatrán
percentage	25.9%	51.9%	22%
toxicity	3.3%	6.7%	10%
thrombotic episodes	1.6%	2.5%	6%
percentage out of therapeutic range	8.4%	24.5%	22%
prolonged aPTT	8.4%	2.5%	80%
prolonged PT	16.9%	21%	4%
Bleeding	(35) 15.4%		
percentage	34.2%	20.8%	7.8%
prolonged aPTT	8.3%	22.2%	100%
prolonged PT	25%	33.3%	80%
median therapeutic range	177	142	154
median serum creatinine	1mg/dl		
median age	81		
median weight	71		

PB2210

HEREDITARY RISK FACTORS OF VENOUS THROMBOEMBOLISM IN YOUNG WOMEN TAKING ESTROGEN DRUGS

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Background: Estrogens are recognized as the most common risk factor of venous thromboembolism (VTE) in young women. The cumulative risk of VTE in patients taking estrogens is significantly increased in carriers of inherited thrombophilia. However, the known hereditary risk factors – mutations FV Leiden and FII G20210A could be detected in only 20-30% of patients with VTE.
Aims: To identify the most common hereditary risk factors of VTE in young women taking estrogen drugs.
Methods: We examined 133 young women with acute VTE (mean age 37.4 years; 16-45), who were genotyped by PCR-RFLP method for DNA polymorphism in 9 genes: FI-A Thr312Ala, FI-B -455 G/A, FII 20210 G/A, FV 1691 G/A, FXII 46 C/T, FXIII-A Val34Leu, PAI-1 -675 4G/5G, EPCR Ser219Gly, TPA 311bp Ins/Del. We compared the distribution of studied genotypes in three groups of patients with VTE: taking estrogens (n=30, group 1), with idiopathic VTE (n=42, group 2) or having other risk factors (n=61, group 3). Intergroup differences in genotype frequencies were assessed by Fisher's exact method. Odds ratios (OR), their 95% confidence intervals (CI) and p-values were calculated with SPSS software version 17.0 (SPSS Inc, Chicago, IL, USA).
Results: The frequencies of prothrombotic genotypes in groups 1, 2 and 3, respectively, were: FV 1691GA – 20.0%, 21.4% and 13.1%; FII 20210GA – 10.0%, 9.8% and 7.1%; FI-B –455AA – 10.0%, 2.4% and 1.6%; FI-A 312Ala/Ala – 13.3%, 14.3% and 13.1%; TPA 311bp Ins/Ins – 16.7%, 28.6% and 31.1%; PAI-1 -675 4G/4G – 36.7%, 42.9% and 27.9%; EPCR 219Ser/Gly – 16.7%, 19.0% and 23.0%; EPCR 219Gly/Gly – 3.3%, 7.1% and 0.0%; FXII 46TT – 13.3%, 0.0% and 9.8%; FXIII-A 34 Leu/Leu – 3.3%, 21.4% and 9.8%. Significant differences between the groups have been detected only for the FXIII-A 34Leu/Leu variant, which was more frequently found in patients with idiopathic VTE than in the group with estrogens (OR=6.5; 95% CI: 1.2-63.4; p=0.012) and women having other risk factors (OR=2.2; 95% CI: 0.8-7.6; p=0.05). The frequency of FI-B –455AA genotype in young women with DVT developed after taking estrogen drugs was 4- and 6-fold higher, respectively, when compared to the group with idiopathic VTE and patients having other risk factors (10.0% *vs* 2.4%; OR=4.1, 95% CI: 0.02-2.2, p=0.16 and 10.0% *vs* 1.6%; OR=6.6, 95% CI: 0.7-67.0, p=0.067).
Summary/Conclusions: FV Leiden and FII 20210G/A mutations as well as the FI-B -455AA genotype are frequently seen in young women with DVT developed after taking estrogen drugs. Further studies are needed to clarify genetic risk factors contributing to VTE development in this group.

PB2211

Abstract withdrawn.

PB2212

Abstract withdrawn.

PB2213

KNOWLEDGE AND ATTITUDE OF MEDICAL DOCTORS ON ANTICOAGULATION THERAPY IN TERTIARY HOSPITALS IN NIGERIA

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Background: Thromboembolic and hypercoagulable diseases are common life-threatening but treatable problems in hospital practice. The most effective and economical approach to decreasing the burden of VTE is to prevent the development of DVT and PE in patients especially in acutely ill hospitalized medical patients. Health care providers in Nigeria may have significant gaps in their anticoagulation knowledge that could affect their decision to prescribe anticoagulation therapy as there are no national guidelines on the use of anticoagulation in Nigeria.
Aims: The purpose of this present study was to examine the knowledge and attitude of medical doctors on anticoagulation in tertiary hospitals in Nigeria.
Methods: The present study is a multicentre survey of the use of anticoagulants among clinicians in South East Nigeria. A pretested questionnaire was administered to clinicians in six tertiary hospitals in the south-east of Nigeria. The following institutions participated in the survey: University of Nigeria Teaching Hospital Enugu, Federal Medical Centre, Abakaliki, Federal Medical Centre Umuahia, Abia State Teaching Hospital, Aba, Amaku Specialist Hospital, Awka and Nnamdi Azikiwe Teaching Hospital, Nnewi. The Likert scale which is in grades from one to five: 1 strongly disagree, 2 disagree,3 neutral,4agree,5 strongly disagree was used. To determine the agreement degree three levels were identified (high medium and low).
Results: There were 528 respondents. 378 of them were males (71.6%) and 150 were females (28.4%). 31.1% of the respondents, were junior residents and the consultants represented only 20.6% of the respondents. Most of the respondents,189 (35.8%) had less than 5 years clinical experience while the least of the respondents (8.7%) had between 16-20 years clinical experience We observed that most respondents irrespective of their job grades didn't know about Fondaparinux and the DOAC (except those in the specialist - registrar job grades) as the overall p=(0.000),<0.05 and were significant. We also observed that responses were divided on malignancy as an indication for anticoagulation. The overall P=0.002, <0.05 and was significant. The p valve for other indications for anticoagulation >0.05 and was not significant. The majority knew of prothrombin test and p valve was 0.03, less than alpha value of 0.05 and was significant. On the contrary, Majority does not know about anti-Xa assay, p-value=0.02, <0.05, was also significant. Their affirmative response on the mode of action as one of the differences showed a p=0.000, <0.05, was significant. On the contrary, the non-affirmative response to drug and food interaction, p=0.03, was also significant. Based on results of the statement analysis, the variables were ranked according to the value of their mean. All except one variable had p-values of <0.05. The statement "Do you think anticoagulation therapy/prophylaxis is clinically important" had the highest mean of 4.60 and had a high degree of agreement. The statement "Should hospital inpatient with >3 days admission routinely receive anticoagulation?" had the lowest mean of 2.27 with a p-value of 0.015 had a low degree of agreement.
Summary/Conclusions: There is a need to upscale knowledge attitude and practice of the use anticoagulation agents especially the NOACs through well-articulated CME educational activities. A limitation of this study is the relatively small number of study participants and some subspecialties that were not reflected in this survey.

PB2214

INTERLEUKIN -10 GENE POLYMORPHISMS AND THE RISK OF UNPROVOKED DVT IN EGYPTIAN PATIENTS

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Background: Thrombosis is often multifactorial, caused by both genetic and acquired risk factors. The inflammatory process is linked to pathogenesis of venous thrombosis. Venous thrombosis is considered to be mediated by an imbalance in proinflammatory as compared with anti-inflammatory molecules. One of the important anti-inflammatory cytokine is interleukin-10 (IL-10) with important immunoregulatory functions. Primarily, IL-10 counterbalances the potentially harmful effects of tumor necrosis factor α (TNFα) and other pro-inflammatory mediator such as IL-1b, IL-6, and IL-8 from monocytes/macrophages. Three important single nucleotide polymorphisms (SNP) affect IL-10 expression, including: 1082 A/G, 819 C/T, and 592 C/A. Studying the association between genetic polymorphisms of anti-inflammatory cytokines such as IL-10, and venous thrombosis may suggest using of polymorphisms as a predictive genetic marker of future VTE.
Aims: The objective of this study was to evaluate a possible association between IL-10 -1082A/G, and -592C/A polymorphisms with DVT.
Methods: The study was conducted on 115 patients with symptomatic DVT proved by venous duplex ultrasound; divided into two cohorts: group A included

60 patients with unprovoked DVT, and group B included 55 patients with provoked DVT. Gene mutations for IL-10 -1082A/G, and -592C/A were performed using PCR-restriction fragment length polymorphism assay. We studied the association between IL-10 gene polymorphisms and occurrence of either provoked or non-provoked DVT. We also investigated the link between these polymorphisms and the recurrence of DVT and family history of DVT.
Results: in our study IL101082AG gene analysis revealed that mutant genotypes distribution is statistically significant different compared to the wild genotype distribution, being higher in group A (with unprovoked DVT) than in group B (with provoked DVT); as GG genotype was detected in 14 patients (63.6%) *versus* 8 patients (36.4%) in group A and B respectively (P value=0.037); AG genotype was detected in 30 patients (63.8%) compared to 17 patients (36.2%) in group A and B respectively (P value=0.007). However, there is no correlation was found between IL101082 mutant genotypes distribution and VTE recurrence (P value= 0.738 and 1 respectively) or positive family history of VTE (P value= 0.101 and 0.714 respectively), compared to wild genotype. IL10592AC gene analysis showed that mutant genotypes (GG and AG) distribution showed no statistically significant difference (p value= 0.43 and 0.687 for GG and AG genotypes respectively) compared to wild genotypes distribution, also there is no correlation was found between IL10592AC mutant genotypes distributions and VTE recurrence (P value= 1 and 0.284 for GG and AG genotypes respectively) or positive family history of VTE (P value= 0.67 and 1 for GG and AG genotypes respectively), compared to wild genotype (AA).
Summary/Conclusions: I IL101082AG gene polymorphism is associated with risk of unprovoked DVT, however it is not associated with either risk of recurrence or positive family history.

PB2215

CATASTROPHIC ANTI-PHOSPHOLIPID SYNDROME TRIGGERED BY SEPSIS. A PROSPECTIVE CASE STUDY HIGHLIGHTING BIOLOGICAL CONCEPTS AND MANAGEMENT STRATEGIES IN THIS COMPLEX AND LIFE THREATENING DISEASE
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Background: Catastrophic antiphospholipid syndrome (CAPS) is a rare and life threatening event characterized by widespread intravascular thrombosis and multi-organ failure. Rarely is CAPS associated with bleeding diathesis, but has been reported in cases with severe thrombocytopenia and acquired prothrombin inhibitors. APS auto-antibodies are heterogeneous and may undergo post-translational modification during antigen stimulation altering its pathogenicity and thrombotic risk. Sepsis and associated disseminated intravascular coagulation is a known phenomenon where cytokines influence pro-coagulant and anti-coagulant pathways on multiple levels to induce haemostatic chaos.
Aims: Demonstrate the role of sepsis in triggering life threatening CAPS, and highlight the management strategies used in this highly complex and fatal disease.
Methods: Prospective case study illustrating two separate atypical CAPS presentations and the management strategies employed. *1st episode (2015):* 54F with long standing 27 years of triple positive APS, pro-thrombotic history with recurrent thrombosis despite optimal anticoagulation. Her pro-thrombotic equilibrium was altered after a respiratory tract infection where she presented with severe headaches. Subsequent investigations demonstrated multiple atraumatic intra-cranial haemorrhages followed by concurrent extensive cerebral venous thrombosis. *2nd episode (2017):* She presented with subdural haemorrhage, preceded by fevers and respiratory symptoms. She then developed pleuritic chest pain and dyspnoea after temporary cessation of anticoagulation for 24 hours. Imaging confirmed multiple pulmonary emboli with areas of infarction. Respiratory symptoms worsened with progressive interstitial ground glass changes on CT consistent with atypical pulmonary infection. Shortly after low therapeutic anti-coagulation she developed acute abdominal pain and hypotension. CT confirmed significant bilateral adrenal haemorrhages. *Management Strategies:* (A) Rapid reduction in APS pathogenic auto-antibodies via plasma exchange, B cell depletion therapy and immune modulation. (B) Treatment of underlying infectious trigger. (C) Judicious anticoagulation with anti-Xa monitoring and (D) long term hydroxychloroquine and statin therapy.
Results: The two life threatening presentations of CAPS were triggered by an infectious event, supporting the biological concept that anti-phospholipid antibodies can be immune modulated altering his pathogenic capabilities creating haemostatic havoc. There are similarities and a degree of overlap with sepsis and the pathophysiology behind disseminated intravascular coagulopathy. Rapid reduction in the pathogenic auto-antibodies using combination plasma exchange, immune modulation and B cell depletion therapy is effective in this acute setting. Judicious anticoagulation and treatment of the precipitating infection is important in turning off the immune response driving this life-threatening coagulopathy.
Summary/Conclusions: CAPS is rare and life threatening, often triggered by an infectious event, trauma or temporary cessation of anticoagulation. It requires prompt recognition and timely commencement of therapy.

PB2216

HAEMATOLOGICAL CORRELATES OF ISCHEMIC STROKE AND TRANSIENT ISCHEMIC ATTACK : LESSONS LEARNED
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Background: Haematological abnormalities are known to cause Ischemic Stroke or Transient Ischemic Attack (TIA). The identification of haematological correlates plays an important role in management and secondary prevention
Aims: The objective of this study was to describe haematological correlates of stroke and their association between stroke profile. The haematological correlates screened were Lupus Anticoagulant, Dysfibroginemia, Paroxysmal nocturnal haemoglobinurea (PNH), Sickle cell disease, Systemic Lupus Erythematosis (SLE) and Myeloploriferative Neoplasms (MPN).
Methods: A cross sectional descriptive study was conducted in a sample of 152 stroke patients referred to haematology department of National Hospital of Sri Lanka for thrombophilia screening. Following tests were performed to assess each hematological correlates (Table 1).

Table 1.

Hematological correlate	Tests performed
Lupus anticoagulant	Diluted Russels Viper Venom Test and Kaolin clotting time
Sickle cell disease	Full blood count (FBC), blood picture and sickling test and High Performance Liquid Chromatography
Paroxysmal nocturnal haemoglobinurea	FBC, blood picture, Ham test and flowcytometry
Myeloploriferative neoplasms	FBC , blood picture, Janus Kinase 2 (V617F) mutation analysis, erythropoietin level and bone marrow examination
Dysfibrinogenaemia	TT, fibrinogen antigen test, clot observation and clauss test
Systemic lupus erythematosis	Anti nuclear antibodies

Results: Among study sample, 134 patients had strokes and only 18 had TIA. The recurrence of stroke/TIA was observed in 13.2% of patients. The majority of patients (94.7%) have had radiological evidence of thrombotic event. One fourth of patients had past thrombotic events while 12.5% had family history of thrombosis. Out of haematological correlates screened Lupus anticoagulant was the most common haematological correlate (n=16) and dysfibrigonaemia(n=11) had the next high prevalence. One patient was diagnosed with Essential thrombocythaemia and one with SLE. None of the patients were positive for screening tests done for sickle cell disease and PNH.
Summary/Conclusions: The Haematological correlates were identified in 19% of our study sample. Among stroke profile only presence of past thrombotic history was statistically significantly associated with haematological disorders (P= 0.04). Therefore hematological disorders appear to be an important factor in etiological work up of stroke patients particularly in patients with past thrombotic events.

PB2217

ANTIPLATELET AND FIBRINOLYTIC EFFECTS OF ARGININE-CONTAINING PEPTIDES IN HEALTHY RATS AND RATS WITH METABOLIC SYNDROME
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Background: Currently, the number of diabetes, hypercholesterolemia, metabolic syndrome (MS) patients has increased sharply in the world. MS is metabolic disorders with increase of cholesterol and glucose levels, dyslipemia, endothelial dysfunction. This is accompanied by an increase in blood clotting, including platelet aggregation strengthening and reducing the activity of the plasminogen activator. Thus, the MS may predispose to venous thrombosis. It is known that, regulatory oligopeptides involved in the conservation normal functional activity of coagulation, anticoagulation, insular systems of the organism, fat metabolism. It is also known that some amino acids, particularly arginine, improve rheological properties of blood and reduce platelet aggregation.
Aims: To study the effect of tripeptides Pro-Arg-Gly and Gly-Arg-Pro, containing arginine in the molecule, on platelet aggregation and tissue plasminogen activator (t-PA) activity in a healthy organism and the development of the experimental MS.
Methods: Experiments were carried out on Wistar rats weighing 300-350 g in accordance with the ethical principles of the Helsinki Declaration. Two groups of animals were used: healthy rats and rats with experimental MS. Peptides were intranasal injected in doses of 1 mg / kg once daily for 5 days. 0.85% NaCl solution was injected to control rats in the same time frame. MS in rats was caused by a hyper-cholesterol fat-rich diet (FD) for 6 weeks. Blood samples were taken from the jugular vein 1 hour after the last drug administration. Activity of t-PA (fibrin plate method) and ADP-induced platelet aggregation (standard method) were measured in blood plasma.
Results: The intranasal administration of peptides Gly-Arg-Pro, and Pro-Arg-Gly to healthy animals resulted a reduction of platelet aggregation by 23% and 52% compared with the control. Both peptides induced enhancement t-PA activity of 2 or 3.5 times respectively. In rats with experimental MS these effects were preserved, besides, platelet aggregation was decreased by 27% (Pro-Arg-Gly) and 38% (Gly-Arg-Pro) compared with the control.

Summary/Conclusions: We concluded that intranasal administration of tripeptides Pro-Arg-Gly and Gly-Arg-Pro to organism of healthy rats and in rats with experimental MS show antiplatelet and fibrinolytic effects of the blood. Thus, arginine-containing peptides could potentially be used as antithrombotic drugs that protect the organism from the blood coagulation and thrombus formation.

PB2218

THE PRINCIPAL COMPONENT ANALYSIS USING CALIBRATED AUTOMATED THROMBOGRAM PARAMETERS AS A POTENTIAL QUALITY CONTROL FOR MEASURING PROCOAGULANT ACTIVITIES OF IMMUNOGLOBULINS

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Background: The calibrated automated thrombogram (CAT) is a method to monitor the generation of thrombin. It can be described by four variables; lag time, peak thrombin, time to peak, and velocity index. Currently, due to thromboembolic event related risks of immunoglobulins, the CAT is widely used to quantify the thrombogenic potential associated with immunoglobulin manufacturing processes and products. However, there is currently no officially approved method for such assessments and even this results are highly variable in inter-laboratories comparison. In this study, to obtain a summary score, we applied the principal component analysis (PCA) for these four outcomes measured from CAT method. The PCA is a statistical procedure concerned with elucidating the covariance structure of a set of variables. In particular it allows us to identify the principal directions in which the data varies.
Aims: In this study, our interest is to apply PCA method in order to find appropriate dose related with CAT variables and to reduce variation of procoagulant values in Immunoglobulin products.
Methods: The CAT are measured in a 96 well plate fluorometer equipped with a 390/460 filter set and a dispenser. Usually experiments are carried out in triplicate. During the measurement, a dedicated software program, Thrombinoscope compares the readings from the trigger wells and the calibrater wells, calculates thrombin concentration and displays the thrombin concentration in time. Outcomes from CAT were analyzed in the principal component anaysis (PCA) which is a statistical procedure that allows us to summarize high dimensional data with a smaller number of representative variables that collectively explain most of the variability. Statistical analyses were performed with R 2.5.
Results: Four variables measured from CAT have different sistribution and too large variations. For example, the mean(sd) of each variable (lag time, peak thrombin, time to peak, and velocity index) are 24.66(8.01), 80.16(94.52), 31.28(9.78), 19.08(28.86), respectively. Therefore, to remedy such high variability among variables and to find a score, PCA method is applied. Then the dose values calculated based on the PCA scores have mean 0.393 and a much smaller variation (sd=0.583) (Table 1).

Table 1.

CAT Parameters Range of commercial IVIG products. (show in mean±2SD)

Sample	n	Lag Time(min)	Peak Height(nM)	Time to Peak(min)	VelIndex(nM/min)
A	3	27.70±6.32	25.68±22.04	35.04±6.50	3.53±3.14
B	3	28.07±9.12	24.78±25.12	35.33±10.02	3.51±3.82
C	3	26.30±7.66	35.27±32.92	33.15±8.06	5.22±5.12
D	3	25.07±6.42	40.81±34.90	31.59±6.66	6.31±5.62
E	3	23.82±5.46	33.00±25.68	30.89±5.90	4.72±4.04
F	3	23.45±0.22	44.60±43.34	30.53±1.22	6.48±6.80
G	3	25.86±2.18	26.08±29.90	33.98±4.08	3.37±4.32
H	3	26.22±2.12	27.29±2.16	32.77±2.30	4.20±0.40
I	3	26.11±2.00	25.74±3.30	32.48±1.78	4.07±0.78
J	3	23.45±1.02	47.91±8.00	29.18±0.70	8.39±1.74
K	3	26.44±1.94	24.23±2.38	32.78±1.56	3.84±0.68
L	3	26.11±3.42	24.45±3.38	32.37±3.16	3.91±0.72

The dose levels estimated based on the PCA scores.

Sample	n	dose (mU/mL)
A	3	0.73±0.12
B	3	0.63±0.24
C	3	1.17±0.30
D	3	1.43±0.12
E	3	1.30±0.60
F	3	1.53±0.46
G	3	0.50±0.20
H	3	1.03±0.46
I	3	0.97±0.42
J	3	2.43±0.64
K	3	0.87±0.21
L	3	0.90±0.26

Summary/Conclusions: The PCA value showed a good agreement with four CAT outcomes and less variation. The PCA method could be used to monitor the process of immunoglobulin manufacturing.

PB2219

PRIMARY THROMBOPHILIA IN MÉXICO XII: MISCARRIAGES ARE MORE FREQUENT IN PERSONS WITH THE STICKY PLATELET SYNDROME

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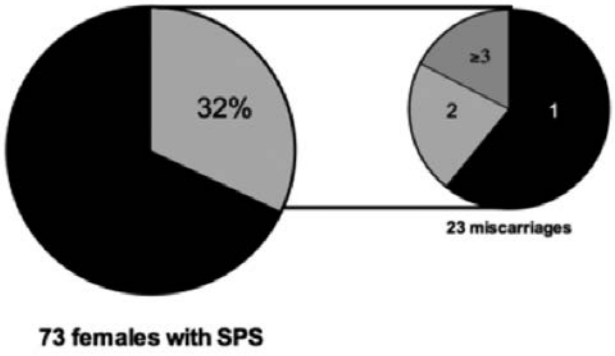
Background: The sticky platelet syndrome (SPS) is an inherited condition which leads into arterial and venous thrombosis. There is scant information about the association between the SPS and obstetric complications.
Aims: To assess the relationship of the SPS and fetal loss in a single institution.
Methods: The obstetric history of all the consecutive female patients prospectively studied along a 324 month period, in a single institution with a history of thrombosis and a clinical marker of primary thrombophilia was reviewed.
Results: Between 1989 and 2016, 268 consecutive patients with a clinical marker of primary thrombophilia and a history of arterial or venous thrombosis were studied; of these, 108 were female patients. Within this subset of thrombophilic female persons, 77 (71%) had been pregnant at some moment. Twenty eight of these 77 patients (37%) had had a spontaneous abortion and 24 out of these (86%) were found to have the SPS. On the other hand, in a subset of 73 female patients with the SPS who had been pregnant, 32% had miscarriages. These figures are significantly higher than the prevalence of abortions in the Mexican general population of pregnant women, which is 12-13% (*chi square*=7.47; p=0.0063). Accordingly, the relative risk of having a miscarriage is 2.66 times higher in female patients with the SPS than in the general population (p=0.0014) (Figure 1).



Twenty-three of 73 (32%) female patients with the sticky platelet syndrome (SPS) had an abortion at some time. 14 patients had one abortion, 5 had two and 4 had three or more.

Figure 1.

Summary/Conclusions: In México, female patients with the SPS experience significantly more spontaneous abortions than the general population. Since the treatment of the SPS is simple and effective and could in turn prevent adverse obstetric outcomes, its investigation in women studied because obstetric complications may be useful and deserves further research.

PB2220

CROSS-SECTIONAL ANALYSIS OF VENOUS THROMBOEMBOLISM IN YOUNG INDIAN MALES; NEW INSIGHTS INTO AN OLD PROBLEM

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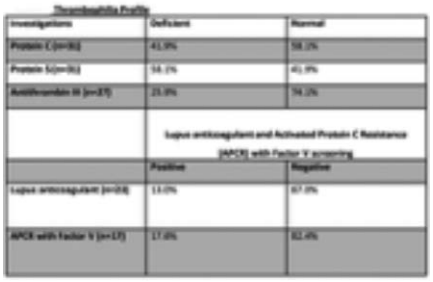
Background: Venous thromboembolism (VTE) comprising of deep vein thrombosis (DVT) and pulmonary embolism (PE) is one of the major cardiovascular causes of death along with MI and stroke. Though earlier works has suggested that DVT is rarer in Asian population, recent studies have revealed that this might not be so. Most of the studies conducted in Asia in general and India specifically has been on hospitalized patients with minimal representation of young healthy individuals.
Aims: We aimed at studying the disease variables of VTE in young healthy males of Indian origin and compare the same with other Indian studies as well as the global statistics.
Methods: Hospital records of 176 Color Doppler Flow Index (CDFI) and /or Contrast Enhanced Computed Tomography (CECT) proven VTE patients being followed up in a tertiary care hospital was analyzed retrospectively to document cause (provoked/ unprovoked), venous systems involved, thrombophilia profile, duration of anti-coagulation and recurrence.
Results: Among the study population, 49.8% had a provoked VTE. 90.9% subjects had DVT, mostly of the lower limb, 15.3% had PE with DVT, 2.8% had PE alone and 6.2% had splanchninc vein thrombosis including portal vein thrombosis. In the subjects who had undergone thrombophilia profile, 41.9% had Protein C, 58.1% Protein S and 25.9% Antithrombin III deficiency. Lupus anticoagulant screen was positive for 13% of the screened subjects. The average duration of anti-coagulation was 18 months with majority (98.2%) patients on Vitamin K antagonist. The recurrence rate in our study population was found to be 11.4% (Table 1).
Summary/Conclusions: Young Indian males have different disease variables

for VTE as compared to western population. The exact pathophysiology of such differences needs to be studied further to formulate strategies for effective screening and prevention.

Table 1.



PB2221

A PRELIMINARY STUDY ON THE EFFECTS OF AMPHIBIAN CRUDE SKIN SECRETIONS ON SOME PARAMETERS OF HEMOSTATIC SYSTEM

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Background: A lot of bio-chemical compounds from secretion of the amphibian skin glands with various biological activities have been isolated and characterized. Several recent studies indicate that amphibian skin secretions can be a source of molecules affecting the platelet activity. We are interested to look for other bioactive components of the amphibian skin which exhibit ability to influence on diverse parameters of hemostatic system.
Aims: We performed a preliminary study of the some effects of amphibian crude skin secretions on hemostasis.
Methods: Adult specimens (both sexes) of *Bombina bombina, Bombina variegata, Bufo bufo*, and *Bufotes viridis* were collected from outdoors in Kyiv region of Ukraine. The crude skin secretions were collected by washing with ultrapure water and centrifuged to remove debris. The supernatants were lyophilized and kept at −20 °C till use. In the experiments we used fresh prepared water solution of lyophilized skin secretions. Protein concentration was determined by Bradford method with BSA as a standard. Rabbit platelet-rich plasma (PRP, $2x10^5$ cells/μL) and platelet-poor plasma were obtained following standard protocols. Platelet fraction (PF) was purified by gel-filtration on Sephadex G 50 column. Platelet aggregation was measured by aggregometer AT-02 (Medtech, Russia). Coagulation parameters (prothrombin time (PT), thrombin time (TT), as well as activated partial thromboplastin time (APTT)) were measured by coagulometer (Rayto, RT-2201C) using corresponding commercial kits (Renam, Russia).
Results: The lyophilized *B. bufo* skin secretions in dose-dependent manner induced platelet aggregation in both PRP and purified PF; its final concentration of 50 mg of total protein/mL caused the same effect as $5x10^{-6}$M ADP. These results indicated that skin components acted directly on platelets, maybe through their surface receptors. The lyophilized skin secretions of *B. variegata* and *B. viridis* also activated platelet aggregation but their effects were lower than *B. bufo* skin secretions. The skin secretions from all studied amphibian did not influence on PT and TT except *B. viridis* which prolonged TT by 40%. The values of APTT were significantly enhanced in 3.4 and 2.3 times under the influence of crude skin secretions (final concentration of 0.2 mg total protein/mL plasma) of *B. bombina* and *B. variegata*, respectively.
Summary/Conclusions: The obtained results indicate the prospects of the search for potential modulators of hemostatic system among the amphibian skin bioactive compounds. To establish their physiological and functional mechanisms of action, the further purification and characterization of components from the skin gland secretions are necessary.

PB2222

PLASMINOGEN-DEFICIENT PATIENTS

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Background: Congenital plasminogen (Plg) deficiency is a rare autosomal recessive disorder that leads to the development of ligneous membranes on mucosal surfaces.
Aims: Here we report our experience with local and intravenous fresh frozen plasma (FFP).
Methods: Our cohort consisted of 14 patients and their 8 first-degree relatives. The patients have been diagnosed between 3 months and 18 years of age, and the median age at the time of first clinical manifestation was 4.5 months (range 3 days to 12 months).
Results: Conjunctivitis is the main complaint, hydrocephalus and hearing loss follow. In 10 patients, ligneous membranes were surgically removed but all recurred. Nine patients were treated with intravenous and conjunctival FFP. Two patients had no complaints after treatment. Most patients needed transfusion with FFP every three weeks. Only one patient had severe endophthalmitis and lost vision in one eye before treatment. Two female patients and one male patient had undergone multiple surgeries for ligneous conjunctivitis despite being treated with FFP. The response rate to FFP treatment was 6/9 (66%). Another 8-year-old female with severe bronchial membranes was treated with FFP and t-PA through bronchoscopy. Venous thrombosis did not occur in any of the patients. Nine have consanguineous parents. The genetic evaluation of our patients revealed heterogeneous mutations as well as polymorphisms.
Summary/Conclusions: The diagnosis and treatment of Plg deficiency is challenging, and there is no consensus on treatment. Topical and iv FFP may be used with clinical outcome.

PB2223

THE TREATMENT OF HEREDITARY TROMBOPHILIA DURING PREGNANCY

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Background: Thrombophilias are genetic conditions that increase the risk of thromboembolic disease. The use of anticoagulant therapy during pregnancy is challenging because of the potential for both fetal and maternal complications. The most common complication is venous thromboembolism.
Aims: This study is conducted in order to assess the importance of treatment during pregnancy for women with hereditary thrombophilia, the risks of not treating the disease or treating incorrectly.
Methods: This study includes a total of 207 women, from which 83% were treated with low molecular weight heparin and Aspirin during pregnancy regardless if it was their first pregnancy or not and the rest 17% remained untreated during pregnancy. The success of the treatment is based on the completion of the pregnancy and the good health of the fetus.
Results: A total of 207 women were included into the study, 172 were treated with low molecular weight heparin and Aspirin while 35 were treated with just Aspirin. Out of 172 patients in the low molecular weight heparin group 155 managed to give birth which accounts for a 90% success rate with a reported case of fetal growth restriction and 2 cases of abruption while the remaining 17 women which represent the 10% of the treated patients were unsuccessful in completing their pregnancy with 14 women presenting pregnancy loss on the first trimester and 2 having late fetal loss, only one case of preeclampsia was recorded. Out of the 35 women who did not receive treatment with low molecular weight heparin and only with Aspirin, 21 managed to complete their pregnancies representing the 60% out of which 2 cases presented with Abruption and 4 cases with fetal growth restriction, out of the 14 women who represent the 40% who were unsuccessful in completing their pregnancies 7 cases were recorded during the first trimester while 3 more had late fetal loss and 4 cases of preeclampsia.
Summary/Conclusions: Women treated for thrombophilia had a lower percentage of fetal loss than their no treatment group counterparts. There is an urgent need for appropriate guidelines for these patients in our medical center.

PB2224

LEARNING ABOUT VALIDATIONS OF THE DVT SCREENING TEST IN PATIENTS WITH SUSPECTED UPPER LIMB THROMBOSIS: A PERSPECTIVE FROM THE CLINICAL PRACTICE

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Background: Deep vein thrombosis (DVT) of the upper limbs represents 1-4% of DVT, most of them related to central venous catheter and / or malignancy. Thrombosis involving the deep veins (ie, subclavian, axillary, brachial) can lead to complications as pulmonary embolism (PE) and long-term sequelae. PE from upper extremity sources accounts for about 6% of cases. Initial treatment in acute context include fibrinolysis and subsequent anticoagulation (Grade 2C). When symptomatology is mild and/or onset of symptoms undetermined (>2 weeks), minimum anticoagulation 3 months is recommended. If there are associated anatomical abnormalities, the possibility of surgical vascular thoracic decompression must be assessed.

Aims: To ascertain D-dimer diagnostic accuracy for upper extremity DVT.
Methods: A retrospective audit was undertaken to determine the aetiology and clinical presentation on patients which UDVT at presentations. Patients wich a formal malignacy confirmed before the diagnosis was excluded. A D dimer (DD) with a cutt off cut off levels *validated for lower limb* DVT was performed.
Results: A total of 18 patients was identify in the period of 2012 to 2016. All the cases investigations included Doppler US or CT/MRI and in 30% of the patients the thrombosis was confirmed via contrast venography as a reference standard test. The gender predominan was male in this group the symptomatology were related to physical efforts in a 60% (Paget-Shroëtter Syndrome) whereas in female serie the predominant was thrombophilic defects (factor V Leiden). The average age was 33 years (ranging from 21 to 68 years) and 2 elderly patients a new diagnosis of cancer was **c**onfirmed (thyroid and lung) (odds ratio, 3.24; 95% CI, 1.13-9.38). The 85% of the patients had an unprovoked event; four patients have a diagnosis of catheter related thrombosis and four cases a thrombofilic defect (factor V Leiden) precipated by anticonceptive. Two patients had a diagnosis of SLE. We had four cases of positve DD screening (both were marginally elevated, P <0.01) . The risk of re-thrombosis was non significative but in the subanalysis of relapsing thrombotic event populations the risk of relapse increased proportionally in relation of thrombofilic defect and high BMI. A trend towards a higher rate of recurrent thrombosis (was observed among patients with BMI>25 (42.6%) compared to those with a BMI <25 (33%). This difference reached statistical significance in women with BMI>25, who had recurrent event in 51.7% of the cases *vs* those with BMI <25 (29.7%) (p <0.05 CI 0.03, 0.41).
Summary/Conclusions: In the unprovoke serie the relation of DD was positive in less tan 30% of the cases and non stadistically significative (p <0.01). In the case of subclavian vein oclusion this is result in limited clot burden (which explain the correspondence with negative DD value). The risk of re-thrombosis is asociated with thrombophilic defect and high BMI esclusively. The DD adjusted to age and specific young population need to be adressed (age adjusted DD cut off adpated of the specific population). A prospective studies of DD in suspected UDVT need to be adressed.

PB2225

THE INFLUENCE OF HEPARINOID FROM THE PEONY ROOTS ON THE THROMBUS DISSOLUTION

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Background: Many plants have an effect on the blood clotting system. It is known that there are heparin-like substances in some types of peony roots (Paeonia lactiflora, Paeonia suffruticosa). It proved that there is an anticoagulant activity in extracts from such roots.
Aims: The intention is to show the inhibitory effect of the extract of Paeonia lactiflora roots (EA) on processes fibrin and thrombus formation.
Methods: We used the standard coagulologic methods for determining anticoagulant activity by APTT test, antiplatelet, total fibrinolytic activity (TFA), fibrindepolymerizing activity (FDPA). Experiments were carried out in accordance with ethical principles and documents recommended by the Declaration of Helsinki of the humane treatment of animals. We used an animal model with prothrombotic condition caused by intravenous administration of subthreshold doses of tissue thromboplastin at a dose of 0.6 -0.7 ml per 200 g body weight in rats. After 30 min after injection of thromboplastin, we injected intraperitoneally 0.1 mL of 1% of extract of EP and after 30 minutes we determined parameters of hemostasis in the blood plasma.
Results: It was shown that after administration of the indicated doses thromboplastin occurs hypercoagulability in blood plasma of animals (APTT decreased by 23% SFA - 15%, FDPA -12%; increased platelet aggregation by 18% compared to control animals not receiving thromboplastin). Normalization of blood clotting is installed in the experimental rats after application EP (recovery of platelet aggregation to 98%, APTT- to 100%, up to 95% SFA- FDPA and - up to 67% compared with control). The high degree of FDPA indicates the ability of EP to obstruct the process of the formation of fibrins and thrombosis. Heparin components in EP interact with fibrin monomers which do not participate in their conversion to fibrin polymer. As a result, stable fibrin polymer or thrombus is not formed.
Summary/Conclusions: Consequently, the extract of Paeonia lactiflora roots containing heparinoid contributes to the restoration of coagulation properties in blood of animals in prothrombotic condition and prevents thrombosis. In the initial stages of fibrin formation, it causes the thrombus dissolution.

PB2226

LOW MOLECULAR WEIGHT HEPARIN AND HIGH MOLECULAR WEIGHT HEPARIN: COMBINATION WITH ADRENORECEPTOR ANTAGONISTS AND PREVENTION OF THROMBUS FORMATION

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Background: Rethrombosis and thromboembolia are the most common side effects of thrombolytic therapy. One of the possible causes of thrombosis is the entering of thromboplastin in the blood stream. Marker of thromboplastin is an intrinsic membrane glycoprotein 5'-nucleotidase (5'NT) that is present as an enzyme in a wide variety of cells. Recently it was shown that compensatory reaction of haemostasis system by using different fibrinolytic drugs was connected with the stimulation of the sympathetic nervous system. Besides, it is known that selective and nonselective α–adrenoreceptor blocking agents have fibrinolytic and antiplatelet effects. The prevention of thrombosis complication is very important field of pathophysiology and medical practices. Therefore, we studied effects of different α–adrenoreceptor antagonists and the influence of these substances combinations with various anticoagulant and fibrinolytic agents on blood coagulation during many years.
Aims: The study of the influence of low molecular weight heparin (LMWH, 4.4 kD) and high molecular weight heparin (HMWH) and their combinations with different α –adrenoreceptor antagonists (AA) on experimental thrombosis prevention.
Methods: Experiments were carry out on 50 white laboratory rats weighing 200-230 g according to the ethical principles of the Helsinki Declaration. Anticoagulant and antithrombotic effects of LMWH or HMWH were studied in two rat models of thrombosis – thrombosis in v. jugularis (Wessler) and thrombosis in arterio-venous shunt (direct registration of blood pressure). The α–AA digydroergotoxin (DET – 1mg/kg), α_1–AA prazosin (PZ – 2mg/kg), LMWH or HMWH (40 USP/kg) were injected in v.jugularis. Saline was administrated in control rat groups. The thrombus were formed 15 or 180 min after substances injected. The degree of thrombus formation (TF) was detected in ball (Wessler model) and by time of TF (arterio-venous shunt model). In blood plasma the activity of 5'NT was detected. The results were processed statistically.
Results: The increase of anticoagulant and antithrombotic effects of LMWH or HMWH by pretreatment of DET or PZ were shown in both animal models of venous thrombosis. The degree of TF by Wessler model may be estimated as 3.7 (saline), 1.3 (LMWH), 1.8 (HMWH), 0.9-1.1 (DET+ LMWH or PZ+LMWH) and 1-1.3 (DET+ HMWH or PZ+HMWH). Besides, it has been shown that TF was accompanied with significant hypercoagulation of blood: 5'NT activity was increased in 2 time comparatively with normal level. LMWH or HMWH combinations with DET or PZ administration led to normalization of 5'NT level in blood plasma. In arterio-venous shunt model it has been shown that the time of TF was 2 min (saline), that was accompanied with the decrease of blood pressure (on 40-50 mmHg). In this case the time of TF was prolonged in 4 time (LMWH) or 2 time (HMWH) comparatively with saline group 15 min after injection; in 4.5-5 time (DET+ LMWH or PZ+LMWH) or 3-3.5 time (DET+HMWH or PZ+HMWH) comparatively with saline group 180 min after injection.
Summary/Conclusions: Thus we confirmed that LMWH (as one, as in combination with α–adrenoreceptor antagonists) has definite advantages over HMWH. Besides our results show that α–adrenoreceptor antagonists significantly improvement antithrombotic effect of anticoagulant agents (LMWH and HMWH). Therefore the combination of LMWH with selective and nonselective α–adrenoreceptor antagonists may be effective used for prevention of venous thrombosis development and thromboembolia.

PB2227

THE POLICY AND PRACTICE OF ANTICOAGULATION THERAPY AMONG CLINICIANS IN SOUTHEAST NIGERIA.

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Background: In the absence of anticoagulation therapy, the risk of Venous thromboembolism; deep-vein thrombosis (DVT) and pulmonary embolism(PE)in medically ill patients is comparable to that in moderate-risk surgical patients. Previous studies have revealed grossly inadequate knowledge and a dismal practice of anticoagulation among healthcare workers in some resource poor countries. Prophylactic anticoagulation is under-prescribed in Nigeria, South Africa, as well as in many other countries in Africa.
Aims: The aims of the study are to evaluate the practice of anticoagulant therapy. It will also document the frequency of drug-induced complications resulting from the use of anticoagulants and presence of an anticoagulation policy in the hospitals surveyed.
Methods: This is a multicentre cohort survey of the practice of anticoagulant therapy among clinicians in South East Nigeria. A pretested validated questionnaire was administered to clinicians in five tertiary hospitals in the southeast of Nigeria. The questionnaire was designed to assess their practices anticoagulation therapy. The questionnaire was administered consecutively on clinicians in the participating centers. The following institutions participated in the survey: University of Nigeria Teaching Hospital Enugu, Federal Medical Centre, Abakaliki, Federal Medical Centre Umuahia, Abia State Teaching Hospital, Aba and Amaku Specialist hospital Awka. Statistical package for Social Science (SPSS) software, version 18 (SPSS Inc., Chicago, IL) was used for analysis.

Results: A total of 528 clinicians were involved in the survey. There were more males 378 (71.6%) than females, 150 (28.4%) the clinicians who practiced for less than 5years are in the majority 189 (35.8%) and those with 15-20 years of practice 46(8.7%) are in the minority. Only 52 of the respondents (9.8%) claimed their institutions had an anticoagulation policy while 274 (51.9%) of them said there was no such policy and 168 (31.2%) do not know of any policy. Unfractionated heparin was the most frequently used (96.8%) and fondaparinux was the most infrequently used (42%). Most of the prescriptions were done by younger clinicians who are the highest in number. The consultants prescribed heparin and warfarin most, with the newer anticoagulants taking the rear position. Only 193 (36.6%) of the respondents routinely prescribed anticoagulation therapy when indicated. 412(78%) of respondents believe the risk of anticoagulation outweighs the benefits while 439 (83.1%) identified cost is an important variable in prescribing anticoagulation agent. Anti-coagulation prophylaxis was the most frequently used for patients immobilized or bedridden (94.1%); malignancy and atrial fibrillation were the most infrequent reasons for using anticoagulation agents (50.6%) . A total of 63 respondents (11.9%) were not satisfied and 219 (41.5%) were not very satisfied with the laboratory monitoring tool available in their institutions. Bleeding is the most common complication of anticoagulation while the least encountered complications are skin and jaw necrosis among the respondents 492(93.2%), 1(0.2%) respectively.
Summary/Conclusions: This survey has shown the lack of anticoagulation policies among the centers that participated. Our survey has also shown deficiencies in the areas of practice of anticoagulation among the clinicians in the Southeast of Nigeria. These gaps can be remedied by continuous medical education and by the establishment of anticoagulation policies.

Transfusion medicine

PB2228

UMBILICAL CORD BLOOD PLASMA INFUSION PROMOTES BLOOD CELL RECOVERY IN INPATIENTS WITH ACUTE LEUKEMIA UNDERGOING CHEMOTHERAPY

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Background: Umbilical cord blood plasma(UCBP) is separated from umbilical cord blood. UCBP contains a variety of hematopoietic growth factors which can stimulate hematopoiesis.
Aims: The aim of this work is to explore the influence of UCBP infusion on blood cell recovery in patients with acute leukemia undergoing chemotherapy.
Methods: Patients with the diagnosis of acute leukemia were included in this study and they were randomly distributed to experimental group and control group. Patients in experimental group received infusion of 100ml UCBP with the same ABO and Rh blood type every day after chemotherapy for five days and patients in control group received placebo for the same time. Blood routine were tested every day until WBC >4.0×10^9/L and PLT >20×10^9/L.
Results: 25 patients were included in the study of which 23 were brought into statistics. 13 patients were in experimental group and 10 in control group. There were no difference in age, gender and dose intensity of chemotherapy between the two groups (P>0.05). The average recovery time of the blood neutriphil granulocyte >0.5×10^9/L in experimental group and control group were respectively (8.53± 3.26) days *versus* (12.92± 4.75) days (P<0.05) and that of PLT >20×10^9/L was respectively (9.24± 3.68) days *versus* (13.15± 5.76) days (P<0.05). No UCBP transfusion-related side effects were found.
Summary/Conclusions: UCBP administration is safe as treatment for cytopenia and could promote blood cell recovery in patients with acute leukemia undergoing chemotherapy.

PB2229

TOWARD BETTER BLOOD TRANSFUSION PRACTICE: A SUCCESSFUL RED BLOOD CELL UTILIZATION TOOLS IN A TERTIARY CARE HOSPITAL

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Background: The need for blood in hospitals continues to exceed the volume collected by the transfusion services. The gross over-ordering of blood, in excess of actual and anticipated needs leads to substantial costs and a burden to the transfusion services. In addition, over-ordering leads to non-availability of cross-matched units for other patients who might be in urgent need of transfusion.
Aims: We are aiming to reduce the Cross-match-to-transfusion ratio (C:T ratio) & improve blood utilization in Mafraq Hospital.
Methods: In 2011 the ordering practice at Mafraq Hospital, a designated Trauma Centre, had been evaluated. Data collected retrospectively over a one year period and a C:T ratio adopted by the American Association of Blood Bank was calculated for all various subspecialties including Surgery, Internal Medicine, Pediatrics and Obstetrics and Gynecology. All procedures related to hospital transfusion practice been retrieved and re-evaluated to address gaps. Policy of maximum surgical blood ordering (MSBO) was implemented based upon both results of audits and by discussion and agreement between medical teams. Focused training and education has been followed to increase the awareness of the health care workers. Plus monitoring of C:T ratio on monthly basis, blood bank team had arranged meetings with the departments that were over-ordering cross-matches to explain that group & save test is a safe, effective and financially beneficial strategy. Communicating with the physicians had been the most challenging aspect of implementing the policy changes.Regular audits had been conducted to measure the compliance and effectiveness of the blood management practice.
Results: Compared to the international guidelines, C:T ratios in 2010 was beyond the acceptable target and ranged between 2.5 to 3.2 highlighting the over-ordered cross-matched blood in certain sub-specialties. This practice of ordering was probably because of the fear that blood will not be available, if needed. Following implementation of control and continuous monitoring measures while establishing proper procedures such us transfusion guidelines, administration of blood and blood products and Maximum Surgical Ordering Practice, Mafraq blood bank ,supported by the Transfusion and Tissue & Quality & Patient Safety Committees, achieved a great success in reducing C:T ratio <2 all through 2016 Figure 1. The reduction of C:T ratio had improved blood inventory control and reduced the workload of the blood bank staff. Because fewer units of cross-matched PRBC are being ordered, the blood bank has been able to decrease the number of units of blood stocked in house and hence decreasing the number of expired units &reducing money loss Figure 1. The savings in technologist time is particularly significant since the blood bank is most of the time at a minimal staffing level.

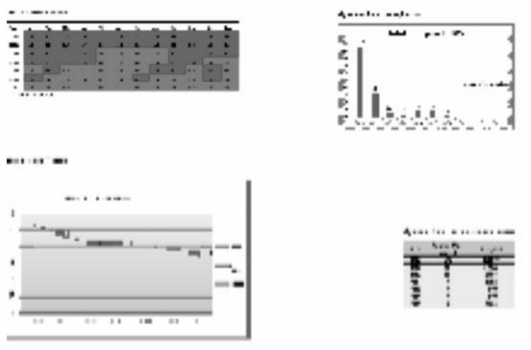

Figure 1.

Summary/Conclusions: There is a tendency to order blood in excess, either by asking for an increased number of units or as a standby precautionary measure. Adherence to MSBO & transfusion guidelines, hospital can achieve C:T ratios below 2.0. The introduction of strategies for improved blood utilization has been shown to be cost effective and safe. Future comparison analysis with facilities with well-established blood management program can enhance the current program and ensure continuous proper utilization.

PB2230

SAFETY AND EFFICACY OF A PROTHROMBIN COMPLEX CONCENTRATE IN VKA REVERSAL AND OFF-LABEL INDICATIONS

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Background: Prothrombin complex concentrates (PCC) are highly purified mixtures of plasma coagulation factors that contains vitamin K dependent and anticoagulation factors, they are approved for urgent reversal of vitamin K antagonists (VKA). Massive bleeding-associated coagulopathy guidelines include PCC in their management, although as an off-label indication.
Aims: The aim of the present work is to evaluate safety and efficacy of PCC in a case series of VKA reversal and refractory coagulopathy associated with major bleeding.
Methods: Retrospective review of cases treated with a four-factor PCC between January 2010 to January 2016 in two tertiary Universitary Hospitals. As safety endpoints we evaluated infusion reactions and incidence of thromboembolic events by self reported registry. The efficacy endpoints were studied in two separate cohorts: 1) INR correction for VKA reversal and 2) coagulopathy correction and early mortality (24 hours) in major bleeding coagulopathy.
Results: 328 patients were included (47.25% male), median age 78 years (range 19-102). PCC was used in the following cases: 1) 66.67% in VKA reversal indication (181 patients due to hemorrhage and 33 prior to emergent surgery), mean dose of PCC 1333.51 IU; 2) 30.54% in refractory coagulopathy in major bleeding (30 patients due to massive bleeding protocol activation, 43 patients in hepathopathy coagulopathy and 25 patients in bleeding not related with any of previous reasons) a mean dose of PCC 1681.63 IU was used. Safety endpoint: Two infusion reactions were reported potentially related to PCC use, they were not specified neither as anafilaxia nor as pulmonary edema, and 8 thrombotic episodes were observed (2.4%): 5 pulmonary embolism, 2 deep venous thrombosis and 1 portal thrombosis, 75% of the events appear in the group of VKA reversal. Efficacy endpoint: VKA reversal in bleeding patients was effective in 97% of them, 76.5% with complete reversal of INR value (INR<1,5), 34,25% of patients required red blood cell (RBC) transfusion, with a mean of 1.32 RBC. Prior to invasive procedure VKA reversal was effective in 83% of patients, all procedures taking place with no bleeding complication, 36,3% of patients needed RBC with a mean of 1.12 units. 24 hours mortality in refractory coagulopathy associated to major bleeding was 31,6%, having a worse outcome (40% rate of death) those who suffer a massive bleeding coagulopathy, all death related with absence of bleeding control. A global INR correction happen in 76.7% of patients, complete correction in 40.7%. 63,26% received previous to PCC fresh frozen plasma.
Invasive hemostatic procedures were required in 20% of the whole series.
Summary/Conclusions: A four-factor prothrombin complex was safe and effective as adjuvant treatment in refractory coagulopathy due to major bleeding as well as for the emergent reversal of VKA.

PB2231

TRACEABILITY OF RED BLOOD CELLS IN A HOSPITAL TRANSFUSION LABORATORY

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Background: According to European legislation (2002/98/EC, 2005/61/EC) as a requirement of hemovigilance system traceability (confirmation of final destination of blood components in hospitals) information should be kept for 30 years, improving the quality and safety of the transfusion process. Various methods are available from simple paper–based procedures to full electronic blood tracking systems. The ideal goal is to trace the final fate of 100% of the red blood cell (RBC) units, from donor to recipient and vice versa.
Aims: To check the ability to trace each individual unit from donor to recipient or disposal in our hospital.
Methods: To ensure compliance, the minimum traceability data set for retention is a mix of 1) Wards' paper files (file of transfusions and/or patient records: 14/2 wards respectively). 2) HTL electronic records and paper records. The transfusion practitioner is responsible for the collection and maintenance of traceability data.
Results: During the year 2016, the number of RBC units transfused in our hospital was 2128. The traceability status of the transfused units is shown in the Table 1.

Table 1.

Traceability of transfused units			
Traceability	**Description**	**N**	**N total: 2128**
Confirmed	Full match of data	2067/2128 (97.13%)	2067
Presumed	Wrong number of unit in the ward	25/2128 (1.18%)	32
Presumed	No number of the unit in the ward	7/2128 (0.33%)	
Unknown	No data in the ward*	29/2128 (1.36%)	29

* Patients' data has been archived for long- term retension.

Summary/Conclusions: Although we are satisfied that the results represent a reasonably accurate working model of the current situation, the trail of a unit is less reliable after blood has left the HTL. 1. Patients' notes to provide traceability are not totally reliable. It is apparent that the ward staff plays a key- role part in the chain and this highlights the need for them to receive training to emphasize the importance of their contribution to hospital compliance. 2. The indications are that the essential requirements on traceability are not fully met by the current laboratory computer system. A configuration is needed to produce a report which lists components which have been assigned for use but do not have an entry for return to stock or final fate. Ongoing problems will be referred to the Hospital Transfusion Committee. 3. For the longer term ultimately only effective IT system in both wards and HTL can ensure total traceability and we recommend the inclusion of electronic tracking system in the National Blood Donor Registry Programme (EMA)

PB2232

NON-HEMOLYTIC FEBRILE POST-PLATELET-TRANSFUSION REACTIONS IN HEMATOLOGICAL PATIENTS

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Background: Platelet concentrate (PC) transfusions are the main method of thrombocytopenia correction in hematological patients, but multiple transfusions could trigger alloimmunity and refractoriness to transfusions.
Aims: Comparison of post-transfusion reactions in hematological patients with individual matching and without individual matching receiving PC transfusion support.
Methods: In 2015-2016, we observed 948 hospitalized patients, who received 12.344 PC transfusions. Individual matching of PCs was performed by cross-matching on the Galileo-Neo (Immucor) analyzer. Statistical processing was performed using the chi-squared test with Yates' correction.
Results: 107 of 948 patients developed refractoriness to PC transfusions (12% of total patients). Out of them, 21 patients received 389 PC transfusions without individual matching. 86 patients received 1705 PC transfusions with individual matching. During transfusions without individual matching to non-refractory patients, 0.003% of non-hemolytic febrile reactions (NHFR) have been record-

ed, after matching to refractory patients the frequency was 0.002%. Before matching to refractory patients, the frequency of NHFR was (0.03%) (Table 1).

Table 1.

Status	Patients	Transfusions	Post-transfusion reactions	% of all transfusions	P
Refractory with matching	86	2027	6	0,002	*P<0,01
Refractory before matching	21	389	15	0,03	
Non-refractory without matching	841	9928	30	0,003	*P<0,01

Summary/Conclusions: The frequency of NHFR in groups with refractoriness with individual matching is significantly lower (10 fold) compared to groups with refractoriness before the matching (P<0,01)*.

PB2233

RARE DONORS AND MALARIA

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Background: Migratory flows of sub-saharan (SSA) persons throughout the world are expected to continously increase. A significant proportion of SSA citizens are affected by Sickle Cell Disease (SCD), condition requiring repeated blood transfusions. Many centuries of malaria pressure have induced in SSA natives a homogeneous selection of peculiar haematologic characteristics, such as the absence of high frequency red cell antigens (defining a rare blood) that cannot be found in donors of European descent so that many SCD transfused patients experience the fearful occurrence of red cell alloimmunization. For these reasons haematologists are expecting to access to Rare Blood Banks in order to assure a full match between donor and recipient's blood, that may be obtained from donors sharing the same ethnicity. Unfortunately SSA donor recruitment is counteracted by the widespread diffusion of infections contracted before migration: one of these is malaria. In SSA malaria may occur sublinically and is characterized by a slow antibody clearance. This peculiar condition, the so-called semi-immunity, has been induced by a strong genetic pressure, and is a kind of co-evolutionary process characterized by the co-exhistence and persistence of small entity of *Plasmodium* genome with relative antibodies. Molecular techniques are unreliable to detect a small number of Plasmodia, which may otherwise be sufficient to induce a transfusion transmitted malaria (TTM). The serologic assessment, despite the low specificity, remains the most sensitive and reliable method to detect the semi-immune status in blood donors (1).
Aims: The aim of this study was to assess the prevalence of malaria immunity in a cohort of healthy SSA citizens.
Methods: Since 2010 in our Department of Haematology and Transfusion Medicine we recruited 184 SSA citizens, in good health, who agreed to underwent clinical and laboratory investigations to become a blood donor. All of them were born in SSA Africa and lived there for at least the first 5 years of life. 70% of subjects didn't recognize any previous malaria fever. The last travel/stay in Africa was 1-20 years (median 3 yrs), and 48% of returning people had received prophylaxis. Malaria serology was determined by a commercial enzyme immunoassay kit (Malaria EIA Ab, BioRad).
Results: Overall 75% of persons were positive for malaria antibodies. Serologic positivity was found in 75% of persons no more exposed in 5 recent years and even in 83% (19/23) persons settled in Italy since 10-20 years. Serologic positivity was present in 100% of people from Benin, 85% from Burkina Faso, 78% from Ivory Coast and Cameroon, 63% from Senegal. We followed antibody concentration in 50 persons (136 assays), and we observed a slightly negative trend that, in most cases, was followed by a prolonged phase of low antibody levels. 4/50 became negative after three years.
Summary/Conclusions: The identification of malaria antibodies is essential in SSA native donors and, by far, irrepleaceble in order to avoid the risk of TTM. Until pathogen inactivation techniques will become available, we have a very low expectation to introduce SSA blood in Blood Bank inventories. Haematologists have to wait some years for the forthcoming SSA second generation that will allow to fully match the entire SCD patient community.

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PB2234

EFFICACY AND INFLUENCE OF IRON CHELATION THERAPY ON RED BLOOD CELL TRANSFUSIONS

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Background: Chelation therapy is recommended for transfused patients that have an elevated serum ferritin level (over 1000 microg/l), evidence of iron overload or received over 20 units of red blood cell transfusions (RBCT). Deferasirox showedefficacy and safety in maintaining or reducing body iron (assessed by liveriron concentration or serum ferritin).Iron chelation therapy was associated with hematopoiesis improvement in transfusion-dependent patients and interruption of Deferasirox treatment of transfusions dependent myelodisplastic patients produced loss of erythroid response.
Aims: Aim of the study: to assess the results of Deferasirox efficacy, side effects and to study if the number of RBCT decreased after starting Deferasirox.
Methods: We have done a retrospective, transversal study including all the adult politransfused patients treated with Deferasirox in three counties Hematology Departments of Nord-West Romanian hospitals. Criteria of Deferasirox treatment: over 20 RBCT, serum ferritine level over 1000 microg/l.
We created a data collection sheet including: demographics, information on patients' disease, serum ferritine level at start of and during treatment, Deferasirox dose, data about dose modification, adverse effects of Deferasirox and their management, reasons for treatment discontinuating, evaluation of commorbidities that could increase serum ferritine level, number of RBCT before and after starting the treatment.
Results: We included 40 politransfused patients treated with Deferasirox, age average 63. The diagnosis included mielodysplastic syndromes (most of patients), thalassemia, other anemias. Myelodysplastic patients were treated with low dose chemotherapy, epigenetic treatment, RBCT and bethatalasemic/anemic patients were transfused. The baseline value of ferritine was between 1075 - 6187 microg/l. Deferasirox dose: 20-30 mg/kg. There was a significant reduction in serum feritine from baseline for all the patients. Ferritine median at start, 3631 microg/l decreases at 1537 microg/l after 6 months of treatment and at 994 microg/l after 12 months of treatment. There were 8 patients that had descendent levels of feritine, but during infectious episodes the ferritine increases for a short period of time. Digestive adverse events appeared in three cases (two cases of diarrhea and one case of digestive hemorrhagic episode). In all these cases the treatment was temporarily discontinued. In three cases, treatment was stopped because low ferritin level (under 500 microg/l). RBCT were administered before (mean 2.43 units/month) and after starting Deferasirox (mean 1.39 units/month), the difference is statistically significant (Student Test, t(39)=6.98, p<0.001). After starting Deferasirox treatment mean number of RBCT decreased, mean of differences (95% CI) was 1.04. We analyzed the group of 23 patients treated with Deferasirox less than 12 months, and the patients treated more than 12 months, 15 patients. In both groups the difference of RBCT means (before and after the start of the treatment) are statistically significant (for the patients treated less than 12 months: Student Test, t(23)=8.12, p<0.001 and for the patient treated more than 12 months: Student test, t(15)=3.03, p=0.008).
Summary/Conclusions: Analyzing our group of 40 patients, Deferasirox proves to be effective and safe. Adverse effects that determined a temporary stop of the treatment were mild/medium short time digestive reactions. The number of red blood cell transfusion significativelly decreased after starting Deferasirox treatment.

PB2235

LIBERAL *VS* RESTRICTIVE COMPARATIVE TRANSFUSIONAL STUDY IN ONCOLOGICAL POPULATION

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Background: Allogeneic transfusion therapy is perhaps one of the most widely used treatments without good evidence support, despite many years of application in clinical practice. This, coupled with blood shortages, the impossibility of achieving zero risk, the lack of evidence that transfusion may increase consumption or decrease tissue oxygen debt and the existence of an association with an increase in morbidity and mortality have favoured that we join efforts towards its optimal use.
Aims: Optimal use in our adult oncological population and evidence that restrictive transfusion (TR, Hb 7-9 grs / dl) is not greater or lowerto the liberal transfusion (TL, Hb 8-10 grs / dl), keeping hemoglobin in safe levels for the patient.
Methods: A research was performed from October 1st, 2015 through December 31st, 2016. We analyzed the proportion of patients receiving packed red cells (CH) and the number of units transfused as well as post-transfusion control in order to describe the outcome of the CH *versus* TL strategies in the cancer population under the study.
Results: See Table 1.
Summary/Conclusions: The results obtained in our series of 311 cancer

patients indicate that the restrictive strategy has been equally effective and probably superior to the liberal one maintaining Hb at a safe level in each patient, as well as quality of life and comfort in a subgroup with advanced and terminal cancer.

Table 1.

Transfusional Therapy	Patients (N°)	Hb Pre (X-)	Hb Post (X-)	yield /CH (grs/dl)
RT	192	8,1	9,1	1,0
LT	97	7,4	9,4	1,0
PWC	22	8,0	-	-
TPT	311	7,8	9,2	1,0
X- RBC transfused: 1,3				

Hb Pre: Pre-transfusional haemoglobin; Hb Post: Post-transfusional haemoglobin; PWC: Patients without post transfusion Hblevel; TPT: Total Patients Transfused; X-: half haemoglobin.

PB2236

HIGH RISK OF HBV INFECTION IN VACCINATED POLYTRANSFUSED CHILDREN

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Background: Children receiving chemotherapy for neoplastic diseases are still susceptible to Hepatitis B virus (HBV) infection despite the national HBV vaccination program coverage for all infants since 1992.
Aims: This study aimed to analyze immunity against HBV and occurrence of HBV breakthrough infections in polytransfused children who had been vaccinated during infancy.
Methods: The study included 89 children with hematological disorders and malignancies, who were categorized into group (A): 37 receiving chemotherapy (M:F 20:17; mean age:7.7±4.0) and group (B): 52 polytransfused children (M:F 31:21; mean age:7.6±3.2). A matched healthy control group (n=162) was also included. All patients and controls had received their primary vaccination against HBV in infancy. Quantitative anti-HBs were tested for patients and controls. Patients' sera were tested for HBsAg, anti-HBc, and HBV-DNA (nested PCR for surface, core & x-regions).
Results: Levels of anti-HBs between 10-100 IU/L and ≥100 IU/L were found among 13.5% and 21.6% [group (A)], 44.2% and 11.5% [group (B)] and 32.1% and 10.5% of controls respectively. There was a significant difference in HBsAb between patients receiving chemotherapy (group A) and both groups B patients (p=0.008) and controls (p=0.032). However, no difference was found between polytransfused children [group (B)] and controls.
HBsAg was positive in 21(67.7%) children under chemotherapy [group (A)] compared to 10 (32.2%) polytransfused children [group (B)] (p<0.0005). Overall, 49 patients (55%) were HBV-DNA positive; 44 c-region positive, 7 s-region positive, 2 positive for both c and s-regions and one positive for c and x-regions. Of those, only 21 patients (42.8%) were also positive for HBsAg; while 28 (47.2%) had occult HBV infection (HBsAg-negative). There was no significant difference between patients receiving chemotherapy [group (A)] and polytransfused children [group (B)] (p 0.157), regarding the rate of HBV DNA. Anti-HBs ≥10 IU/L co-existed in 38.7% (12/31) of HBsAg positive patients and 49% (24/49) of HBV-DNA positive patients.
Summary/Conclusions: Children with neoplastic diseases vaccinated during infancy were at a high risk for HBV infection. The effect of immunosuppression on the HBV protective level favored overt HBV infection in children receiving chemotherapy. The co-existence of anti-HBs with HBsAg and/or HBV-DNA demonstrated a possible residual transfusion-transmission risk with mutant HBV strains.

PB2237

THE ISOHEMAGGLUTININ TITERS OF BLOOD BANK DONORS: THE EXPERIENCE OF ISTANBUL FACULTY OF MEDICINE

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Background: Isohemaglutinins that develop against ABO blood group antigens are very important in transfusion and transplantation medicine. Today, 30-40% of allogeneic stem cell transplantations are ABO incompatible transplantation, 20-25% of which are major, 20-25% are minor and remaining bi-directionally incompatible transfusion. Our study; based on the knowledge that isohemagglutinins play an important role in blood transfusion policies in patients undergoing ABO incompatible hematopoietic stem cell transplantation(HSCT) has been shaped by the assumption that each healthy blood bank donor may be potential transfusion donors for ABO incompatible HSCT transplant recipients
Aims: In this study, we investigated the isohemagglutinin titer values of the individuals with A, B and O blood groups; the distribution of the isohemagglutinin titers according to the decades and gender. Also we examined the posibility of determining the isohemagglutinin cut off value in Turkish society.
Methods: One thousand five voluntary blood donors (48 female, 957 male), randomly chosen from the donors, providing the criteria to be a standard blood donor in Blood Center Department, Istanbul Faculty of Medicine were studied. This study was approved by the Ethics Committee of Istanbul Medical Faculty. In the donor population group; blood group A (%40) was the most common and blood group AB was the rarest blood group. Accoording to the Rh D phenotypes; 85% of the population was Rh D positive and 15% of the population was Rh D negative.The frequency of our blood group was determined similar with other European countries. The most common age range of one thousand five voluntary blood donors, including the same rate individuals with blood group A, B and O, was the age range between 26 and 35 years. Forward and reverse blood group determination were performed to these donors and also we identified the Anti-B Ig M and Ig G isohemagglutinin titer values for blood group A; Anti-A Ig M and Ig G titer values for blood group B; eventually both Anti-A Ig M /Ig G and Anti-B Ig M/ Ig G izohemaglutinin titer values for blood group O by using column agglutination methods. Statistical analysis was performed with NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA).
Results: While the titer value of Anti-A Ig M isohemagglutinin was 1:128 for female individuals with blood group B; the titer values of both Anti-B Ig M (1:128 and 1:256) , Anti-B Ig G (1:1024) and Anti-A Ig M (1:256) isohemagglutinins were statistically significiance in female individuals rather than male ones. The levels of isohemagglutinin in the blood groups A, B and 0 are shown in Table 1A,B. There was no statistical difference in anti-B IgG and IgM titers in blood group A, anti-A IgG and IgM titers in blood group B and anti-A IgG and IgM titers in blood group O between males and females(p>0.05). However Anti-B IgG and IgM antibody titers were higher in females than males in donors with blood group O respectively p=0,017 (p<0,05) and p= 0.001 (p<0,01) (Figure 1 A,B).

Table 1.

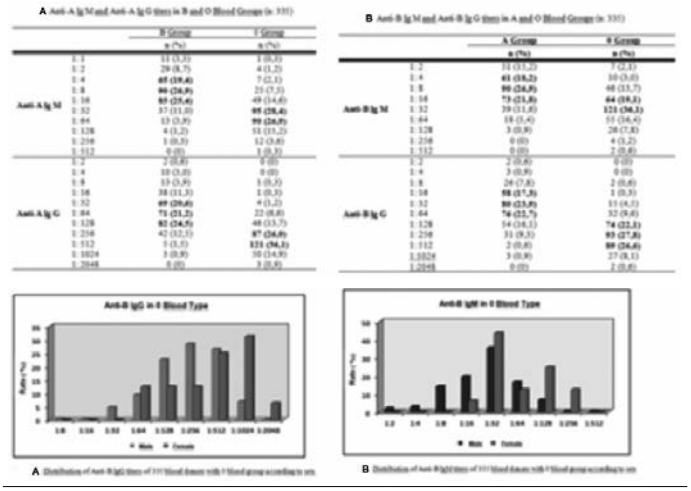

Figure 1.

Summary/Conclusions: Female individuals of blood bank donors participated in our study have higher isohemagglutinin titer values rather then male individuals. Recurrent blood group incompatibility in pregnancy, invasive diagnostic and therapeutical approaches for risk analysis in fetal examination during pregnancy, perinatal complications causing fetomaternal hemorrhage after pregnancy or during birth and lastly autoimmun diseases cause the enchancement of isohemagglutinin titer values in female individuals. Regarding the gender differences; nutrition, vaccination and recurrent blood transfusion history of blood bank donors alsa effect and change the isohemagglutinin titers of individuals. Population spesific isohemagglutinin titer values play a key role in blood donation policy of patients undergoing hematopoietic stem cell transplantation. Consequently; we predict that Turkish community-spesific isohemagglutinin cut off titer values can be identified and we will hope our knowledge on this issue in the future with the increase of research is going to increase further.

PB2238

THE NEW METHOD OF PURIFICATION FACTOR COAGULATION VIII

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Background: The human plasma of blood can be transfused directly to patients or pooled and fractionated into plasma protein products. Plasma contains about 60-80 g/L of protein, of which about 95% are used for many therapeutic prod-

ucts. The main proteins that use for treatment many diseases are albumine (45 g/L), immunoglobulin (8-11 g/L), factors coagulations. The Factor VIII (FVIII) is one of the blood coagulation factor and it deficient causing development of bleeding desorders known as Haemophilia A. The purification of FVIII is generally required for the treatment Haemophilia A or von Willebrand's disease and heavy loss of blood, requires relatively high purity for medical use.
Aims: optimization of a process purification of FVIII by the method of affinity chromatography on the Diasorb-aminopropyl matrix with triazin active dyes as ligands.
Methods: adsorption/precipitation, ion-exchange chromatography on DEAE-Sepharose, affinity chromatography on the Diasorb-aminopropyl matrix with Active Scarlet Damask 4GT as ligands in combination methods of antiviral treatment.
Results: The process plasma fractionation is largest industry segment in manufacture of therapeutic concentrate of plasma proteins. We developed technological scheme that involves fractionation plasma of blood in combinations of classical methods of protein precipitation and two chromatographic steps: ion exchange and affinity chromatography.
Of all plasma fractionation methods, chromatography is the best candidate for purification of factor coagulation, especially FVIII. The methods adsorption/precipitation permits the fractionation of large volumes of plasma, but the quality of the product obtained by chromatography is superior.
We offer: fresh frozen plasma – adsorption of proteins on the barium citrate – adsorption of proteins on $Al(OH)_3$ – adsorption of proteins to PEG-4000 – viral inactivation (solvent-detergent method) – ion exchange chromatography on DEAE-Sepharose – viral inactivation (ammonium thiocyanate) – dye-ligand affinity chromatography (Diasoorb-Active Scarlet Damask 4GT). We got the drug of FVIII with specific activity 69.65±2.24 IU/mg protein.
Summary/Conclusions: we developed technological scheme of plasma fractionation and reached a high degree of purification of coagulation FVIII.

PB2239

PRIMARY TROMBOTIC MICROANGIOPATHIES. REVISION IN A CENTER OF THE LAST 8 YEARS

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Background: Thrombotic microangiopathies are a group of rare diseases characterized by non-immune microangiopathic hemolytic anemia, thrombocytopenia and involvement of organs of varying intensity, mainly renal and CNS damage. TTP and HUS are the most important forms of TMA and without adequate treatment administered early are associated with high morbidity and mortality.
Aims: To review our experience in the management of the primary TMA and to raise a series of questions that perhaps could improve the approach of these pathologies
Methods: We made a retrospective, descriptive analysis of ten cases diagnosed of primary thrombotic microangiopathy (TTP n=5; typical HUS n=3; atypical HUS n=2) over the last eight years, 70% of which were women with an average age between 40-60 years. Only three cases had previous records of autoinmune diseases (MTCD, RA and HIV), all of which would eventually develop TTP. We requested ADAMTS13 levels on all cases, they were low (<5-10%) only in those pacients diagnosed with TTP, and on the other hand confirming the positivity for Shiga toxin in those patients who eventually developed typical HUS.
Results: Regardless of the diagnosis, 10-12 plasma exchanges were performed to improve the biological parameters of hemolysis, requiring the placement of a central catheter, most commonly at the right jugular vein (70%) due to the lower risk of thrombotic and infectious complications. Although renal involvement is frequent in HUS, only two of the patients required dialysis without recovery of baseline renal function. Is to be noted the part played by the aproval in 2011 of eculizumab and how patients eight years ago suffered a torpid course, requiring a greater number of plasmapheresis and the side effects this carries. However, we still do not know its repercussions out of indication. In our study, it was used in a patient with a diagnosis of HUS associated with an infection with good evolution, although perhaps this result is due only to the natural evolution of the disease. Another controversial point is the use of antibiotics, which are known to worsen the clinical course of these processes, but because of a concomitant infection or new positive determination of the Shiga toxin, as occurred with two of our patients diagnosed of HUS had to be used. Finally, 30% of the patients have relapsed after the first episode with a primary diagnosis of a TTP.
Summary/Conclusions: Thrombotic microangiopathies are a group of processes of enormous complexity, in addition to the low frequency with which they are usually present in our usual clinical practice requiring a large deployment of means to reach an early diagnosis and begin treatment as soon as possible given that the unfortunate prognosis of these patients. With this study we have raised a series of questions to improve the management of this type of diseases:
The need to request levels of ADAMTS13 in patients diagnosed with TTP or to repeat the determination of Shiga toxin in patients with typical HUS as part of the disease follow-up and to try to prevent possible relapses.
The use of eculizumab out of indication in typical HUS and whether the improvement in the picture is due to the drug or by natural evolution of the disease. The real benefit of using plasmapheresis in patients diagnosed with typical HUS. The use of antibiotics and possible harm to the diagnosis made.

PB2240

HAEMOVIGILANCE REPORTS OF ADVERSE BLOOD DONOR REACTION AMONG VOLUNTARY BLOOD DONORS IN TERTIARY CARE HOSPITAL IN KATHMANDU, NEPAL

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Background: Voluntary blood donation is widely considered to be safe with very minimum chance of adverse reaction, which may occur during or after the end of phlebotomy procedure
Aims: To identify and understand the complication of adverse donor reactions though the incidence of reactions of blood donation among blood donor in the tertiary care hospital in Nepal
Methods: This is a prospective study done among voluntary blood donors at Grande International Hospital, Kathmandu, Nepal from February 2013 to March 2015. The outlines of reported and communicated adverse donor reaction were also collected after the blood donation from voluntary blood donors in different locations including outdoor and in-house blood donation drive
Results: In the present study 6,955 whole blood donors were included, during the period of 2 years, 105 (1.50%) adverse donor reactions were reported. Majority 89(84.76%) of adverse donor reactions were mild in nature such as, sweating; 27(25.72%), Light headedness; 19(18.09%), nausea and vomiting; 15(14.28), allergy and bruises;11(10.47%), sore arm; 9(8.58%) and hematoma; 8(7.62%) while 16 (15.24%) were severe adverse reactions similarly, anaphylaxis;11(10.49%), loss of consciousness; 3(2.85%) and convulsive syncope;2(1.90%). Markers of the adverse donor reaction were age, sex, pulse, weight, blood pressure and donation status. Age and first time status were related with significantly higher risk of adverse reaction with 18-23 years old at higher risk compared to 24-55 years old. First time donors were at higher risk compared to repeated volunteer donors
Summary/Conclusions: The results of the study are helpful to identify and understand the complication of adverse donor reactions though the incidence of reactions in the blood donors is lower than in other studies. Donor age and donation status were strong possibilities of complications.

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Late Breaking Oral Session

LB2600 *This abstract is part of the Presidential Symposium*

NOVEL SMALL MOLECULE INHIBITORS CO-TARGETING CK1A AND P-TEF-B DISRUPT SUPER-ENHANCERS AND ERADICATE ACUTE MYELOID LEUKEMIA IN A MOUSE MODEL

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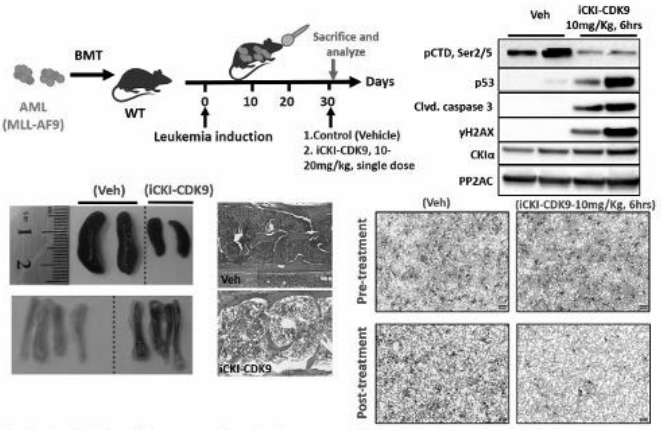
Background: Whereas p53 is mostly non-mutated in AML, various oncogenic pathways, frequently through enhancing the activity of its major antagonist Mdm2, suppress its activity. We have previously showed that genetic ablation of CKIα robustly activates p53 *(doi:10.1038/nature09673*). However, with no selective CKIα inhibitors for *in vivo* use, the therapeutic value of CKIα inhibition in hematological malignancies cannot be validated.
Aims: To develop small molecule CKIα inhibitors and assess their effect in mouse models of human leukemia.
Methods: CKIα inhibitors were identified via cell-based screening based on p53 activation. We focused on a small class of pyrazole-pyrimidine scaffolds, which through extensive medicinal chemistry yielded derivatives with high affinity binding, validated by crystallography studies, potent CKIα inhibitory activity and a good pharmacokinetic profile. Anti-leukemic activity was assessed by oral treatment in mouse models of AML: MLL-AF9 and Bcr-Abl Blast Crisis
Results: We first demonstrated the inhibitors' anti-leukemic effect by single oral dose treatment, robustly inducing p53 activation and blast cell cytoreduction (Figure 1).



Single-dose inhibitor effects treated for 6hrs (BM Western blot and blood smear) and 16hrs (tissue records), in heavily leukemic mice, showing strong cytoreduction in the spleen and bone marrow and pro-apoptotic signaling of combined inhibition of P-TEFb (CDK9) and CKIa, evident by RNA Pol II phospho-CTD inhibition and activation of DNA damage response (gH2AX) and p53

Figure 1.

These inhibitors distinguished leukemic from normal hematopoietic stem cells: they did not affect normal hematopoietic CFUs, but eliminated leukemic CFUs at an IC50 <9nM. We tested the long-term oral therapeutic effects of the inhibitors in MLL-AF9 leukemic mice. Whereas all vehicle-treated mice succumbed to the disease within a month, 40-50% of inhibitor-treated mice survived with no signs of disease up to 5 months' observation, nor had the surviving mice any sequela of long-term treatment; all had normal blood counts and normal organ morphology and histology. Long-term leukemia control with possible cure, attesting to eradication of LSCs and preservation of normal HPSCs was demonstrated by transplanting leukemia-treated BM into lethally irradiated mice: all transplanted mice recovered and none showed any evidence of residual disease within 6 months. To elucidate the mechanisms by which the inhibitors distinguished leukemia from normal hematopoietic cells, we profiled the kinome affinity of the inhibitors and further studied their signaling effects *in vitro* and *in vivo*. We found that CKIα inhibitors having potent anti-leukemia activity are distinguished from less active analogues by their capacity to co-target CDK9 and suppress the RNA Pol II elongation factor P-TEFb (CDK9-CyclinT1 complex). This property, validated by co-crystallography studies, enables the inhibitors to disrupt super-enhancers (SE), demonstrated by suppression of chromatin H3K27 acetylation and Brd4 association. As a result, transcription of SE-dependent major anti-apoptotic leukemia oncogenes including Mdm2, Bcl-2 and Mcl-1 was nearly abolished and inhibitor-treated leukemia cells underwent apoptosis. Strikingly, brief drug exposure (10mins *in vitro*; 2hrs *in vivo*) results in prolonged (24hrs) SE suppression. This unique property, which is at variance with the current occupancy-driven pharmacological paradigm, likely, contributes to the dramatic therapeutic effect of co-targeting CKIα and P-TEFb in leukemia.
Summary/Conclusions: We developed a new class of small molecule inhibitors that co-target CKIα and P-TEFb. These inhibitors induce very rapid, robust activation of p53 in synergy with shutdown of leukemic super-enhancers, resulting in a lasting, powerful and specific anti-leukemic therapeutic effects in-vivo, with cure potential.

LB2601

CRYPTIC INSERTIONS OF IMMUNOGLOBULIN LIGHT CHAIN ENHANCER REGIONS ACTIVATE CCND3 AND CCND2 IN CYCLIN D1-NEGATIVE MANTLE CELL LYMPHOMAS

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Background: Mantle cell lymphomas (MCL) are characterized by the primary translocation t(11;14)(q13;q32) involving *CCND1* and IGH genes in virtually all cases. Recently, a small subset of cyclin D1-negative (cyclin D1$^-$) MCL has been recognized. About half of these cases have *CCND2* gene rearrangements and overexpression of this gene. However, the primary oncogenic events in cyclin D1$^-$/cyclin D2$^-$MCL still remain elusive.
Aims: To identify potential mechanisms driving the pathogenesis of cyclin D1$^-$/cyclin D2$^-$MCL.
Methods: We investigated 66 cyclin D1$^-$/SOX11$^+$MCL cases by a combination of fluorescence *in situ* hybridization (FISH), gene expression profiling by Affymetrix U133+2.0 and qPCR (n=51), and copy number arrays (n=47) (Agilent CGH 1M, Affymetrix Oncoscan and 500K). Six cases were investigated by genome-wide sequencing including 4 mate-pair whole-genomes, 4 whole exomes, and 1 whole-genome sequencing. The male/female ratio was 2.5:1 and median age at diagnosis 66 years.
Results: Most cyclin D1$^-$MCL (49/51, 96%) showed overexpression of other G1 cyclins: *CCND2* in 33/50 (66%), *CCND3* in 12/51 (24%), and moderate overexpression of both *CCNE1* and *CCNE2* in 4/35 (11%). *CCND2* rearrangements were detected by FISH in 25/33 cases (76%) with *CCND2* overexpression, but the remaining *CCND2*$^+$ cases and those with *CCND3* overexpression did not show *CCND2*, *CCND3* and IG rearrangements using currently used break-apart probes. Interestingly, by mate-pair whole-genome and whole-exome sequencing analyses we discovered cryptic insertions of IG light chain regions including the enhancer regulatory elements (2 IGK and 1 IGL) near *CCND3* gene in the three cases with cyclin D3 overexpression. These rearrangements were confirmed by Sanger sequencing and FISH with specifically designed probes to recognize the cryptically rearranged regions. Furthermore, using these probes we detected 6 additional cases with cryptic IGK-*CCND3,* as well as 3 cases with IGK-*CCND2* juxtaposition in tumors with high levels of *CCND3* and *CCND2,* respectively. Taken together, 74% and 18% cases corresponded to cyclin D2$^+$ and cyclin D3$^+$ MCL, respectively, whereas 6% showed overexpression of *CCNE1* and *CCNE2* without gene rearrangements. The whole-genome analysis of one cyclin D1$^-$ MCL with *CCNE1* and *CCNE2* overexpression identified 29 somatic protein-coding mutations, 42 complex structural variants and 24 copy number alterations (including *CDKN2A* and *RB1* homozygous deletions) but not rearrangements involving any of the IG genes or G1 cyclins. The global genomic profile of 47 cyclin D1$^-$ MCL was highly complex (mean 13 alterations/case) and similar to 102 conventional SOX11$^+$ MCL, with significantly more gains at 7p and 18q in the cyclin D1$^-$ MCL. Moreover, 32% cases had chromothripsis at least in one chromosome.
Summary/Conclusions: We have identified a novel IG light chain locus-associated rearrangement, consisting of cryptic insertion of IG enhancer near *CCND3* gene that leads to cyclin D3 overexpression. Similarly, we found cryptic insertions of IGK enhancer region into *CCND2* gene. Both aberrations were undetectable by cytogenetics and FISH break-apart approaches. Overall, 65/66 (98%) MCL had G1 cyclin overexpression. The detection of these rearrangements with cus-

tom FISH probes or that of high levels of *CCND2* or *CCND3* by qPCR, together with SOX11 expression, are helpful diagnostic tools to recognize cyclin D1− MCL and provide insights on the pathogenesis of this rare subgroup.

LB2602

ARNT/HIF-1BETA LINKS POOR CLINICAL OUTCOME TO MICROENVIRONMENTAL HYPOXIA AND HIGH-RISK 1Q GAIN IN MULTIPLE MYELOMA

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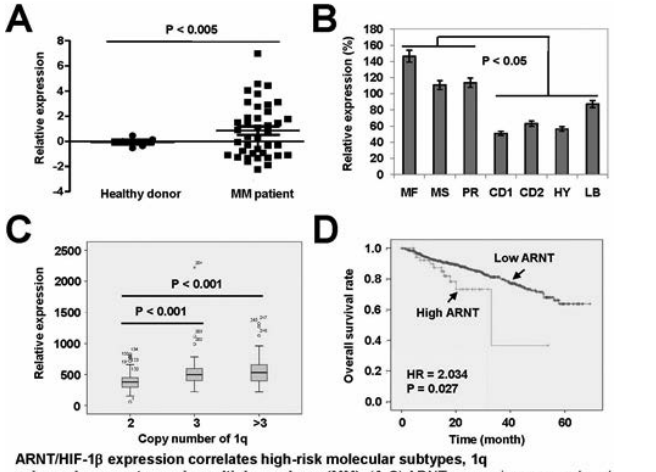
Background: 1q (1q21 gain) is a common high-risk subtype of multiple myeloma (MM), which drives MM progression, confers drug resistance, and correlates with inferior outcome. However, the molecular mechanism underlying the adverse prognostic roles of 1q remains largely unclear. Recently, 1q has been linked to hypoxia and resulting drug-resistant gene expression.
Aims: To understand the function and clinical significance of hypoxia-induced factor-1β (HIF-1β), a gene located in the 1q21 region, in 1q MM and hypoxic microenvironment.
Methods: The relationship between 1q or HIF-1β and Btz response or overall survival (OS) was analyzed in patients with newly-diagnosed MM (NDMM). Western blot and qPCR analyses were performed to determine expression of HIF-1β and other 1q21 genes in 1q+ *vs* 1q− or drug-resistant MM cells, or under hypoxia. The function of HIF-1β was evaluated using genetic means and pharmacological inhibitors.
Results: In a cohort of 180 NDMM patients, median OS (mOS) was 29 and 43 months for cases with (w) or without (w/o) 1q (P=0.038), among which 24.3, 43.3, and 43.8 months for 1q copy number >3,=3, and=2 (P=0.030), respectively; whereas Btz-based therapy displayed a marked increase in response rate ≥VGPR, it failed to improve mOS of 1q patients significantly (28.5 and 33.9 months for patients w or w/o Btz treatment, P=0.983); in contrast, Btz treatment dramatically prolonged mOS in patients w/o *vs* w 1q (53.7 and 28.5 months, P=0.016). To explore the molecular basis for the adverse effect of 1q on prognosis, expression of the 1q21 genes related to drug resistance was examined. Notably, robust expression of HIF-1β at protein level was found in 1q+ MM cells, while no difference observed in CKS1B, a biomarker widely used for 1q MM, or PSMB4 and MCL-1. Further, analysis of additional 40 NDMM patients revealed that HIF-1β mRNA level was significantly higher in MM patients, compared to normal donors (n=5, P <0.005); analysis of the microarray database UAMS "Multiple Myeloma DataBase" (University of Arkansas) also showed that HIF-1β expression was higher with MM progression, in high (*e.g.*, MF, MS, PR) *vs* low risk (*e.g.*, CD1, CD2, HY, LB; P <0.05) subtypes, or in w 1q *vs* w/o 1q (P <0.001 for copy number ≥3), as well as correlated to shorter OS (P=0.027). In the *in vitro* study, HIF-1β was markedly upregulated in MM cells acquired drug-resistance against Btz and lenalidomide, while no changes observed in other 1q21 genes (*e.g.*, PSMD4, CKS1B). Ectopic expression of HIF-1β in 1q− cells reduced sensitivity of Btz. Hypoxia (1% O_2) or its chemical mimetic lactic acid induced HIF-1β expression and Btz resistance, an event reversed by shRNA knockdown of HIF-1β. Furthermore, hypoxia-induced HIF-1β expression was associated with activation of NF-κB, which was prevented by the IKK inhibitor parthenolide, leading to restoration of Btz sensitivity in Btz-resistant cells (Figure 1).



ARNT/HIF-1β expression correlates high-risk molecular subtypes, 1q gain, and poor outcome in multiple myeloma (MM). (**A-C**) ARNT expression was analyzed in newly-diagnosed MM patients vs normal donors (**A**), in high- (MF, MS, PR) vs low-risk (CD1, CD2, HY, LB) molecular subtypes of MM patients (**B**); and in patients with (copy number = or > 3) vs without 1q (copy number = 2) MM. (**D**) The relationship between ARNT expression and overall survival of MM patients was examined by Kaplan-Meier analysis.

Figure 1.

Summary/Conclusions: Together, these findings argue that HIF-1β represents a novel biomarker for risk stratification and prognostic prediction of MM patients, especially those with high-risk cytogenetics such as 1q. They also suggest that HIF-1β might play a critical role in drug resistance related to microenvironmental factors (particularly hypoxia) and 1q21 gain, therefore serving as a potential therapeutic target for development of agents or therapy to overcome intrinsic and acquired drug resistance in MM.

LB2603

ANTI-CD69 MAB TREATMENT INCREASES THE CAPACITY OF NK CELLS TO ELIMINATE HYPER-REACTIVE ALLOGENIC T CELLS AND PREVENTS ACUTE GRAFT *VERSUS* HOST DISEASE

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Background: Hematopoietic stem cell transplantation remains the best therapeutic option for blood malignancies. Acute graft *versus* host disease (aGvHD) is one of the main potentially fatal complications of this treatment with an incidence as high as 50%. The NK cell population has been extensively studied as a potential target for treatments, as these cells have the capacity to potentiate the graft *versus* leukemia effect with a minimum risk for graft *versus* host reactions. Indeed, the abundance of circulating NK cells has been inversely correlated with the probability to develop (aGvHD). CD69 is a C-type lectin expressed on the surface of certain immune cell progenitors as well as activated mature leukocytes. CD69$^{-/-}$ NK cells were previously shown to eliminate tumour cells more effectively than WT NK cells.
Aims: We wished to examine whether CD69$^{-/-}$ NK cells would have a higher cytolytic capacity against activated allogenic T cells and whether this would lead to successful aGVHD prevention.
Methods: We took advantage of a fully allogenic aGvHD mouse model in which wild type (WT) or CD69$^{-/-}$ BALBc mice were lethally irradiated and reconstituted with C57/BL6 HSCs and naïve T cells. Results were confirmed by *in vivo* killing assays as well as by use of CD69 neutralizing antibodies. Mouse strains deficient in T cells, B cells and NK cells were used to establish the NK cells as the population responsible for the observed phenotype. Mass cytometry was employed for extensive phenotyping of WT and CD69$^{-/-}$ NK cells and RNAseq analyses were used to elucidate the molecular mechanisms implicated.
Results: CD69$^{-/-}$mice were highly resistant to aGvHD and significantly more efficient at eliminating hyper-reactive allogenic T cells *in vivo*. This phenotype was reproduced in WT mice treated with a CD69 neutralizing monoclonal antibody during disease induction. Mass cytometry analyses showed that NK cells lacking CD69 expression upregulate the Ly49D and Ly49G2 receptors, responsible for self/non-self discrimination. Further, expression of inhibitory receptors such as CD94/NKG2A was downregulated in CD69$^{-/-}$ NK cells. Finally, *in vivo* data and RNAseq analyses indicated that CD69$^{-/-}$ NK cells are resistant to apoptosis. Preliminary data on NK cell chimerism from HSCT patients indicate that host NK cells can persist shortly after conditiong and transplant, and could be targeted with anti-CD69 mAb to avoid clonal expansion of highly reactive donor T cells.
Summary/Conclusions: NK cells treated with anti-CD69 mAb show a higher capacity to eliminate hyper-reactive allogenic T cells and confer resistance to aGvHD. This data could pave the way for novel therapeutic strategies to optimize allogenic HSCT.

LB2604

GLOBAL PIVOTAL PHASE 2 TRIAL OF THE CD19-TARGETED THERAPY CTL019 IN ADULT PATIENTS WITH RELAPSED OR REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA – AN INTERIM ANALYSIS

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Background: CTL019 is an investigational chimeric antigen receptor (CAR) T-cell therapy with a high rate of durable complete responses (CRs) and a manageable safety profile in a previously reported single-center trial in adult patients (pts) with R/R DLBCL.
Aims: Results of a planned interim analysis of a single-arm, open-label, multicenter, global phase 2 trial of CTL019 in pts ≥18 y with R/R DLBCL (JULIET; NCT02445248) are reported.
Methods: Industry-manufactured CAR T cells were provided to pts at 27 centers on 4 continents using a global supply chain. Pts had received ≥2 lines of chemotherapy and had disease progression after or were ineligible for autologous stem cell transplant (autoSCT). Autologous T cells were transduced with a lentiviral vector encoding an anti-CD19 CAR, expanded, cryopreserved, shipped, and infused at study sites. The primary endpoint (centrally reviewed by an independent review committee) was best overall response rate (ORR: CR + partial response [PR]).
Results: 141 pts were enrolled. Following restaging, bridging therapy, and lymphodepleting chemotherapy (fludarabine 25 mg/m^2/cyclophosphamide 250 mg/m^2/day × 3 days or bendamustine 90 mg/m^2/day × 2 days), 85 pts received a single dose of CTL019 transduced cells (median, 3.1 × 10^8 [range, 0.1-6.0 × 10^8] cells). Median time from infusion to data cutoff (20 December 2016) was 3.7 mo. Median age was 56 y (range, 24-75) and median prior lines of antineoplastic therapy, 3 (range, 2-7). 51% of pts had prior autoSCT. Among 51 pts with ≥3 mo follow-up or earlier discontinuation, best ORR was 59% (95% CI, 44% to 72%) with 43% CR and 16% PR; the primary endpoint was met. CR and PR rates at 3 mo were 37% and 8%, respectively. All pts in CR at 3 mo remained in CR at data cutoff. Efficacy was observed across prognostic subgroups. Median duration of response was not reached. CTL019 was detectable in peripheral blood by quantitative PCR for up to 355 days in responders. Cytokine release syndrome (CRS) was graded using the Penn scale and managed by a protocol-specific algorithm. CRS occurred in 57% of infused pts (17% grade 3; 9% grade 4); no CRS-associated deaths occurred. 16% of pts received tocilizumab for CRS management. 13% of pts had grade 3/4 neurologic adverse events (AEs), managed with supportive care; no cerebral edema was reported. Grade 3/4 cytopenias lasting >28 days and grade 3/4 febrile neutropenia occurred in 21% and 14% of pts, respectively. 3 pts died from disease progression within 30 days of infusion. No deaths were attributed to CTL019.
Summary/Conclusions: This planned interim analysis of a global study of CTL019 in adults with R/R DLBCL confirms the high response rates and durable CRs observed in the previous single-center experience in a cohort of highly pretreated patients. Centralized manufacturing was feasible. CTL019 was generally tolerated without instance of treatment-related mortality. CRS and other AEs could be effectively and reproducibly managed by appropriately trained investigators.

LB2605

INDUCTION OF HEMOGENIC REPROGRAMMING IN HUMAN FIBROBLASTS

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Background: Hematopoietic stem cells (HSCs) are multipotent stem cells capable of sustaining all mature blood cells throughout life. During development, HSCs arise directly from specialized endothelial cells called hemogenic endothelial (HE) cells within the developing aorta-gonad-mesonephros (AGM) region, in a process termed endothelial-to-hematopoietic transition (EHT). However, despite extensive studies in various animal models, the genetic program driving human HSC emergence remains largely unknown.We have previously reported the generation of hemogenic precursor cells from mouse fibroblasts with the expression of the transcription factors (TFs) Gata2, cFos, Gfi1b and Etv6. These TFs induce a dynamic, multi-stage hemogenic process that progresses through an endothelial-like intermediate, recapitulating developmental hematopoiesis *in vitro*.
Aims: Here, to better understand the molecular events underlying human HE cell specification we expressed hemogenic TFs in human fibroblasts and mapped the TF binding sites at initial stages of reprogramming.
Methods: To determine the transcription factors binding sites we used Chromatin Immunoprecipitation coupled with sequencing (ChIP-seq).
Results: We demonstrate that human fibroblasts can be reprogrammed into hemogenic cells by the expression of GATA2, GFI1B and FOS. Induced cells express CD34 and CD49f and display dynamic endothelial to hematopoietic transcription programs. In addition, reprogrammed fibroblasts repopulate immunodeficient NSG mice and generate hematopoietic progeny of multiple lineages, including T-cells and myeloid cells. Mechanistically, GATA2 display dominant and independent targeting activity during the early phases of reprogramming while GFI1B depends on GATA2 to bind most of its targets. Interestingly, GATA2 and GFI1B interact and co-occupy a cohort of target sites engaging sites preferentially with AP-1 motifs, including the RUNX1 locus. This cooperative binding is reflected by the engagement of open enhancers and promoters marked by H3K4me3, H3K4me1 and H3K27ac in the fibroblast genome that initiates the silencing of fibroblast genes while activating the hemogenic program.
Summary/Conclusions: Together, these findings uncover a collaborative TF interaction that specify a human hemogenic program and EHT. These findings shed light on the processes controlling human HSC specification and provide means to generate human reprogrammed HSCs at high efficiency for transplantation.

LB2606

BONE MARROW SITES DIFFERENTLY IMPRINT DORMANCY AND CHEMORESISTANCE TO T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: T-cell acute lymphoblastic leukemia (T-ALL) is a disease of T-cell progenitors, which mainly affects children and young adults. Numerous genomic alterations such as *NOTCH1/FBXW7* mutations, *TLX1/3* overexpression or *SIL-TAL1* deletion are known to induce survival, proliferation and differentiation block in T-ALL cells. Interactions between leukemic cells and their microenvironment also contribute to T-ALL pathogenesis. Cell-cell contacts - Delta-Like/Jagged-Notch1, integrin LFA1/ICAM1 - and secreted factors - such as interleukin 7 and 18 or CXCL12 - are key players in T-ALL development. In the course of the disease, T-ALL cells settle in various environments such as thymus, blood, bone marrow (BM), pleura or lymph nodes, which differ in terms of cell content, extracellular matrix and secreted factors. To which extent these distinct niches imprint niche-specific features on T-ALL cells is not well understood.
Aims: Compare the growth of leukemic cells from human and mouse T-ALL in various BM sites. Uncover novel mechanisms of chemoresistance, in relation with the BM microenvironment.
Methods: We used grafts of human and mouse T-ALL in immune-deficient and normal mice, respectively. We explored the behavior of leukemic cells ex-vivo and *in vivo* after they had engrafted different BM sites of the mouse body (femurs, Thorax and Tail vertebraes). We tested their respective chemoresistance to conventional drugs (dexamethasone, vincristine, cytarabine).
Results: We observed that mouse and human T-ALL develop slowly in tail vertebrae BM compared to thorax vertebrae and femur BM. T-ALL recovered from tail BM display lower cell surface marker expression and decreased metabolism and cell cycle progression, demonstrating a dormancy phenotype. Functionally tail-derived T-ALL exhibit a deficient short-term *ex vivo* growth and a delayed *in vivo* propagation. These features are non-cell autonomous as T-ALL from tail and thorax share identical genomic abnormalities and functional disparities disappear *in vivo* and in prolonged *in vitro* assays. Importantly tail-derived T-ALL display higher intrinsic resistance to cell cycle-related drugs, *i.e.* vincristine sulfate and cytarabine, but not to dexamethasone. T-ALL recovered from gonadal adipose tissues or from co-cultures with adipocytes share metabolic, cell cycle and phenotypic or chemoresistance features with Tail-derived T-ALL.
Summary/Conclusions: These results demonstrate that BM sites differentially orchestrate T-ALL propagation. T-ALL derived from adipocyte–rich BM are associated with quiescence and decreased response to cell cycle dependent chemotherapy indicating that adipocyte-rich aged BM or pathologies enhancing BM adipocyte content may help leukemia escaping drug treatment.

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